

**Fig. 3** Correlations between SN-38 AUC and ANC nadir in patients in irinotecan monotherapy (a) and combination therapy with cisplatin (b).  $r$  Spearman's rank correlation coefficient

metabolites in Caucasians treated with irinotecan monotherapy [18] and to lower the incidence of grade 3 diarrhea in Koreans treated with a combination therapy of irinotecan and cisplatin [24]. In the current study, no significant association of *ABCC2*\*1C/G on PK/PD was observed in the monotherapy. Although a high incidence of grade 3/4 neutropenia was observed in patients with *ABCC2*\*1C/G in the combination therapy with cisplatin, most patients also had *ABCG2*\*IIB (data not shown); thus, the effect of *ABCC2*\*1C/G remains obscure.

For *ABCG2*, the current study examined the association with the combinatorial haplotypes consisting of the three previously defined block haplotypes [28]. *ABCG2*\*IIB contains the non-synonymous SNP 421C>A (Q141K), which was detected at higher frequencies in Asians and was reported to cause reduced expression of BCRP in vitro [36, 39–41]. In clinical studies, the association of 421C>A (Q141K) with higher plasma levels of diflomotecan was shown in Caucasians [42]. However, an association of this SNP with irinotecan PK/PD had not been shown [19, 24]. An association of 421C>A (Q141K) alone with irinotecan PK/PD was not significant in our hands (data not shown), but \*IIB containing both 421C>A (Q141K) and IVS12 + 49G>T showed a moderate association with neutropenia. It is unclear whether the additional SNP IVS12 + 49G>T itself or another unknown linked SNP is causative for the reduced function. *ABCG2*\*IIC contains a non-synonymous SNP 34G>A (V12M) which has no influence on BCRP expression or activity in vitro [36, 39–41]. Our study showed no influence of *ABCG2*\*IIC on the SN-38 AUC/dose levels and neutropenia in the irinotecan monotherapy (data not shown), but did show a decreasing trend in grade 3/4 neutropenia in the combination therapy with cisplatin. In contrast, a report on Korean patients

suggested the association of *ABCG2* 34G>A (V12M) with a higher incidence of grade 3 diarrhea in a combination therapy of irinotecan and cisplatin [24].

Among *SLCO1B1* polymorphisms, 521T>C (V174A), a tagging SNP of \*15 · 17, was demonstrated to reduce in vitro SN-38 influx [7], and clinical studies in Asians also showed its relevance to a higher SN-38 AUC and severe neutropenia in combination therapy of irinotecan with cisplatin [22–24]. Our results support these previous findings. Note that our \*15 · 17 mainly consists of \*17 [containing -11187G>A, 521T>C (V174A) and 388A>G (N130D)].

Taken together, the clinical data on transporter genotypes show variability among the studies. The reasons for these conflicting findings might be partly attributed to the ethnic differences in transporter genotypes and the regimens used. In addition, non-genetic factors, such as disease status and inflammation [43, 44], hepatic or renal function [45], and co-administered or pre-administered drugs, may also influence the clinical outcome.

The current study suggests combined effects of multiple haplotypes/variants on neutropenia. From clinical aspects of irinotecan therapy, the benefit of additional genotyping of transporters to predict severe toxicities should be clarified. Regarding grade 3 and 4 neutropenia, positive prediction values for two or more candidate genotypes including *UGT* (+) (Fig. 2) were 46 and 89% in the monotherapy and the cisplatin-combination therapy, respectively, which are low compared with *UGT*+/+ (80 and 100%, respectively). Regarding grade 4 neutropenia, positive predictive values for these candidate genotypes were 15 and 41% in the monotherapy and the cisplatin-combination therapy, respectively, while for *UGT*+/+, they were 0 and 43%, respectively. Further studies using a

larger population size are needed to further elucidate the roles of these candidate markers.

In conclusion, the current study suggests there are additive effects for several transporter genotypes on the SN-38 AUC level and the reduction of neutrophil counts in irinotecan therapy. The clinical benefits of additional genotyping of these candidate markers should be further delineated.

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## Sequential chemotherapy with methotrexate and 5-fluorouracil for chemotherapy-naïve advanced gastric cancer with disseminated intravascular coagulation at initial diagnosis

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### Abstract

**Purpose** Advanced gastric cancer (AGC) rarely presents with disseminated intravascular coagulation (DIC) at the time of diagnosis before treatment with no current standard chemotherapy (CTx) regimen. However the prognosis is extremely poor without CTx. We investigated the effectiveness of sequential CTx with methotrexate and 5-fluorouracil (MF) in chemotherapy-naïve AGC patients with DIC.

**Methods** We retrospectively examined AGC patients who received first-line CTx and selected those who were diagnosed with DIC before starting CTx to investigate clinical characteristics and responses.

**Results** From July 1999 to January 2007, 1,365 patients with unresectable or recurrent AGC received first-line CTx at the National Cancer Center Hospital in Tokyo, Japan. DIC was diagnosed in 22 (1.6%) patients (16 men and 6 women; median age, 56 years) and the performance status of all the patients was 1/2/3 = 9/10/3. Nineteen patients (86%) had histologically diffuse-type adenocarcinoma and 18 (82%) had bone metastasis. Patients received sequential MF every week until progressive disease was confirmed, with DIC improving in 17 (77%) patients. The median time-to-treatment failure for AGC and overall survival

were 98 days [95% confidence interval (CI), range 50–146 days] and 154 days (95% CI, range 126–180 days), respectively. Grade 3 or greater toxicities consisted of neutropenia (4 patients, 18%), anemia (9 patients, 40%), thrombocytopenia (4 patients, 18%), and bilirubinemia (1 patient, 5%).

**Conclusions** MF was an effective and well-tolerated regimen for improving DIC in chemotherapy-naïve AGC patients with DIC; however, the prognosis of the patients remained poor even with improved DIC parameters.

**Keywords** Gastric cancer · Disseminated intravascular coagulation · Chemotherapy · Methotrexate · 5-fluorouracil

### Introduction

Disseminated intravascular coagulation (DIC) is a clinical condition in which various underlying diseases pathologically activate the coagulation system. DIC is characterized by multiple thrombi in microvessels (Levi and Ten Cate 1999; Sase et al. 2003). Subsequent microcirculation failure can induce organ injury, while exhaustion of coagulation factors and platelets induces a bleeding tendency. Underlying diseases causing DIC include hematological malignancies, infection, sepsis, and trauma. Solid tumors can be complicated by DIC (Al-Mondhry 1975; Sallah et al. 2001), which occurs in approximately 10% of patients with solid tumors between the time of diagnosis and death (Okajima et al. 2000).

The prognosis of advanced gastric cancer (AGC) patients with DIC is extremely poor, and life expectancy without any intervention is only 1–3 weeks (Al-Mondhry 1975; Sallah et al. 2001). DIC treatment includes chemotherapy (CTx) to control the underlying disease. However,

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only a few studies have examined the effectiveness of CTx for AGC with DIC (Chao et al. 2000; Hironaka et al. 2000; Huang et al. 2008; Tokar et al. 2006).

One of the standard systemic chemotherapeutic regimens for unresectable or recurrent gastric cancer is 5-fluorouracil (5-FU) combined with cisplatin (CDDP) (Kim et al. 1993; Koizumi et al. 2008). However, when AGC is complicated by DIC, the patient's systemic condition is often poor with accompanying thrombocytopenia. Anemia is often detected and may be caused by microhemolysis (Jiang et al. 1997; Tsuchiya et al. 1989). An increased bleeding tendency due to thrombocytopenia is also often observed. These abnormal bleeding conditions make CDDP administration to AGC patients with DIC difficult.

The rationale for the use of methotrexate (MTX) in combination with 5-FU (MF) is based on biochemical modulation. Pre-administered MTX inhibits purine synthesis, which causes elevated levels of intracellular phosphoribosyl pyrophosphate that facilitate 5-FU metabolism, thereby enhancing its antitumor effects (Cadman et al. 1979; Fernandes and Bertino 1980). The effectiveness of MF against various cancers, particularly metastatic colon cancer, has been studied worldwide. A meta-analysis of 5-FU monotherapy and MF confirmed the efficacy of MF for colorectal cancer (Advanced Colorectal Cancer Meta-Analysis Project 1994) as well as for other unresectable and recurrent gastric cancers (Konishi et al. 1994; Perez et al. 1998).

MF is associated with only mild hematologic and non-hematologic toxicities and thus this regimen has been administered to patients in poor general condition, including those with AGC-induced ascites or peritoneal dissemination (Hamaguchi et al. 2008; Konishi et al. 1999; Tahara et al. 2001; Yamao et al. 2004). Based on some reports, in Japan, MF is considered one of effective and safety regimens for AGC patients in poor general condition. A randomized phase III study of 5-FU continuous infusion versus MF in chemotherapy-naive gastric cancer patients with peritoneal metastasis is currently being conducted by the Gastrointestinal Oncology Study Group of the Japan

Clinical Oncology Group. In our hospital, we have been using MF as a first-line CTx for AGC patients with DIC.

In this study, we retrospectively investigated the therapeutic effects and toxicity of MF therapy in chemotherapy-naive AGC patients with DIC.

## Patients and methods

Chemotherapy-naive AGC patients with DIC were identified among those receiving CTx for AGC at the National Cancer Center Hospital between July 1999 and January 2007. Chemotherapy-naive AGC patients included those with recurrent tumors for more than 6 months following completion of oral adjuvant fluoropyrimidine CTx. We analyzed patient background, treatment courses, response to MF therapy for DIC and AGC, time-to-tumor progression, and overall survival (OS). All study participants provided written consents before participating in the study.

### Definition of DIC

We defined DIC according to the Japanese criteria issued in 1988 (Table 1) with individual patient scores based on underlying disease, bleeding symptoms, organ symptoms, and essential laboratory data, including elevated fibrin degradation product (FDP), decreased platelet count, decreased serum fibrinogen levels, and prolonged prothrombin times. DIC was diagnosed in patients with a total score of  $\geq 7$  points, and DIC was considered to improve when a patient's DIC score dropped to  $< 5$ .

### Chemotherapy regimen

MTX (100 mg/m<sup>2</sup>) was administered intravenously by bolus infusion followed by a bolus infusion of 5-FU (600 mg/m<sup>2</sup>) 3 h later. Six courses of leucovorin rescue (10 mg/m<sup>2</sup>) were administered orally or intravenously every 6 h commencing 24 h following MTX administration.

**Table 1** Diagnostic criteria of disseminated intravascular coagulation

Items/points	0	1	2	3
Basic disease	–	+		
Bleeding symptoms	–	+		
Organ symptoms	–	+		
FDP (mg/ml)	<10	≤10 to <20	≤20 to <40	≤40
Platelets ( $\times 10^4/\text{mm}^3$ )	<12	<8 to ≤12	<5 to ≤8	≤5
FIBG (mg/dl)	<150	<100 to ≤150	≤100	
PT (ratio)	<1.25	≤1.25 to <1.67	≤1.67	
Diagnosis	Total $\geq 7$	Certain DIC		
	Total 6	Suspicion of DIC		
	Total $\leq 5$	No DIC		

In an effort to prevent MTX-associated renal toxicities, acetazolamide (250 mg) was given intravenously immediately following MTX infusion, and sodium bicarbonate (33.3 mEq) was added to 500 ml of electrolyte solution and administered by drip infusion for urine alkalinization during the 3 h interval between MTX and 5-FU administrations. Treatment was repeated every week until progressive disease was observed in the patients.

#### Toxicity assessment

We evaluated each patient's physical examination records and laboratory tests at least every week during treatment, and a toxicity assessment was performed using the Common Terminology Criteria for Adverse Events version 3.0.

#### Evaluation of efficacy outcomes

Tumor response to CTx was assessed based on tumor reduction according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria for patients with measurable lesions. This objective response was evaluated every 4–8 weeks using a computerized axial tomography scan. The time-to-treatment failure (TTF) was measured from initiation to the final day of CTx, and OS was measured from CTx initiation to either the last date of follow-up or death and was censored as of the last date of contact. The TTF and OS were estimated using the Kaplan–Meier method, and statistical analyses were performed using Dr. SPSS II for Windows 11.0.1J software (SPSS Japan, Inc., Tokyo, Japan).

## Results

### Patients

A total of 1,365 chemotherapy-naive patients received CTx for recurrent or unresectable AGC from July 1999 to January 2007; 22 (1.6%) of these patients, including 16 men and 6 women, were diagnosed with DIC.

The background data of the 22 patients are summarized in Table 2. The median age was 56 years (range 26–75 years), the performance status (PS) was  $\geq 2$  in 13 of 22 cases (59%), and many patients had a poor clinical condition. In terms of histological type, diffuse-type adenocarcinomas were identified in 19 patients (86%) and macroscopic type 3 or type 4 tumors were seen in 16 patients (73%). Bone metastasis was found in 18 patients (82%).

MF was administered to all 22 patients. The median number of doses was eight (range 1–17) with 15 of the 22 patients (68%) receiving four or more doses of MF. Treatment was terminated because of disease progression in 21 patients, but one patient was still on MF at the time of this study.

**Table 2** Patient characteristics

Characteristics	No. of patients	
	(n = 22)	(%)
Sex		
Male	16	73
Female	6	27
Age, years		
Median	56	–
Range	26–75	–
Histology		
Intestinal type	3	14
Diffuse type	19	86
Macroscopic type of primary tumor		
Early	3	14
Type 2	1	5
Type 3	8	36
Type 4	8	36
Unknown	2	9
ECOG performance status		
1	9	41
2	10	45
3	3	14
Gastrectomy		
No	10	45
Yes	12	55
Metastatic site		
Bone	18	82
Lymph node	11	50
Liver	3	13
Peritoneum	2	9

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### Safety and toxicity

Toxicities related to MF are summarized in Table 3. Hematological toxicities  $\geq$  grade 3 included neutropenia in four patients [(18%) although neutropenia-induced fever was not observed], anemia in nine patients (41%), and thrombocytopenia in four patients (18%). In terms of non-hematological toxicities  $\geq$  grade 3, one patient (5%) showed an elevated bilirubin level. However, none of the patients exhibited symptoms of gastrointestinal toxicities such as nausea, vomiting, or diarrhea.

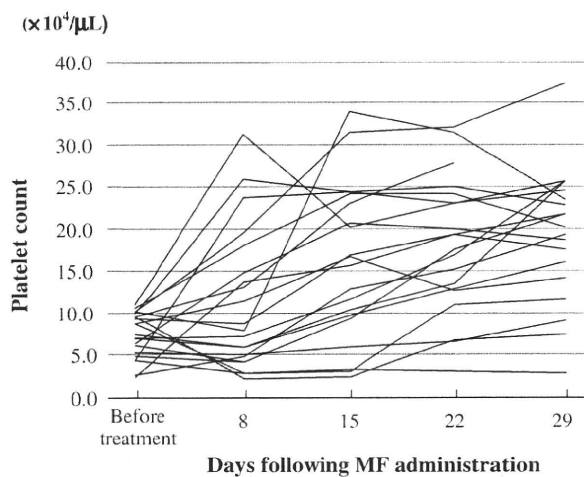
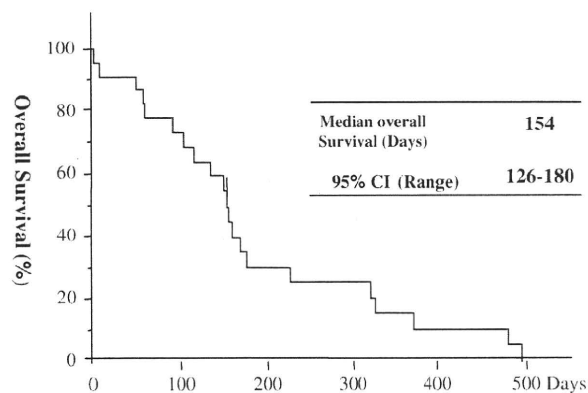
### Therapeutic outcomes

DIC improvement with a DIC score  $< 5$  was observed in 17 patients (77%). Following MF administration, most of the patients showed improvement of hematological data within

**Table 3** Toxicities of MF regimen (no. of patients)

	Grade 1	Grade 2	Grade 3	Grade 4	Grade $\geq 3$ (%)
Leukopenia	4	5	1	1	2 (9)
Neutropenia	4	4	3	1	4 (18)
Anemia	2	2	4	5	9 (41)
Thrombocytopenia	0	0	1	3	4 (18)
Bilirubinemia	1	1	1	0	1 (5)
Elevated AST/ALT	6	1	0	0	0
Elevated Creatinine	4	0	0	0	0
Nausea	10	1	0	0	0

2 weeks. Changes in platelet count, an important indicator in DIC assessment, are shown in Fig. 1. Tumor response based on the RECIST criteria could be assessed in only nine of these patients, and three of them (33%) showed evidence of a partial response. Median TTF was 98 days [95% confidence interval (CI), range 50–146 days] and median OS was 154 days (95% CI, range 126–180 days) (Fig. 2).

**Fig. 1** Changes in platelet count during MTX + 5-FU**Fig. 2** Kaplan–Meier overall survival plot

Eleven of 17 patients (65%) whose DIC improved following MF administration later showed DIC recurrence at the time of disease progression. Despite MF administration, DIC showed no improvement in 5 of the 22 patients (23%). Weekly paclitaxel (PTX) was administered immediately as a second-line CTx to two of these five patients. Both responded to the PTX treatment, showing improvement in their DIC. Overall, CTx resulted in DIC improvement in 19 of 22 patients (86%). All patients who recovered from DIC were discharged and started outpatient chemotherapy. DIC showed no response to CTx in the remaining three patients (14%). One patient died of an acute subdural hematoma 2 days after starting MF, and another died of pulmonary carcinomatous lymphangiosis 8 days after starting MF. The third patient received MF four times. However, DIC showed no improvement, and the patient died from cancer progression 60 days after starting MF. We determined that these three patients were not treatment-related death but disease progression.

## Discussion

To the best of our knowledge, this investigation is the largest single-institutional study of AGC complicated by DIC. Several other case series involving 6–19 patients (Chao et al. 2000; Hironaka et al. 2000; Huang et al. 2008; Tokar et al. 2006) are summarized in Table 4.

With respect to other solid tumors, DIC usually develops after diagnosis during the follow-up period; however, DIC can sometimes be detected during the initial diagnosis of metastatic gastric cancer (stage IV) or during recurrence after a curative surgical operation (Pasquini et al. 1995). In some patients, screening for DIC can lead to a diagnosis of AGC. There are several distinctive characteristics of AGC with DIC. In previous reports, relatively young patients were diagnosed with AGC with DIC. However, the patients in this study had a median age of 56 years. In terms of histological type, most of these patients had diffuse-type adenocarcinoma, ranging from 68 to 100%. Interestingly, there was a high frequency of bone metastasis or bone marrow involvement, ranging from 50 to 100%. In our study, bone

**Table 4** Summary of case series previously reported

Author	Regimen	n	Median age (range)	Diffuse-type adenocarcinoma	Bone metastasis	DIC response	MST (weeks)
				No. of patients (%)	No. of patients (%)	No. of patients (%)	
Chao et al.	Weekly EEPFL	6	38 (36–71)	–	3 (50)	6 (100)	30
Hironaka et al.	MF	9	–	–	9 (100)	8 (89)	16
Tokar et al.	5-FU	6	48.5 (32–56)	6 (100)	–	5 (83)	14.5
Huang et al.	5-FU/Leucovorin	19	53 (31–72)	13 (68)	13 (68)	14 (74)	12
The present study	MF	22	56 (26–75)	19 (86)	18 (82)	17 (77)	22

DIC disseminated intravascular coagulation, MST median survival time, EEPFL etoposide and epirubicin and cisplatin and 5-FU, MF methotrexate and 5-fluorouracil

metastasis was diagnosed by bone scintigraphy and/or magnetic resonance imaging. Since such tests were performed only when symptoms were evident, the actual frequency of bone metastasis may have been higher. Although we did not conduct bone marrow tests, we suspect that most patients probably had bone marrow infiltration with the resultant bone marrow dysfunction leading to a predisposition to DIC development. In this case, AGC with DIC would be quite different from AGC without DIC.

When AGC is complicated by DIC, anti-cancer agents may not be used because of the poor general condition of the patient or the presence of thrombocytopenia and severe anemia. Unfortunately, the prognosis of untreated AGC with DIC is extremely poor, and patients generally live for only 1–3 weeks without CTx (Al-Mondhiry 1975; Okajima et al. 2000). Here, DIC improved in 19 of 22 patients following CTx with an OS of 167 days (95% CI, range 141–192 days), indicating that CTx was at least somewhat effective in treating AGC complicated by DIC. However, DIC showed no improvement in 3 of 22 patients, who died 2, 8, and 60 days following MF. Based on our analysis, we believe that MF provides a survival benefit for AGC patients with DIC. And 19 of 22 patients (86%) were discharged and continued outpatients chemotherapy, these data suggest that MF provide better QOL.

Several reports have been published examining the control of DIC by CTx in patients with AGC. Chao et al. administered etoposide, epirubicin, CDDP, and 5-FU to six AGC patients with DIC (Chao et al. 2000). Hironaka et al. (2000) administered MF to AGC patients with bone metastasis and reported DIC in nine of these patients. Tokar et al. reported that 5-FU administered alone and in combination with CDDP and epirubicin stopped the bleeding tendency in six AGC patients with DIC (Tokar et al. 2006). Finally, Huang et al. (2008) administered 5-FU and leucovorin to 19 AGC patients with DIC. Although each of these reports involved only a relatively small number of patients, the successful control of DIC by CTx was achieved in most patients. In our study, MF improved DIC in 17 of 22

patients (77%). Based on our results, AGC patients with DIC may respond favorably to CTx with accompanying improvement in DIC. However, even if DIC improves in these patients, their prognosis still appears to be worse than that of AGC patients without DIC.

One of the primary reasons for selecting MF is because of its mild toxicity (Hamaguchi et al. 2008; Konishi et al. 1999; Tahara et al. 2001; Yamao et al. 2004). However, the frequency of both anemia and thrombocytopenia as adverse events was higher in the present study patient group than in past clinical study patient groups. Anemia may have been caused by microhemolysis or the bleeding tendency associated with DIC. CTx improved thrombocytopenia (Fig. 1), and the other toxicities were mild and well-tolerated by the patients.

In conclusion, MF for the treatment of chemotherapy-naïve AGC patients was an effective and well-tolerated regimen for improving DIC; however, the prognosis of the patients remained poor even with improvement in DIC parameters. Although this was a retrospective study where concrete conclusions based on our findings are not possible, the results are nonetheless significant in terms of their implications for clinical practice.

**Conflict of interest statement** We received no financial support for this study and report no conflicts of interest. Informed consent was obtained from all patients before initiating chemotherapy. This study was approved by the president of National Cancer Center Hospital.

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## Effects of bevacizumab on plasma concentration of irinotecan and its metabolites in advanced colorectal cancer patients receiving FOLFIRI with bevacizumab as second-line chemotherapy

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### Abstract

**Purpose** Bevacizumab (BV) prolongs the survival of colorectal cancer patients when combined with irinotecan (CPT-11)-based regimens. In the AVF2107g study, the area under the curve (AUC) ratio for bolus CPT-11/5-fluorouracil (5-FU)/leucovorin (LV) (IFL) with the BV arm to bolus IFL with placebo indicated that SN-38 concentrations may have been increased in subjects receiving BV. However, the mechanism underlying such increase remains unclear, and the difference might be caused by an imbalance between the two arms and a possible inter-subject variability of CPT-11 metabolism. Within-subject comparisons were used to evaluate the effect of BV on advanced colorectal cancer patients when administered with the FOLFIRI regimen as second-line chemotherapy.

**Methods** Ten advanced colorectal cancer patients received the FOLFIRI regimen every 2 weeks. At cycle 1, BV was administered following FOLFIRI administration to allow baseline pharmacokinetic (PK) analysis of CPT-11 and its metabolites. From cycle 2, BV was administered just before FOLFIRI administration. Plasma samples were collected under the same condition (at cycle 3).

**Results** There were no significant differences in the  $C_{\max}$  and  $AUC_{0-\infty}$  of CPT-11, SN-38, and SN-38G between cycle 1 (without BV) and cycle 3 (with BV). PK parameters of CPT-11, SN-38, and SN-38G were not significantly

affected by BV. There were no significant differences in the changes in the AUC ratio of CPT-11 to SN-38 between cycles 1 and 3, as well as in the ratio of SN-38 to SN-38G. **Conclusion** BV does not affect the plasma concentration of CPT-11 and its metabolites on FOLFIRI regimen.

**Keywords** Bevacizumab (BV) · Irinotecan · Pharmacokinetics · Colorectal cancer

### Introduction

Bevacizumab (BV) is a humanized monoclonal antibody against vascular endothelial growth factor, an important regulator of physiologic and pathologic angiogenesis [1]. A large, randomized, controlled Phase III clinical trial (AVF2107g) has demonstrated that BV addition to standard chemotherapy with the bolus irinotecan (CPT-11)/5-fluorouracil (5-FU)/leucovorin (LV) (IFL) regimen improves survival of patients with previously untreated metastatic colorectal cancer [2]. Subsequently, CPT-11/bolus 5-FU/continuous 5-FU/LV (FOLFIRI) + BV conferred a significant survival benefit compared with IFL + BV in the BICC-C study [3]. Thus, CPT-11 with BV demonstrated significant survival benefits in patients with colorectal cancer. CPT-11 has a complex metabolism requiring activation into SN-38 by carboxylesterase [4, 5] and glucuroconjugation for catabolism [6]. As shown in the AVF2107g study, SN-38 concentrations were on average 33% higher in patients receiving bolus IFL in combination with BV compared with bolus IFL alone [7]. However, the underlying mechanism of such increase remains unclear, and the difference might be caused by an imbalance between the two arms and a possible inter-subject variability of CPT-11 metabolism. Thus, we investigated the potential pharmacokinetic (PK) interaction between CPT-11 and BV in advanced

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colorectal cancer patients when administered with the FOLFIRI + BV regimen as second-line chemotherapy.

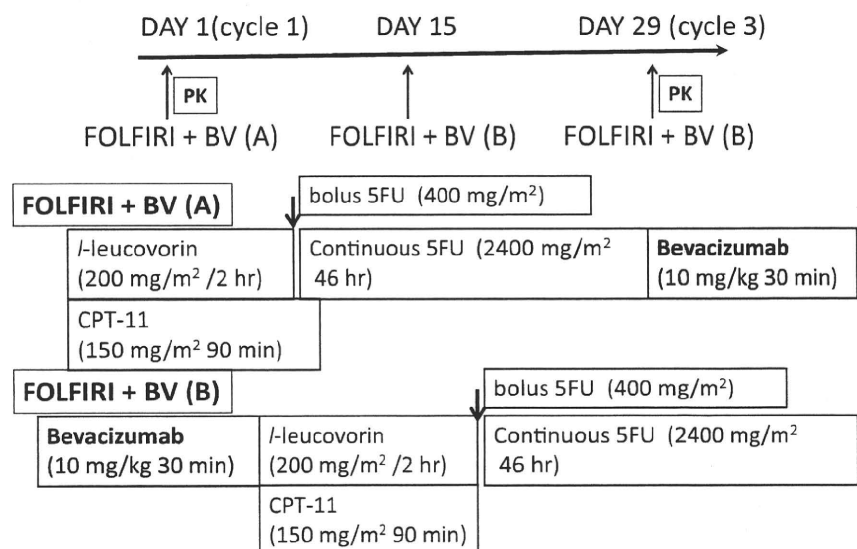
## Methods

### Inclusion and exclusion criteria

Patients meeting the following inclusion criteria were eligible: histologically proved colorectal cancer (e.g., adenocarcinoma, mucinous carcinoma, and signet-ring cell carcinoma); failure of first-line treatment containing 5-FU-based chemotherapy (almost an adjuvant setting and recurrence were found in the chemotherapy period or after the end of chemotherapy within 24 weeks) or oxaliplatin-based chemotherapy (all FOLFOX regimens) without BV and CPT-11; Eastern Cooperative Oncology Group performance status of 0–2; age: 20–74-year-old; no previous exposure to BV or CPT-11; adequate bone marrow function (leukocyte count  $\geq 3,000$  and  $\leq 12,000/\mu\text{l}$ ; hemoglobin  $\geq 8.0$  g/dl, and platelet count  $\geq 10 \times 10^4/\mu\text{l}$ ); serum creatinine level  $\leq 1.5$  mg/dl; total bilirubin level  $\leq 1.5$  mg/dl; AST and ALT  $\leq 100$  IU/l; qualitative urine protein  $\leq (1+)$ ; measurable disease according to response evaluation criteria for solid tumors (RECIST); and written informed consent.

Patients were excluded if they had the following: known central nervous system metastasis; other active double cancer; inadequately controlled hypertension, diarrhea, diabetes, or heart disease; severe peritoneal metastasis; interstitial pneumonia or pulmonary fibrosis; previous history of vascular thromboembolism or severe drug hypersensitivity; bleeding tendency; hepatic B or C virus infection; underwent any form of surgery within 4 weeks before study enrollment; pregnant or lactating.

**Fig. 1** At cycle 1, CPT-11 was administered before BV to allow baseline pharmacokinetic (PK) analysis of CPT-11 and its metabolites. At cycle 3, plasma samples were collected for PK analysis of CPT-11 when administered in combination with BV



### Study design

Ten patients were treated with the FOLFIRI regimen preceded by BV every 2 weeks. At cycle 1, CPT-11 was administered before BV to allow baseline PK analysis of CPT-11 and its metabolites. At cycle 3, plasma samples were collected for PK analysis of CPT-11 when administered in combination with BV. The PK investigations were used intra-patients comparison.

### Pretreatment and follow-up examination

Complete medical history evaluation, physical examination, laboratory tests (complete blood count, creatinine, serum electrolytes, calcium, uric acid, total protein, albumin level, hepatic, and coagulation tests) and urinalysis were performed to obtain baseline data and repeated biweekly.

Toxicity was evaluated biweekly and graded using the National Cancer Institute's Common Toxicity Criteria, version 3.0. Tumor responses were evaluated and measured as baseline data and reassessed every 4 cycles using RECIST.

### Drug administration

The FOLFIRI regimen consisted of CPT-11 (180 mg/m<sup>2</sup> IV over 90 min), l-LV (200 mg/m<sup>2</sup> IV over 2 h), and 5-FU (400 mg/m<sup>2</sup> IV bolus), followed by 5-FU (2,400 mg/m<sup>2</sup> IV over a 46-h infusion), and repeated every 2 weeks. BV was administered as a 30-min intravenous infusion at a biweekly dose of 10 mg/m<sup>2</sup> before the FOLFIRI regimen (only in the cycle 1, BV was administered after the FOLFIRI regimen for PK analysis of the non-BV phase) (Fig. 1).

**Table 1** Patient characteristics

Age (years)	
Range	38–74
Median	60
Gender	
Male	9
Female	1
Previous chemotherapy	
5-FU-based regimen <sup>a</sup>	5
FOLFOX	5
Total cycles of treatment	
Range	7–19
Median	11

<sup>a</sup> As adjuvant chemotherapy

**Pharmacokinetic analysis**

Plasma samples were collected at cycles 1 and 3 before the start of chemotherapy, and 0, 1, 2, 4, 7, and 24 h after CPT-11 infusion. Whole blood (4.0 ml) samples were collected in heparinized tubes and centrifuged at 3,000 rpm for 10 min at 4°C. Then, 2.0 ml of plasma was transferred into tubes with 2.0 ml of phosphate buffer (0.1 M) and stored at –80°C before analysis. Thereafter, quantitative analysis of CPT-11 and its metabolites was performed using high-performance liquid chromatography [8]. The lower limit of quantification was 0.002 µg/ml for CPT-11 and its metabolites. Maximum plasma concentration (C<sub>max</sub>), area under the plasma/serum concentration time curve (AUC) and terminal half-life were determined. The AUC calculation is limited up to 24 h or to infinite (∞). Changes in the ratios of CPT-11 to SN-38 and SN-38 to SN-38G were estimated as AUC<sub>SN-38</sub>/AUC<sub>CPT-11</sub> and AUC<sub>SN-38G</sub>/AUC<sub>SN-38</sub>, respectively.

**Statistical analysis**

Correlation between related species were all carried out using the paired *t* test (Microsoft Excel 2000 SP-3), and *P* values <0.05 with a two-tailed distribution were considered significant.

**Results**

**Patient characteristics**

Ten patients received the treatment regimens (Table 1), and all the patients completed the PK program and were assessable for drug safety and anti-tumor activity. A total of 120 cycles of treatment was administered (median number of cycles: 11 (range 7–19)).

**Pharmacokinetic analysis**

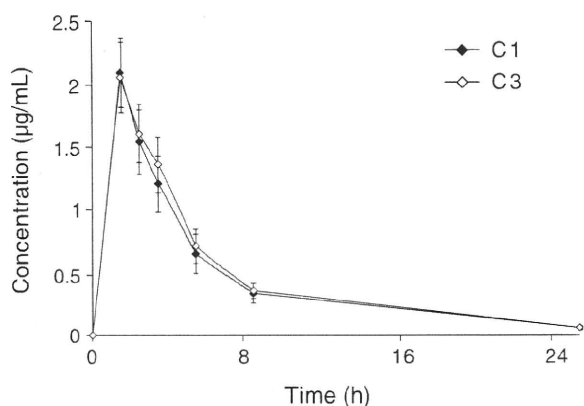
Analysis of the PK parameters showed no significant difference between the parameters of cycle 1 (non-BV phase) and cycle 3 (BV phase) (Table 2). This indicates that BV had no effect on the pharmacokinetics of CPT-11. The mean AUCs for CPT-11 were 12.2 ± 2.3 µg h/ml at cycle 1 and 12.8 ± 1.7 µg h/ml at cycle 3. The half-lives of CPT-11 were 6.0 ± 0.6 h at cycle 1 and 5.7 ± 0.6 h at cycle 3. Mean CPT-11 concentrations versus time profiles either alone or in combination with BV were nearly superimposed (Fig. 2).

The mean SN-38 PK parameters showed no significant differences between cycles 1 and 3 (Table 2). The mean AUCs for SN-38 were 0.40 ± 0.44 µg h/ml at cycle 1 and 0.22 ± 0.16 µg h/ml at cycle 3. Mean SN-38 concentrations versus time profiles either alone or in combination with BV were nearly superimposed (Fig. 3). In SN-38G, significant differences in the PK parameters were also not found between cycles 1 and 3 (Table 2), and mean SN-38G concentrations versus time profiles either alone or in

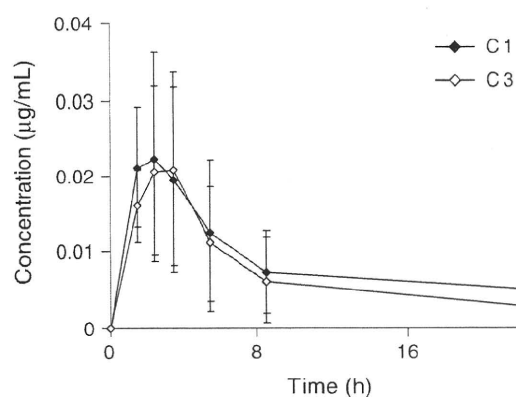
**Table 2** Pharmacokinetic parameters

Analyte		C <sub>max</sub> (mg/ml)	T <sub>max</sub> (h)	t <sub>1/2</sub> (h)	AUC <sub>0-∞</sub> (mg h/ml)	MRT <sub>0-∞</sub> (h)	Vd (L)	CL (L/h)
CPT-11	BV (–)	2.1 (0.3)	1.5 (0)	6.0 (0.6)	12.2 (2.3)	6.1 (0.6)	185 (43.3)	21.6 (5.6)
	BV (+)	2.1 (0.3)	1.5 (0)	5.7 (0.6)	12.8 (1.7)	6.1 (0.5)	164 (34.6)	19.7 (3.0)
SN-38	BV (–)	0.024 (0.013)	2.0 (0.7)	14.3 (16.6)	0.40 (0.44)	–	–	–
	BV (+)	0.022 (0.012)	2.8 (0.8)	8.3 (7.6)	0.22 (0.16)	–	–	–
SN-38G	BV (–)	0.14 (0.030)	2.4 (0.3)	12.9 (4.7)	1.98 (0.70)	–	–	–
	BV (+)	0.14 (0.030)	2.6 (0.6)	11.4 (3.5)	1.81 (0.26)	–	–	–

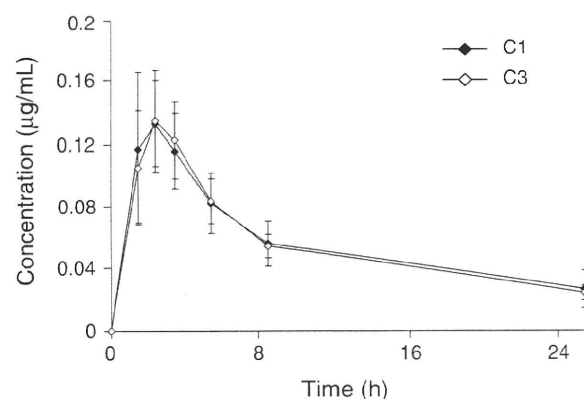
Values are expressed as mean (±SD). There are no significant differences in the C<sub>max</sub> and AUC<sub>0-∞</sub> of CPT-11, SN-38, and SN-38G between cycle 1 (BV–) and cycle 3 (BV+); paired *t* test



**Fig. 2** Mean CPT-11 concentrations versus time profiles either alone or in combination with BV were superimposed



**Fig. 3** Mean SN-38 concentrations versus time profiles either alone or in combination with BV were nearly superimposed



**Fig. 4** Mean SN-38G concentrations versus time profiles either alone or in combination with BV were superimposed

combination with BV were also nearly superimposed (Fig. 4).

There were no significant differences in the changes in the ratio of CPT-11 to SN-38 between cycles 1 and 3 (Table 3), as well as in the ratio of SN-38 to SN-38G.

**Table 3** Changes in ratio of CPT-11 to SN-38 and SN-38 to SN-38G

Patient No.	AUC ratio of SN-38/CPT-11(%)		AUC ratio of SN-38G/SN-38	
	BV (-)	BV (+)	BV (-)	BV (+)
1	3.1	4.1	3.8	3.9
2	2.2	2.2	7.9	5.9
3	2.5	1.8	8.7	8.0
4	9.2	1.8	2.3	7.7
5	0.6	0.7	23.1	22.3
6	0.3	0.3	51.7	76.0
7	4.4	1.4	3.5	8.8
8	1.0	0.5	13	23.8
9	1.0	0.8	13.5	14.6
10	5.6	4.3	2.3	3.2

There were no significant differences in the AUC ratios of SN-38/CPT-11 and SN-38G/SN-38 between cycle 1 (BV-) and cycle 3 (BV+); paired *t* test

The results indicate that the CPT-11 and BV combination had no effect on the extent of conversion of CPT-11 into its metabolites SN-38 and SN-38G.

We also observed a larger inter-patient variability for the changes in the ratios of CPT-11 to SN-38 and SN-38 to SN-38G (Table 3).

## Discussion

In the present study, we found no significant differences in the mean AUCs,  $C_{max}$  and CPT-11 clearance after BV addition. Our results demonstrate that BV addition to CPT-11 (in the FOLFIRI regimen) showed no effect on the drug disposal of CPT-11 and its metabolites. This is the limited sample size study, but this is the first report clarifying the effect of BV on CPT-11 metabolism in humans.

Gaudreault et al. previously reported on the effect of BV on CPT-11 metabolism and safety using cynomolgus monkeys as subjects. Their report was the only published study available in the literature search regarding the effect of BV on CPT-11 metabolism. In their study, monkeys received bolus IFL with or without BV, and blood samples were collected for PK analysis of CPT-11 and 5-FU. They concluded that BV had no effect on the metabolism of either agent, although the number of animals tested in each group was small [with BV ( $n = 5$ ); without BV ( $n = 4$ )] and no statistical comparison between groups was performed [9].

As previously shown, in the AVF2107g study, CPT-11 metabolism was characterized in a small PK study (results are presented only in the package insert of BV

[7]). In the results, SN-38 concentrations were on average 33% higher in patients receiving bolus IFL in combination with BV compared with bolus IFL alone. But it might be caused by an imbalance between the two treatment arms and a possible inter-subject variability of CPT-11 metabolism. Inter-patient variability of CPT-11 metabolism was previously reported [10], and such variability appears to be caused by inter-individual variability of carboxylesterase activity [4, 5], or glucuroconjugate activity correlated with UGT1A1 polymorphism [6]. In the present study, we could indeed observe a large inter-patient variability of CPT-11 catabolism, which is another good area for future investigation. This was not performed here since investigations into metabolic enzymes or genetic polymorphism with inter-patient comparison were not the specific aims of the present study. Here, we used intra-patient comparison to exclude inter-patient variability. As a result, we were able to clarify that BV has no effect on CPT-11 catabolism. Moreover, BV appeared to exert no effect on the conversion ratios of CPT-11 to SN-38 and SN-38 to SN-38G (Table 3). The explanation of the lacking pharmacokinetic interaction between BV and CPT-11 may be caused by different pathways of clearance: IgGs are cleared through Fc/Fc/Rn systems, whereas CPT-11 are primary enzymatically transformed in the liver [11, 12]. The analysis of PK parameters failed to provide any explanation for the observed supra-additive clinical efficacy of the CPT-11 and BV combination [2, 3]. The absence of PK interaction between CPT-11 and BV has been recognized to indicate the safety of this combination therapy for further clinical study and general practice.

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## Adipocytokines and squamous cell carcinoma of the esophagus

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### Abstract

**Purpose** Adipocytokines are adipocyte-secreted hormones associated with some malignancies. It has been reported that the impaired response of adipocytokines to body weight loss may play a role in the pathogenesis of cancer-induced cachexia. We investigated the association between adipocytokines with squamous cell carcinoma of the esophagus (SCCE).

**Methods** The levels of body mass index (BMI) and adiponectin, leptin, resistin, visfatin and C-peptide in the blood at diagnosis were measured in 117 SCCE patients and 117 age- and sex-matched controls. Logistic regression models were employed to estimate odds ratio. One-way analysis was performed to examine the prevalence of variables between two or more groups. A non-parametric Spearman

correlation test was conducted to examine the associations between BMI and other variables.

**Results** Adiponectin and BMI levels were significantly lower, and resistin level was significantly higher in the patients on multivariate analysis ( $P = 0.01$ ,  $<0.01$  and  $<0.01$  respectively). BMI gradually decreased with stage progression, and resistin level gradually increased with stage progression ( $P < 0.01$  for both). The inverse correlation between BMI and adiponectin was comparatively strong in the controls, but was weak in the patients. Leptin showed comparatively strong correlation with BMI in the controls, but was weakly correlated in the patients. The correlation between BMI and resistin or C-peptide was demonstrated weakly only in the controls, and visfatin did not correlate with BMI.

**Conclusions** Resistin may be a biomarker for the progression of SCCE. In addition, the impaired responses to body weight loss of adiponectin and leptin in the patients with SCCE were suggested.

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**Keywords** Esophageal cancer · Adiponectin · Leptin ·  
Resistin · Visfatin · C-peptide

### Introduction

Patients with esophageal cancer tend to become cachexic with low body mass index (BMI) levels, because most of them present with gastrointestinal symptoms such as dysphagia, epigastralgia, nausea and anorexia. However, the carcinogenesis of adenocarcinoma of the gastroesophageal junction, of which the incidence has been increasing over the past three decades in Western countries, has been reported to be correlated with obesity (Calle and Thun 2004; Wolk et al. 2001). In Japan, the incidence of

squamous cell carcinoma of the esophagus (SCCE) remains at more than 90%, whereas that of adenocarcinoma of the gastroesophageal junction has remained very low. The risk of SCCE has never been reported to be correlated with obesity.

Adipocytokines, such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, type-1 plasminogen activator inhibitor, heparin-binding epidermal growth factor-like growth factor, hepatocyte growth factor, adiponectin, leptin, resistin, visfatin and apelin, are cytokines secreted by visceral adipose tissue, and they have recently been suggested to be associated with the risk of cancer at various sites (e.g., breast, prostate gland, endometrium and colorectum) as well as Barrett's esophagitis, which is the main risk factor for adenocarcinoma of the gastroesophageal junction (Rubenstein et al. 2008; Wei et al. 2005). There is as yet no case-control study investigating the correlation between adipocytokines and esophageal cancer.

On the other hand, adiponectin levels are inversely correlated with body weight, while leptin levels are directly associated with weight loss (Arita et al. 1999; Pannacciulli et al. 2003; Yang et al. 2001; Wolfe et al. 2004). Although the association between cancer-induced cachexia and adipocytokine level has been recently reported, the association between adipocytokine level and cancer-induced cachexia has not yet been fully elucidated (Kerem et al. 2008; Wolf et al. 2006) (Jamieson et al. 2004). In breast and colon cancer patients, no significant correlation was observed between adiponectin level and cachexia, and leptin level was significantly lower only in female cachexic patients than in female non-cachexic patients (Wolf et al. 2006). In gastric cancer patients, adiponectin, leptin and resistin levels were reportedly higher in cachexic patients than in non-cachexic patients (Kerem et al. 2008). These findings indicate that the impaired response of adipocytokines to body weight loss may play a role in the pathogenesis of cancer-induced cachexia.

We previously reported that resistin and visfatin may be biomarkers of gastric cancer, and adiponectin may have the possibility as a biomarker for the diagnosis of early stage gastric cancer (Nakajima et al. 2009). Moreover, the correlations between BMI and adiponectin were weaker in gastric cancer patients than that in the controls, and the correlation between BMI and leptin was observed both in the patients as well as in the controls. In the present study, we investigated the association between the levels of several blood adipocytokines and SCCE through a case-control study including BMI as a variable to speculate the importance of adipocytokines as biomarkers of SCCE and the association between cancer-induced cachexia and the changes in adipocytokines levels.

## Materials and methods

### Study population

Following the approval of the study protocol by the Institutional Review Board which conforms to the provisions of the Declaration of Helsinki in 1995, patients who underwent upper gastrointestinal (UGI) endoscopy at the National Cancer Center Hospital, Tokyo, from January 1999 to January 2007, who were considered to have no active malignancies except esophageal cancer, and whose blood samples at diagnosis before any treatments for SCCE could be obtained, were identified and invited to participate as patients or controls. Subjects pathologically and newly diagnosed as having SCCE by biopsy using UGI endoscopy and treated at our hospital for SCCE were identified as patients from the enrolled participants. Age- and sex-matched controls (1:1) diagnosed as being free from esophageal cancer by UGI endoscopy were identified from the enrolled participants. BMI at diagnosis was calculated on the basis of the data in medical records as follows: weight (kg)/height (m)<sup>2</sup>. Clinical and pathological information for both groups was obtained from medical records. All subjects (patients and controls) provided an informed consent prior to the collection and analysis of blood samples.

### Adipocytokines and C-peptide measurements

All blood samples were stored at  $-20^{\circ}\text{C}$  until use. None of the samples were previously thawed. Blood levels of adiponectin, resistin, visfatin and C-peptide at diagnosis were measured by SRL Inc., Tokyo, Japan. Adiponectin was determined by enzyme-linked immunosorbent assay (ELISA) (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) with a sensitivity of 1.9  $\mu\text{g/ml}$ , an intra-assay coefficient of variation of 3.5–5.1%, and an inter-assay coefficient of variation of 6.0–8.7%. Resistin was determined by ELISA (BioVender Laboratory Medicine, Brno, Czech Republic) with a sensitivity of 1.1 ng/ml, an intra-assay coefficient of variation of 2.8–3.4% and an inter-assay coefficient of variation of 5.1–6.9%. Leptin was measured using radioimmunoassay kits (LINCO Research, St Charles, MO, USA) with a sensitivity of 0.5 ng/ml, an intra-assay coefficient of variation of 3.4–8.3% and an inter-assay coefficient of variation of 3.0–6.2%. Visfatin was determined by ELISA (Adipo Gen, Seoul, Korea) with a sensitivity of 0.13 ng/ml, an intra-assay coefficient of variation of 4.4–10.4% and an inter-assay coefficient of variation of 6.4–9.9%. C-peptide was determined by ELISA (Fujirebio, Tokyo, Japan) with a sensitivity of 0.04 ng/ml, an intra-assay coefficient of variation of 1.96–2.97% and an inter-assay coefficient of variation of 1.06–2.60%. Duplicate measurements were performed in a single experiment.



**Table 1** Clinical characteristics of patients with esophageal cancer and controls

	Patient (n = 117)	Control (n = 117)	P value
Age (years)	63.6 ± 8.7	63.6 ± 8.8	0.63
Sex			
Female	30 (25.6%)	30 (25.6%)	
Male	87 (74.4%)	87 (74.4%)	1.00
Stage <sup>a</sup>			
0	21		
I	24		
II	24		
III	20		
IV	28		

Data are expressed as mean ± SD

<sup>a</sup> Japanese classification of esophageal carcinoma

Statistical analysis

The results of the comparison of clinical characteristics between patients and controls were evaluated by the  $\chi^2$  test for categorical variables and the two-sample *t*-test for continuous variables. Conditional logistic regression models were used for estimating odds ratio and 95% confidence interval (95% CI) to evaluate the association between each

variable with SSCE. One-way analysis of variance was performed to examine the prevalence of each variable between tumor stage groups. Log transformations were conducted on variables prior to the analysis to achieve normal distribution. A non-parametric Spearman correlation test was conducted to examine the associations between BMI and other variables. Differences with  $P \leq 0.05$  were considered statistically significant. All statistical analyses were carried out using the SAS system, version 9.1.3.

Results

Adipocytokine, C-peptide and BMI levels and SSCE risk

The clinical characteristics of the 117 patients and 117 controls are shown in Table 1. There was no significant difference in age and sex between the two groups. Adipocytokines, C-peptide and BMI levels of the patients and controls are shown in Table 2. The results of the univariate and multivariate logistic regression analyses are shown in Table 3. Adiponectin and BMI levels were significantly lower in the patients than in the controls on multivariate analysis ( $P = 0.01$  and  $<0.01$ , respectively). On the other hand, resistin level was significantly higher in the patients than those in the controls on multivariate analysis ( $P = <0.01$ ).

**Table 2** Adipocytokines, C-peptide and BMI levels in patients with esophageal cancer and controls

	Patient				Control			
	n	Median value	25th Quartile value	75th Quartile value	n	Median value	25th Quartile value	75th Quartile value
Adiponectin (µg/ml)	117	7.9	5.7	11.6	117	8.9	6.3	12.8
Resistin (ng/ml)	117	4.7	3.2	7.7	116	3.0	2.2	4.4
Leptin (ng/ml)	117	2.5	1.6	4.0	116	3.7	2.2	5.5
Visfatin (ng/ml)	117	2.6	1.1	6.7	117	1.4	0.8	2.6
C-peptide (ng/ml)	117	0.1	0.1	0.4	115	0.3	0.1	0.5
BMI (kg/m <sup>2</sup> )	117	20.5	18.3	23.0	117	23.3	21.2	25.7

**Table 3** Univariate and multivariate analyses of adipocytokines, C-peptide and BMI levels in patients with esophageal cancer and controls

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
Adiponectin	0.617 (0.348–1.092)	0.10	0.296 (0.112–0.782)	0.01
Resistin	3.461 (1.997–5.998)	<0.01	3.428 (1.664–7.062)	<0.01
Leptin	0.364 (0.220–0.603)	<0.01	0.708 (0.317–1.581)	0.40
Visfatin	1.390 (1.124–1.719)	<0.01	0.990 (0.712–1.377)	0.95
C-peptide	0.739 (0.597–0.915)	<0.01	0.864 (0.619–1.206)	0.39
BMI (kg/m <sup>2</sup> )	0.738 (0.658–0.828)	<0.01	0.738 (0.626–0.870)	<0.01

**Table 4** Association between adiponectin, resistin, BMI and stage progression of esophageal cancer

Variable	Control		Stage 0		Stage 1		Stage 2		Stage 3		Stage 4		P value
	n		n		n		n		n		n		
Adiponectin*	117	2.2 ± 0.5	21	2.0 ± 0.5	24	2.3 ± 0.7	24	2.1 ± 0.5	20	1.9 ± 0.6	28	2.1 ± 0.5	0.25
Resistin*	117	1.2 ± 0.5	21	1.2 ± 0.6	24	1.4 ± 0.6	24	1.7 ± 0.5	20	1.6 ± 0.7	28	1.8 ± 0.7	<0.01
BMI (kg/m <sup>2</sup> )	117	23.4 ± 3.3	21	21.8 ± 2.8	24	21.3 ± 3.5	24	19.9 ± 2.7	20	21.3 ± 3.3	28	18.8 ± 2.8	<0.01

Data are expressed as mean ± SD

\*Log-transformed

**Table 5** Spearman correlation coefficients between BMI and adipocytokines or C-peptide

Variable	BMI	
	Patient	Control
Adiponectin	-0.26	-0.45
Resistin	-0.14	0.25
Leptin	0.36	0.53
Visfatin	0.00	-0.10
C-peptide	0.18	0.20

#### Correlation of adiponectin, resistin and BMI levels with tumor stage

Linear contrast analysis was conducted to evaluate the correlation between adiponectin, resistin, BMI and tumor stage (Table 4). Because the ranges of adipocytokines and C-peptide levels were wide, we used the log-transformed values for them. BMI levels gradually decreased with stage progression ( $P < 0.01$ ); in contrast, resistin level gradually increased with stage progression ( $P < 0.01$ ).

#### Correlation between BMI levels and other variable levels

The Spearman correlation coefficients between BMI and other variables are shown in Table 5. The inverse correlation between BMI and adiponectin was comparatively strong in the controls but was weak in the patients. Leptin showed comparatively strong correlation with BMI in the controls, but was weakly correlated in the patients. The correlation between BMI and resistin or C-peptide was demonstrated weakly only in the controls, and visfatin did not correlate with BMI.

## Discussion

In this case-control study, we demonstrated that the levels of adiponectin, resistin and BMI were significantly different between SCCE patients and controls. Resistin and BMI levels also significantly correlated with the stage progres-

sion of SCCE. The correlation between BMI and resistin was demonstrated weakly only in the controls, and resistin may be a biomarker of SCCE. An inverse correlation between adiponectin level and BMI was observed in the controls, and was weak in the patients. It indicates that the importance of adiponectin as a biomarker of SCCE cannot be completely evaluated in this study. In addition, the impaired responses to body weight loss of adiponectin and leptin in the patients with SCCE were suggested.

In case-control studies, adiponectin levels significantly decreased in patients with breast, endometrial, prostate, colon and gastric cancers and myelodysplastic syndrome (MDS) (Ishikawa et al. 2005; Wei et al. 2005; Dalamaga et al. 2008). Adiponectin suppresses the secretion of inflammatory cytokines such as TNF- $\alpha$ , and induces the secretion of anti-inflammatory cytokines such as IL-10 in the atherogenic process (Fantuzzi 2005; Kumada et al. 2004; Yokota et al. 2000). Furthermore, it has been reported to inhibit tumor growth by suppressing angiogenesis in vitro and in vivo (Wang et al. 2005). These data suggest that the decreased systemic level of adiponectin may indicate its decreased protective physiological function against inflammation or angiogenesis. The association between leptin level and the risk of cancer has remained controversial in breast, prostate and colon cancers (Chia et al. 2007; Stattin et al. 2004). On the other hand, the normal response of adiponectin or leptin to body weight change was reported as follows: adiponectin level inversely correlates with body weight, and leptin level decreases in response to body weight loss (Arita et al. 1999; Pannacciulli et al. 2003; Wolfe et al. 2004). In the cachexic status, however, these responses might be impaired, in which case adiponectin cannot respond to body weight loss by increasing secretion, and leptin cannot respond to body weight loss by decreasing secretion (Jamieson et al. 2004; Kerem et al. 2008; Wolf et al. 2006). Our results showed the inverse correlation between adiponectin and SSCE. However, an inverse correlation between adiponectin and BMI was observed, and we could not evaluate the importance of adiponectin as a biomarker of SCCE in this study. The correlation between BMI and leptin also existed. Both these

correlations between adiponectin/leptin and BMI were weaker in the SCCE patients than in the controls in agreement with the above-mentioned previous reports and with our previous report for gastric cancer (Nakajima et al. 2009).

Resistin is expressed mainly in the bone marrow, peripheral mononuclear cells, lungs, placental tissue and pancreatic  $\beta$  cells; however, its biological effects have remained unclear to date. Three case–control studies for the risk of MDS, multiple myeloma or colorectal cancer have been reported (Dalamaga et al. 2008, 2009; Kumor et al. 2009). Dalamaga et al. demonstrated the decreased level of resistin in MDS patients, and speculated that it was due to a compensatory response to the upregulation of other inflammatory factors etiologically linked to myelodysplasia. They also reported the decreased level of resistin in patients with multiple myeloma. Kumor et al. reported a decreased resistin level in colorectal cancer patients. Comparatively, we previously reported that the elevated resistin level may be a biomarker of gastric cancer (Nakajima et al. 2009). Here, resistin in SCCE patients was significantly higher than those in controls, and the correlation between BMI and resistin was demonstrated weakly only in the controls. It implicates that resistin may be a biomarker of SCCE and it also gradually increased with tumor stage progression. This may imply that resistin is a biomarker for the progression of SCCE, rather than a risk factor in SCCE carcinogenesis.

Visfatin is also a new adipocytokine found to be identical to a previously known pre-B cell colony enhancing factor expressed by lymphocytes. The clinical correlations of visfatin with cancer have been rarely reported. In the present study, visfatin level showed no difference between SCCE patients and controls. Previous studies have investigated the correlation between the risk of cancer and obesity in reference to insulin resistance involving insulin, C-peptide, IGFBP and IGF (Giovannucci 2001). Insulin resistance has also been investigated in terms of its correlation with cancer-induced cachexia (Kerem et al. 2008). In this study, C-peptide level showed no difference between SCCE patients and controls. Moreover, its correlation with BMI was demonstrated weakly only in the controls.

The results of the present study suggest that resistin may be a biomarker of SCCE, as well as of stage progression of SCCE. In addition, the impaired responses to body weight loss of adiponectin and leptin in SCCE were suggested. A prospective cohort study is needed to completely elucidate the importance of adipocytokines as biomarkers of SCCE and the causative association between cancer-induced cachexia and the changes in adipocytokines levels. Furthermore, an in vitro study will also be necessary to evaluate the direct function of these adipocytokines on cancer cells.

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