Table III. Prognosis of patients in PaP score groups A and B.

	N	MST (months)	P-value
PaP score group			
A	28	11	0.0045
B	12	3	
Ascites			
Absent	31	10	0.7548
Present	9	10	
Surgical intervention			
No	25	10	0.7238
Yes	15	11	

PaP, palliative prognostic; MST, median survival time.

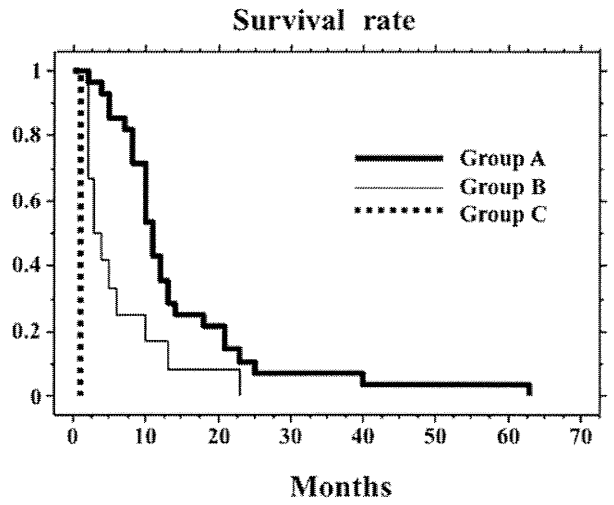

Figure 1. Survival of patients in group A was much better compared to patients in group B or C (P<0.0001).

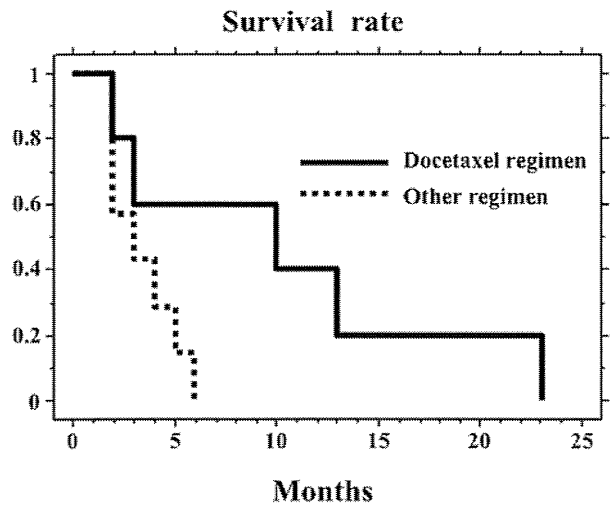

Figure 2. Survival of the 5 patients treated with docetaxel was better compared to the 7 patients treated with other regimens in the PaP group B, with no statistically significant difference (P=0.062).

Table IV. Correlation between PaP score and first-line chemotherapy.

	PaP score group		
First-line chemotherapy	A	B	C
S-1 plus cisplatin	16	1	0
S-1 plus docetaxel	8	5	0
S-1 plus CPT-11	2	2	0
S-1 only	2	4	1
BSC	0	0	3

PaP, palliative prognostic; BSC, best supportive care.

in group B, the docetaxel regimen prolonged the survival from 3 (other regimens, MST, 3 months, n=7) to 10 months (docetaxel regimen, MST, 10 months, n=5, Fig. 2).

Discussion

Large-scale randomized phase III clinical trials may reveal effective chemotherapeutic regimens for patients with advanced cancers, with the exception of those of advanced age or with poor PS. However, in clinical situations, it is difficult to decide the most suitable chemotherapeutic regimen for patients with short life expectancy or poor PS, such as patients with non-resectable gastric cancer. Identifying the patients that may benefit from palliative chemotherapy is quite difficult and its usefulness when controlling symptoms and maintaining QOL has not yet been proven (13,14).

The PaP score contains five parameters (symptom, PS, inflammation, immunity and physician's survival prediction) associated with cancer patient survival. Findings of previous studies have indicated that the PaP score may accurately estimate pre-terminal patient survival (15-17). Using the PaP score in 44 patients with non-resectable advanced gastric cancer, the correlation between PaP score groups and chemotherapeutic regimens was investigated. The findings showed that in the PaP score group A, the S-1 plus cisplatin regimen was commonly used and differences in chemotherapeutic regimens did not affect the survival of the patients in this group. In comparison, the survival of patients in PaP group B was extremely poor, although the S-1 plus docetaxel regimen prolonged the survival of these patients from 3 to 10 months. Although this study is retrospective and the number of objective cases is small, the docetaxel regimen may have a survival advantage in patients with a poor prognosis.

Docetaxel is reported to have a low rate of grade 3/4 leucopenia and neutropenia (19.4 and 10.6%) and rare, severe non-hematologic toxicities (18). Docetaxel chemotherapy with or without S-1 has been a suitable treatment for patients with advanced gastric cancer, advanced age or poor PS (19).

In conclusion, in the treatment of advanced non-resectable gastric cancer, the PaP score should be used to select patients and chemotherapeutic regimens. The S-1 plus docetaxel regimen is expected to improve outcomes in patients with a poor PS.

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Clinicopathologic Characteristics and Prognosis of Gastric Cancer in Young Patients

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To determine the clinicopathologic characteristics and prognosis of gastric cancer in young patients, a total of 1985 gastric cancer patients who had undergone gastrectomy at our hospital were reviewed. The male-to-female ratio was significantly lower in the young patients than in either the middle-aged ($P < 0.0001$) or elderly patients ($P < 0.0001$). Undifferentiated carcinoma was observed more frequently in the young patients compared with either the middle-aged ($P < 0.0001$) or elderly patients ($P < 0.0001$). Furthermore, peritoneal metastasis was observed more frequently in the young patients than in either the middle-aged ($P < 0.005$) or elderly patients ($P < 0.005$). Five-year survival rates were 61.0, 73.6 and 68.1% in the young, middle-aged and elderly patients, respectively. The prognosis of the middle-aged patients was significantly better than that of either the young or the elderly patients ($P < 0.05$). Multivariate analysis indicated that age was an independent prognostic factor. Peritoneal recurrence was more frequently observed in the young patients than either the middle-aged or the elderly patients ($P < 0.05$). Gastric cancer in young patients has unique characteristics, namely, a predominance of female patients and a high frequency of undifferentiated cancer and peritoneal metastasis and recurrence.

Key words: age; gastric cancer; prognosis

Gastric cancer is frequent in middle-aged and elderly populations. Although gastric cancer is rare in young populations (Okamoto et al., 1988; Mitsudomi et al., 1989), it has been reported that gastric cancer in young patients has some unique characteristics compared with that in middle-aged and elderly patients. For instance, the male-to-female ratio shows a predominance of females among younger patients (Bloss et al., 1980; Mori et al., 1985; Sandler and Holland, 1987; Tso et al., 1987). Furthermore, a significantly higher frequency of both Borrmann type 4 and poorly differentiated adenocarcinoma with a scirrhus growth pattern has been noted as a characteristic of gastric cancer in young patients (Bloss et al., 1980; Sandler and Holland, 1987; Tso et al., 1987; Okamoto et al., 1988; Mitsudomi et al., 1989). On the other hand, the prognosis of gastric cancer in young patients remains unclear thus far. The aim of the present study was to clarify the clinicopathologic characteristics and prognosis of gastric cancer in young patients.

Materials and Methods

Patients

The present study was based on a retrospective analysis of 1985 patients with gastric adenocarcinoma who underwent gastrectomy at our institution between January 1975 and December 2000. The clinicopathologic findings were determined according to the Japanese Classification of Gastric Carcinoma

Gastric Cancer
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ORIGINAL ARTICLE

Increased apoptosis and elevated Fas expression in circulating natural killer cells in gastric cancer patients

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Abstract
Background Immune cells undergo extensive apoptosis in patients with cancer, which may be related to immune evasion by cancerous cells. The present study was designed to investigate the relationship between natural killer (NK) cell apoptosis and Fas expression in gastric cancer patients.
Methods NK cell apoptosis and Fas expression were evaluated by multicolor flow cytometry. Soluble Fas ligand (sFasL) was quantitated by enzyme-linked immunosorbent assay.
Results The frequency of apoptotic NK cells in gastric cancer patients was significantly higher than in normal controls ($p = 0.0016$). Moreover, their frequency was related to the progression of gastric cancer. Fas-positive NK cells were significantly more common in gastric cancer patients compared with normal controls ($p = 0.034$). Furthermore, Fas expression was closely related to the frequency of NK cell apoptosis ($r = 0.6$, $p < 0.0001$). The frequency of tumor-infiltrating NK cell apoptosis was significantly higher than that of circulating NK cell apoptosis ($p = 0.035$). Furthermore, Fas-positive NK cells in gastric cancer tissues occurred significantly more often than in peripheral blood ($p = 0.029$). FasL concentration in gastric cancer patients was lower than that in normal controls, and the difference tended to be significant ($p = 0.057$). Apoptotic circulating NK cells significantly decreased after surgery compared to before surgery ($p = 0.023$). Furthermore, Fas expression on circulating NK cells also significantly decreased after surgery compared with before surgery ($p = 0.021$).
Conclusions Upregulation of Fas expression on NK cells is related to increased apoptosis of circulating NK cells in gastric cancer patients.

Keywords Apoptosis · Fas · Fas ligand · Gastric cancer · Natural killer cells

Introduction

Natural killer (NK) cells are effector lymphocytes of the innate immune system that respond to several types of tumors and microbial infections by limiting their spread and subsequent tissue damage [1]. Because NK cells exhibit natural cytotoxicity against a broad range of human solid tumors in the absence of major histocompatibility complex molecules on target cells [2–5], they play an important role in host anticancer defense mechanisms in vitro [6] and in vivo [7, 8]. Nonetheless, NK cell immune responses are not sufficient to eradicate tumors in cases of clinical cancer because tumors develop a mechanism to escape host immune responses [9, 10]. In fact, defects in NK cell activity have been found in various cancers [11].

Gastric cancer is one of the most common malignancies. Although prognoses of patients with gastric carcinoma have improved because of better diagnostic techniques and better intraoperative and postoperative care, death from gastric cancer still ranks second among all cancer deaths worldwide [12]. Decreased NK cell activity has been observed in gastric cancer patients. Furthermore, Takeuchi et al. demonstrated that lower NK cell activity was an independent prognostic indicator in gastric cancer patients,

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suggesting a potential role for NK cells in preventing progression of gastric cancer [13]. However, the mechanism responsible for defective NK cell function in gastric cancer has yet to be defined. One possible mechanism responsible for dysfunction of immune cells in cancer patients is extensive apoptosis, such as T cells [14, 15] and NK cells [16]. Tumor cells express FasL [17], receptor-binding cancer antigen expressed on SiSo cells (RCAS1) [18], and B7-H1 [19], which induce T-cell apoptosis. It has been suggested that the Fas/Fas ligand (Fas/FasL) system plays an important role in establishing tumors with a privileged immune status by inducing Fas-mediated apoptosis in tumor-specific lymphocytes. A number of studies have demonstrated that tumor cells express FasL, which induces Fas-mediated apoptosis in T cells [17, 20, 21]. Furthermore, we have previously demonstrated that Fas expression on CD8+ T cells is closely related to the frequency of CD8+ T-cell apoptosis in gastric cancer patients [22]. On the other hand, Fas expression on NK cells remains relatively undetermined in patients with cancer. In the current study, we therefore sought to assess a potential role of Fas expression on NK cell apoptosis.

Materials and methods

Gastric cancer patients and normal donors

Thirty-eight patients treated at Tottori University Hospital (Yonago, Japan) and pathologically diagnosed with gastric cancer were enrolled in this study. None of the patients received radiotherapy, chemotherapy, or other medical interventions before surgery. International review boards at Tottori University approved the study, and informed consent for blood donations was obtained from all individuals. Patient characteristics are shown in Table 1. Healthy controls ($n = 17$) were age-matched (62.7 ± 16.1 years for the controls vs. 65.5 ± 9.9 years for patients), and each experiment was performed in parallel for controls and patients.

Table 1 Clinicopathological parameters of gastric cancer patients enrolled in the current study

	Gastric cancer patients	Normal controls
Gender		
Male	20	8
Female	18	9
Age	48–82 (65.5)	44–88 (62.7)
Depth of invasion		
T1 (early)	18	
T2/T3/T4 (advanced)	20	
Lymph node metastasis		
Absent	23	
Present	15	

Preparation of peripheral blood mononuclear cells (PBMCs)

Peripheral blood (30 ml) was drawn from each donor before surgery and centrifuged using a Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient. In 10 of the 33 patients, 30 ml peripheral blood was also drawn 1–2 months after surgery.

Isolation of tumor-infiltrating NK cells

Freshly excised tumor tissues were minced and digested with 1.5 mg/ml collagenase D (Wako Pure Chemical Industries, Osaka, Japan). The resulting cell suspensions were filtered through a mesh filter (BD, Franklin Lakes, NJ, USA). Because it is necessary to get a large piece of tissue sample to have enough tumor-infiltrating NK cells for flow cytometry analysis, tumor-infiltrating NK cells were not available for analysis from most early gastric cancer patients. Therefore, tumor-infiltrating NK cells were available in 7 patients of 38 patients in the current study.

Flow cytometry analysis

Fluorescence-activated cell sorting (FACS) analysis was performed on a FACSCalibur (BD Pharmingen, Franklin Lakes, NJ, USA), and cells were classified using the following antibodies: anti-CD3-PE-Cy5, anti-CD56-PE, and anti-CD95-FITC (BD Pharmingen).

Apoptosis in peripheral NK cells

The percentage of apoptotic cells was calculated by scoring annexin V-binding cells after back-gating of CD3−/CD56+ cells. All gated mononuclear cell subpopulations were visualized on forward angle scatter/side angle scatter (FSC/SSC) dot plots. To include all apoptotic cells and avoid debris with a high SCC signal, the gate was set to include a wide boundary of mononuclear cells because apoptotic cells accumulate mainly in the lower FSC/SSC channels. A cutoff was set using unstained control cells.

Measurement of soluble Fas ligand (sFasL)

sFasL in human sera was measured by enzyme-linked immunosorbent assay (ELISA) using human Fas ligand/TNFSF6 immunoassay (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Either paired t tests or Mann–Whitney U tests were used to determine statistical differences between groups. Correlations between NK cell apoptosis and Fas expression were analyzed using the Spearman rank correlation coefficient. $p < 0.05$ was considered significant. GraphPad Prism software (GraphPad Software, La Jolla, CA, USA) was used for statistical analyses.

Results

Apoptosis of circulating NK cells in gastric cancer patients

We first determined the frequency of apoptotic circulating NK cells in both normal controls and gastric cancer patients. The frequency of apoptotic cells in gastric cancer patients (21.3 ± 11.6 %) was significantly higher than that in normal controls (11.2 ± 5.2 %) ($p = 0.0016$; Fig. 1). The frequency of apoptotic circulating NK cells was 17.4 ± 9.8 % and 24.8 ± 12.2 % in early and advanced gastric cancer patients, respectively, and the differences were significant between early and advanced gastric cancer patients ($p = 0.037$). Furthermore, there were significant differences in the frequency of apoptotic circulating NK cells between node-negative (17.0 ± 8.4 %) and node-positive (28.0 ± 12.8 %) gastric cancer patients ($p = 0.0086$).

Fas expression on NK cells in gastric cancer patients

To determine the mechanism responsible for increased apoptosis in circulating NK cells in gastric cancer patients, we compared Fas expression on NK cells obtained from gastric cancer patients with that from normal controls. The mean (±SD) number of Fas-positive NK cells was 41.2 ± 16.9 % in gastric cancer patients compared with 29.5 ± 13.0 % in normal controls ($p = 0.034$; Fig. 2). Furthermore, Fas expression was closely related to the frequency of NK cell apoptosis ($r = 0.6$, $p < 0.0001$; Fig. 3). These findings indicate that upregulation of Fas expression on circulating NK cells may be responsible for increased NK cell apoptosis in gastric cancer patients.

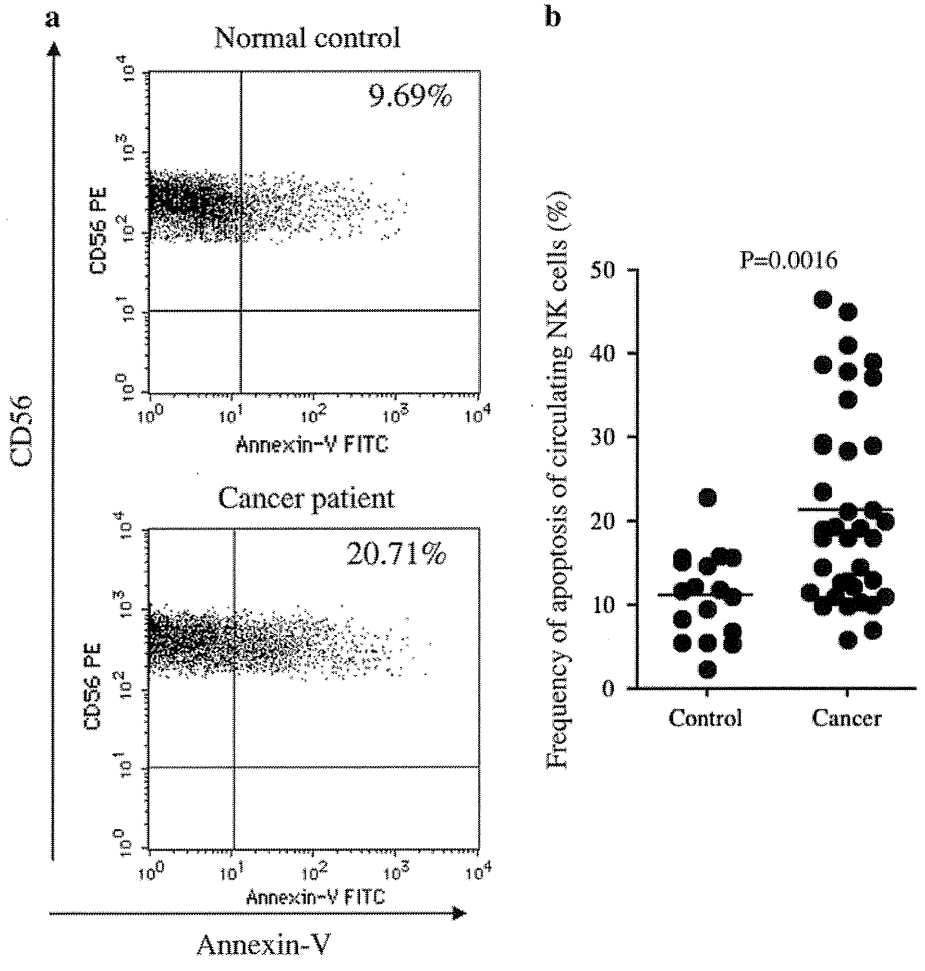

Fig. 1 **a** A representative fluorescence-activated cell sorting (FACS) result showing the frequency of apoptotic natural killer (NK) cells in normal controls and gastric cancer patients. The percentage of apoptotic cells was calculated by scoring annexin V-binding cells after back-gating of CD3−/CD56+ cells. **b** The frequency of apoptotic NK cells in gastric cancer patients was significantly higher than that in normal controls ($p = 0.0016$)

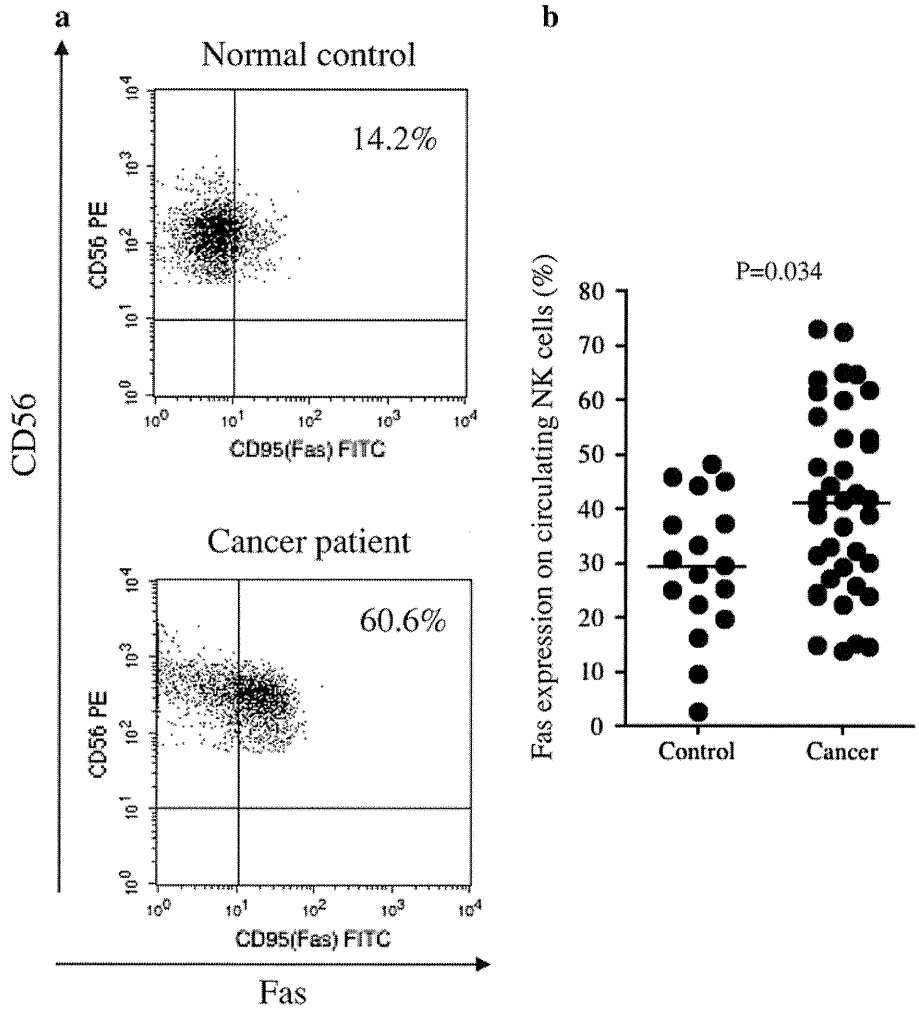

Fig. 2 **a** A representative FACS result showing Fas expression on NK cells from normal controls and gastric cancer patients. **b** Fas+ NK cell frequency in gastric cancer patients was significantly higher than that in normal controls ($p = 0.034$)

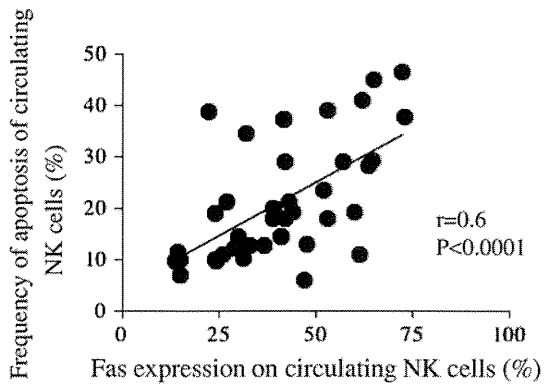

Fig. 3 The frequency of apoptotic NK cells was significantly correlated with Fas expression on NK cells ($r = 0.6$, $p < 0.0001$)

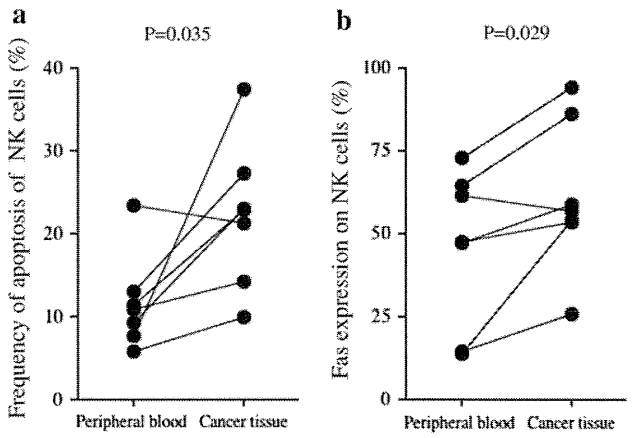

Fig. 4 **a** The frequency of tumor-infiltrating NK cell apoptosis was significantly higher than that of circulating NK cell apoptosis ($p = 0.035$). **b** Fas expression of tumor-infiltrating NK cells was significantly higher than that of circulating NK cells ($p = 0.029$)

Apoptosis and Fas expression of tumor-infiltrating NK cells

We then determined the frequency of tumor-infiltrating NK cell apoptosis and found that the frequency of tumor-infiltrating NK cell apoptosis (22.4 ± 8.9 %) was significantly higher than that of circulating NK cell apoptosis (11.7 ± 5.7 %) ($p = 0.035$; Fig. 4a). Furthermore, the mean (±SD) number of Fas-positive NK cells was

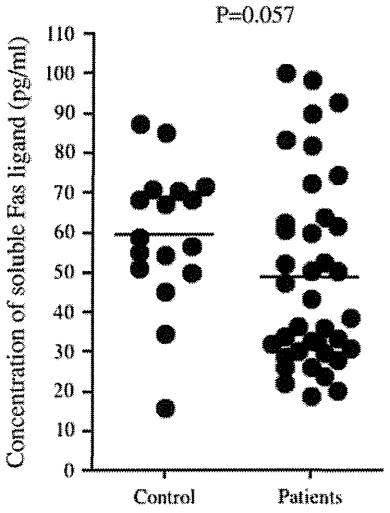

Fig. 5 The concentration of sFasL in patients with gastric cancer tended to be lower than that in normal controls ($p = 0.057$)

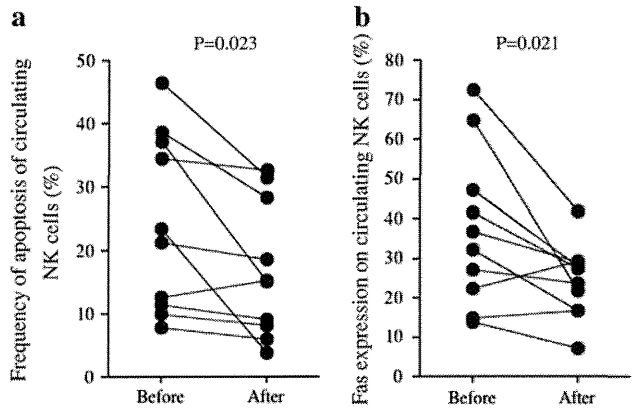

Fig. 6 **a** A significant decrease in the percentage of apoptotic NK cells was observed after surgery compared with that before surgery ($p = 0.023$). **b** A significant decrease in Fas expression on circulating NK cells was observed after surgery compared with that before surgery ($p = 0.021$). The *connecting lines* represent specimens from the same patient

61.6 ± 22.4 % in tissue of gastric cancer compared with 46.2 ± 23.6 % in peripheral blood ($p = 0.029$; Fig. 4b).

Serum concentrations of soluble Fas ligand in gastric cancer patients

To evaluate the contribution of the Fas/FasL pathway to circulating NK cell apoptosis, sFasL concentration was determined. Serum concentrations of sFasL were 48.9 ± 23.7 pg/ml and 59.4 ± 17.7 pg/ml in gastric cancer patients and controls, respectively, and the difference tended to be significant ($p = 0.057$; Fig. 5).

Decreased apoptosis and Fas expression of circulating NK cells after tumor removal

Decreased apoptosis of circulating NK cells was observed after surgery (16.9 ± 10.7 %) compared with before surgery (24.4 ± 14.0 %) ($p = 0.023$; Fig. 6a). Furthermore, Fas expression on circulating NK cells also significantly decreased after surgery (24.2 ± 9.4 %) compared with before surgery (37.4 ± 19.7 %) ($p = 0.021$; Fig. 6b).

Discussion

NK cells use inhibitory receptors to monitor constitutively expressed "self" molecules on susceptible target cells. In particular, NK cells express MHC class I-specific receptors and 'lose' inhibitory signals when encountering MHC class I-deficient hematopoietic cells in several in vitro and in vivo models [23, 24]. Because most cancer cells downregulate MHC class I expression, tumor cells are recognized as NK cell targets [1]. In fact, NK cells may participate in tumor immune surveillance, particularly in leukemia [25], neuroblastoma [26], and gastrointestinal stromal tumors [27]. However, NK cell function is suppressed in gastric cancer patients, even though gastric cancer cells exhibit decreased MHC class I expression [28]. This result suggests other mechanisms are responsible for the NK cell dysfunction observed in gastric cancer.

In the current study, we determined the frequency of NK cell apoptosis to assess one potential mechanism of immune evasion by gastric cancer cells. We observed more frequent NK cell apoptosis in PBMCs obtained from gastric cancer patients than from normal donors. To examine the mechanism responsible for increased apoptosis of NK cells in more detail, we examined Fas expression in these cells. Fas, a member of the tumor necrosis factor (TNF) receptor family, is a type I membrane protein expressed by a variety of cell types. Upon cross-linking by either FasL or an agonistic anti-Fas monoclonal antibody, the Fas-associated death domain and caspase-8 form a death-inducing signaling complex; this activates the caspase cascade, resulting in apoptosis and cell death [29]. In the current study, Fas expression on circulating NK cells from gastric cancer patients was more frequent than that from normal controls. Furthermore, Fas expression was closely related to the frequency of NK cell apoptosis, indicating that increased apoptosis of circulating NK cells might be caused by elevated Fas expression.

Binding of FasL is indispensable for inducing apoptosis of Fas-positive cells. FasL is a type II transmembrane protein expressed by NK cells and activated T cells and within immune-privileged sites, such as the eye and brain [30, 31]. FasL is a member of the TNF family, which also includes TNF-α and CD40 ligand [32]. Membrane-bound FasL is expressed on the cell surface as a 37- to 42-kDa

protein that is proteolytically cleaved by matrix metalloproteinase (MMP) to generate its 26-kDa soluble form (sFasL) [33]. Song et al. [34] demonstrated that sFasL induces apoptosis of Fas+ T lymphocytes in patients with cancer. Moreover, studies have demonstrated that sFasL concentration in cancer patients is elevated compared with normal controls [35, 36]. Therefore, we examined sFasL concentration in the current study. In contrast to previous reports, we observed that sFasL concentration in patients with gastric cancer trended to be lower than that in normal controls. We therefore suspected that sFasL of these patients might be consumed by binding to Fas expressed on circulating NK cells. This result is in line with a previous report on head and neck squamous cell carcinoma, demonstrating that increased lymphocytic apoptosis is associated with low-level sFasL in the serum [14]. To our knowledge, this is the first report to show that Fas upregulation on NK cells, but not an increase in sFasL concentration, correlates with apoptosis of circulating NK cells in gastric cancer patients. Fas expression significantly decreased after surgery. Furthermore, Fas expression in tumor-infiltrating NK cells was significantly higher than that in circulating NK cells. These findings indicate that gastric cancer affects Fas expression in NK cells. However, the factor responsible for upregulating Fas on NK cells remains unclear in gastric cancer patients. In this regard, we added supernatant obtained from five different gastric cancer cell lines and serum from advanced gastric cancer patients to the culture of PBMC to determine the alteration of Fas expression on NK cells and observed no alteration of Fas expression on NK cells, indicating that soluble factor including cytokines might not be responsible for the upregulation of Fas expression on NK cells (data not shown). Further investigations to clarify the mechanisms responsible for Fas upregulation on NK cells in gastric cancer patients are urgently required.

In conclusion, a significant proportion of circulating NK cells in gastric cancer patients is eliminated by apoptosis, thus potentially weakening the antitumor defense in these patients. Our results provide new data suggesting that Fas overexpression on NK cells, but not sFasL secreted from tumor cells, is related to increased apoptosis of circulating NK cells.

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ORIGINAL ARTICLE

The central vein access port and catheter in outpatient chemotherapy for colorectal cancer: a retrospective study of 101 patients

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Abstract
Purpose The central venous access port (CV-port) system was examined in a series of colorectal cancer (CRC) patients.
Methods One hundred and one CRC patients underwent chemotherapy with the 5-fluorouracil + oxaliplatin (FOLFOX) or 5-fluorouracil + irinotecan regimen. The complications of the CV-port system were retrospectively assessed.
Results The CV-port system was placed in a total of 101 patients. The patients received a total of 1035 courses of these regimens. Eight complications occurred in the 101 patients (7.9%). The complications included three instances of catheter rupture, two thrombotic events around the catheter, and three infections at the site of the port or catheter. The complications were identified after a median of nine courses (range 6–16) and 135 days after the placement of the CV-port system. Sixty-six of the 101 patients switched their regimen from FOLFOX to another regimen, and 4 of these 66 patients (6.1%) experienced complications associated with the CV-port system. There were 25 subjects who were admitted to the hospital emergency wing during the chemotherapeutic regimens, and 4 of these patients (16%) had complications associated with the CV-port system.
Conclusions The complications of the CV-port system occurred at a defined rate, therefore the early diagnosis and the appropriate treatment to address these complications is crucial.

Keywords Colorectal cancer · Outpatient chemotherapy · Central venous access port · Complication · Pinch-off

Introduction

Completely implantable port systems were first introduced in the early 1980s. A variety of anticancer agents have been administered while using the devices without difficulty, and the patient acceptance of this system is excellent [1]. Late complications may occur, including catheter rupture and embolization, venous thrombosis, pocket infection, and port-related bacteremia. However, these devices have a long working life and a low rate of patient complications, and are of great value to patients who require long-term or cyclic intravenous treatments [2]. These data support the increasing use in current oncologic medical practices. The gastrointestinal division originally used the central venous access port (CV-port) system, either for administering chemotherapy to patients with gastric cancer, to provide nourishment to patients with short bowel syndrome, or for the treatment of patients with other conditions. The CV-port system has been extensively used since its introduction in colorectal cancer (CRC) patients receiving the 5-fluorouracil + oxaliplatin (FOLFOX) or 5-fluorouracil + irinotecan (FOLFIRI) + bevacizumab [3] chemotherapy.

A summary of this study was presented at the 108th Annual Meeting of the Japan Surgical Society in 2008 and the 46th Annual Meeting of the Japan Society of Clinical Oncology in 2008.

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Patients and methods

Patients and chemotherapeutic regimens

One hundred and three CRC patients underwent FOLFOX or FOLFIRI chemotherapy between April 2005 and March 2008 at our institution. One hundred and one of the 103 patients (98%) underwent CV-port system placement. Two patients could not receive the CV-port, because one patient had a mechanical valve and the other experienced difficulty in the placement of the CV-port. The 101 remaining patients (range 27–82 years of age, with a median age of 62 years) underwent chemotherapy for unresectable metastatic CRC, and also underwent adjuvant chemotherapy following hepatectomy. The regimens consisted of the modified FOLFOX-6 (m-FOLFOX 6), FOLFOX-4, or FOLFIRI regimens. The regimens consisted of a continuous infusion of 5-fluorouracil (5-FU) using a portable disposable pump, which was manufactured by Baxter (Deerfield, IL, USA).

Ports and routes of access to the central vein and maintenance of ports

Central venous access ports were placed by surgeons in the CRC patients. An indwelling catheter was inserted from the right subclavian vein at the lateral side using diagnostic imaging guidance and fluoroscopy to confirm that the catheter was placed in the superior vena cava. The ports were placed at the jugular vein or the inguinal vein if the surgeon experienced difficulty placing it in the subclavian vein. All 101 patients had a single-lumen Groshong 8-F catheter and an MRI-Port (CR Bard, Summit, NJ, USA) implanted. The first one or two courses of the regimen were administered while the patients were hospitalized in order to monitor any adverse events. The CV-port was put in place, and the patients were educated about the chemotherapy. After one or two courses of chemotherapy in the hospital, the patients underwent chemotherapy every 2 weeks as outpatients. Their ports were punctured by a doctor with a Huber-pointed needle. The doctor confirmed whether there was redness, swelling, or pain around the port, and confirmed that the natural drip was smooth before the patient was connected to the pump. The state of the catheter was regularly checked with chest X-rays every 3 months. The needle was removed without a saline flush after chemotherapy by the patients themselves or their family doctor.

The frequency and types of complications involving CV-ports and catheters were retrospectively evaluated. We also examined the instances of emergency hospital outpatient admission during chemotherapy and the reasons for changing to other regimens. The purpose of the present study was to demonstrate the placement methods and maintenance of the CV-port system for preventing and identifying late complications.

Results

A total of 101 patients underwent the FOLFOX regimen, and a total of 750 courses were administered (median 8 courses per patient). Forty of the 101 patients also received the FOLFIRI regimen, and a total of 270 courses were administered (median 6 courses). An overall total of 1035 courses were administered (median 10). Eight patients had central vein access port and catheter complications (7.9%). The complications associated with the central vain access port and catheter occurred at a median of 9 courses (range 6–16) and at a median time of 135 days after putting the CV-port system in place (Table 1).

Table 1 Complications of the central venous access port and catheter

	Total patients	Patients with complications
Number of patients	101	8
Sex, male/female	66/35	6/2
Age, median (range)	62 (27–82)	69 (65–81)
Courses of chemotherapy, median (range)	10 (1–25)	9 (6–16)

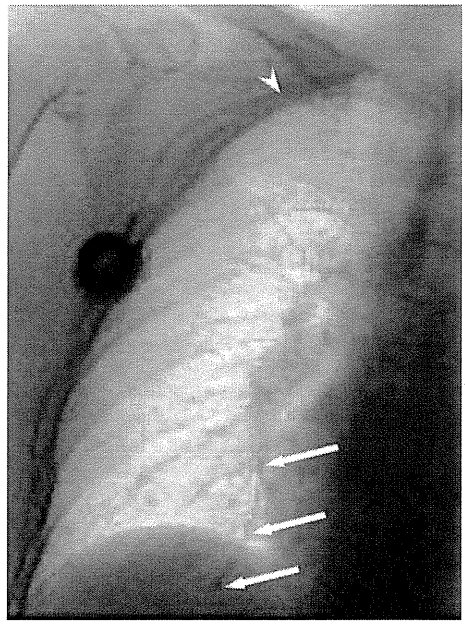

Fig. 1 Pinch-off syndrome and fracture of the catheter. The catheter was transected between the clavicle and the first rib (*arrowhead*), and the tip of the catheter was wedged into the pulmonary artery (*arrow*)

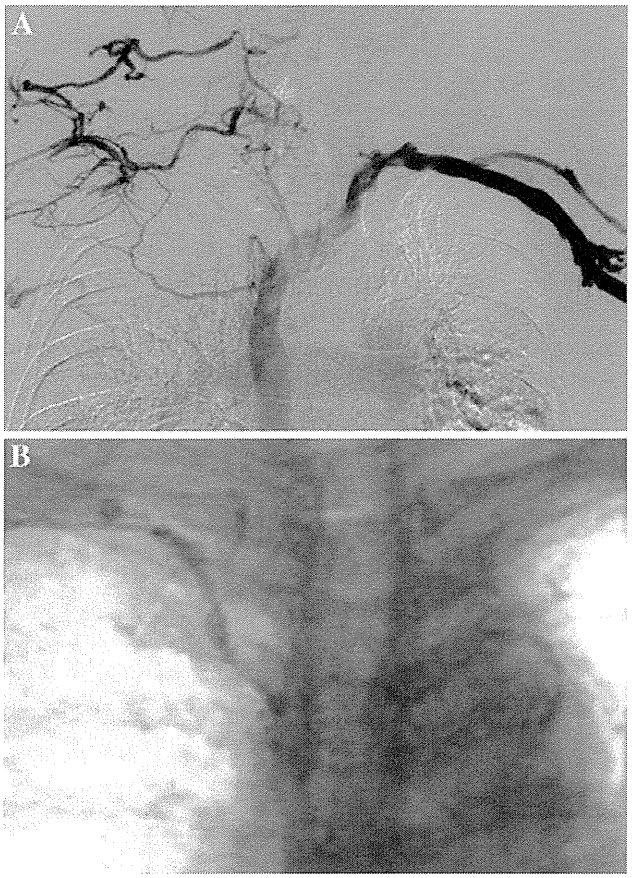

Fig. 2 A case of thrombosis around the site of the catheter (fibrin-sheath formation). **a** Contrast medium was injected from the bilateral median veins; however, the contrasting effect was not seen in the right subclavian vein, and it was concluded that a collateral pathway had developed. **b** There was no outflow of contrast media from the catheter tip, and a light contrasting effect was observed around the catheter

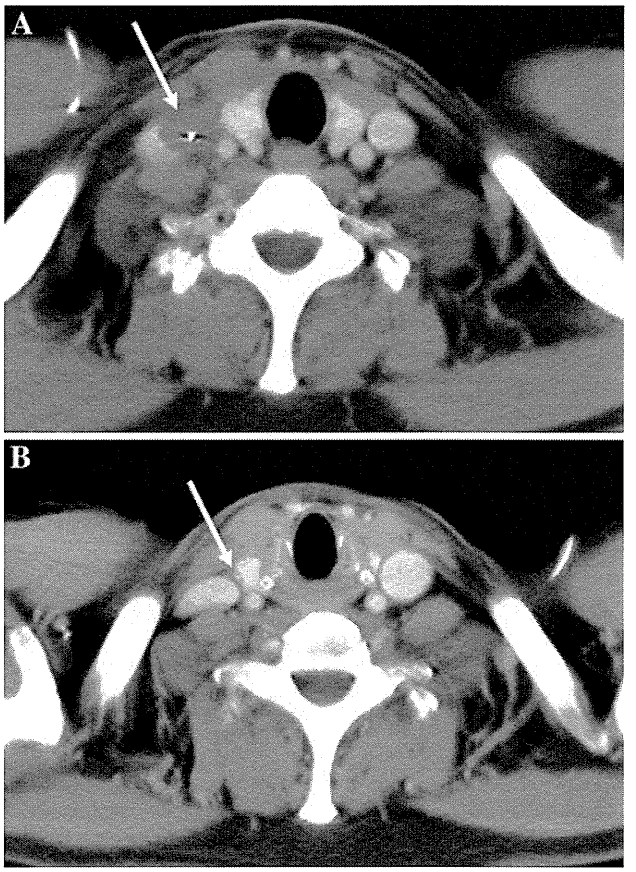

Fig. 3 Cases of thrombosis in the internal jugular vein. **a** The tip of the catheter was detected in an internal jugular vein and there was thrombosis around the catheter (*arrow*), as observed on contrast computed tomography. **b** Thrombosis in the internal jugular vein improved (*arrow*) after 5 months of warfarin treatment

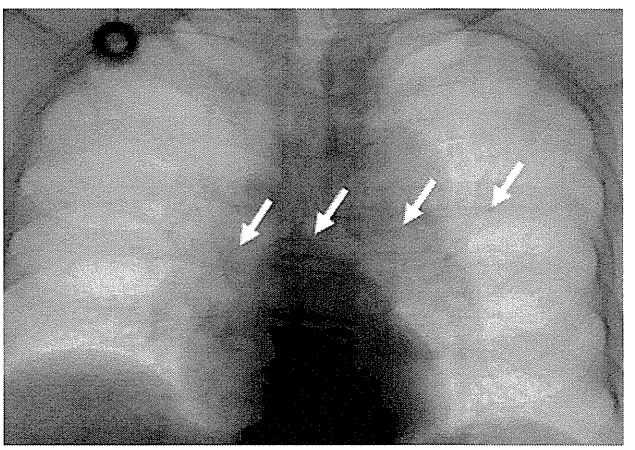

Fig. 4 Port connector rupture, connection portion coming off. The catheter was wedged into the pulmonary artery (*arrow*). The catheter was not fractured, and the rupture was judged to be caused by the catheter separating from the port connector

The incidents involved catheter pinch-off syndrome (POS) and fracture of the catheter ($n = 1$, Fig. 1), thrombosis around the catheter ($n = 2$, Figs. 2, 3), the connection portion of the port and catheter coming off ($n = 1$, Fig. 4), the flexure of the catheter ($n = 1$, Fig. 5), and the infection of the site of the port or catheter ($n = 3$) (Table 2).

Sixty-six of the 101 patients changed their regimen from FOLFOX to other regimens. Thirty-seven subjects were switched because of progressive disease (56.1%), 22 patients switched due to an adverse event (33.3%), and 4 patients were switched because of complications associated with the CV-port system (6.1%). The adverse events included peripheral neuropathy in 13 patients (19.7%), allergy in 5 patients (7.6%), and myelosuppression, interstitial pneumonia, and one patient's request (Table 3).

There were 25 patients admitted to the emergency department during the FOLFOX or FOLFIRI chemotherapeutic regimen, and 3 of 25 patients (12.5%) had adverse effects including pyrexia with neutropenia, severe anorexia, and acute exacerbation of interstitial pneumonia. However,

4 subjects (16.7%) required an emergency hospital admission due to complications associated with the CV-port system (Table 4).

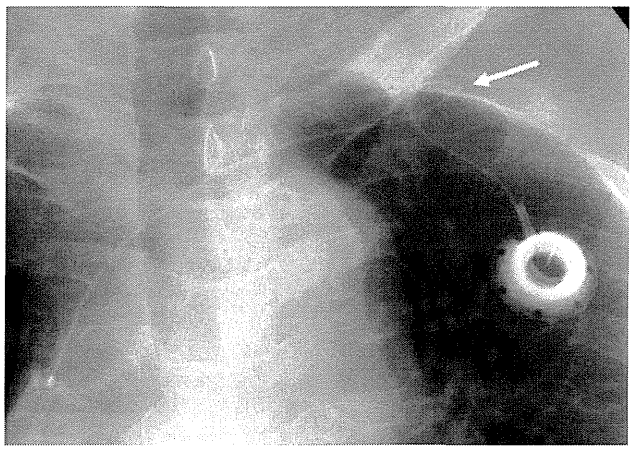

Fig. 5 Flexure and obstruction of the catheter. The catheter was bent in the subcutis (*arrow*), not in the subclavian vein, and was therefore manually repositioned

Discussion

FOLFOX or FOLFIRI regimen administration with a continuous infusion of 5-FU may be switched to a combination of an oral anticancer drug, such as S-1 or capecitabine, with irinotecan or oxaliplatin (IRIS, XELOX, etc.) [4–6]. However, the FOLFOX and FOLFIRI regimens are administered to CRC patients because there is a large amount of evidence indicating the efficacy, safety, and feasibility of these regimens.

Complications have been associated with the long-term placement of a CV-port and catheter [7–10]. The current series demonstrated complications in 8 of 101 patients (7.9%). The frequency of complications that occurred in association with the CV-port system during the chemotherapeutic treatment of outpatients in the present study was consistent with past reports. Several CRC patients required hospitalization for complications associated with the catheter. Furthermore, the complications of the CV-port and catheter caused some patients to change to another regimen (6.1%) or to require emergency treatment (16.7%). Outpatient chemotherapy was safely performed for the

Table 2 Summary of complications of central venous access-ports or catheters, excluding three patients with a catheter infection

Age (years)/sex	Chief complaint	Complication	Treatment
71/F (9)[a]	Pain around the port	Pinch off syndrome	Extraction of the catheter by interventional radiology
		Fracture of the catheter	Change to IRIS regimen
68/M (5)	Pain around the port	Thrombosis	Extraction of the catheter
		Fibrin sheath formation	Change to IRIS regimen
62/M (9)	Right neck pain	Thrombosis, dislocation	Extraction of the catheter
		Right internal jugular vein	Anticoagulant and change to the IRIS regimen
73/M (11)	Swelling around port	Port connector rupture	Extraction of the catheter by interventional radiology
		Connection portion coming off	Catheter replacement
81/M (13)	Poor infusion	Flexure of the catheter	Repositioning: stretch the catheter out
		Bent in subcutis	

IRIS regimen: combination therapy of S-1 and irinotecan

[a] Courses of chemotherapy in parentheses

Table 3 Reasons for changing from the FOLFOX regimen to another regimen

Reason ($n = 66$)	Number	Percentage	Age (years) Median	Sex M/F	Courses of chemotherapy
Progressive disease	37	56.1	61	24/13	8
Adverse events	22	33.3			
Peripheral neuropathy	13	19.7	63	10/3	10
Allergy	5	7.6	55	2/3	10
Myelosuppression	2	3.0	58	2/1	4
Interstitial pneumonia	1	1.5	75	1/0	8
Patient's request	1	1.5	44	0/1	2
Complication of CV-port system	4	6.1	69	3/1	12
Others	3	4.5	61	2/1	10

FOLFOX 5-fluorouracil + oxaliplatin, *CV-port* central venous access port

Table 4 Emergency hospital admissions during FOLFOX or FOLFIRI chemotherapy

Reason ($n = 25$)	Number	Percentage
Progressive disease	9	36
Adverse events	3	12
Peripheral neuropathy	0	0
Allergy	0	0
Myelosuppression	0	0
Interstitial pneumonia	1	4
Pyrexia with the neutropenia	1	4
Severe anorexia	1	4
Complication of CV-port system	4	16
Surgical site infection	2	8
Others	7	28

FOLFOX 5-fluorouracil + oxaliplatin, *FOLFIRI* 5-fluorouracil + irinotecan, *CV-port* central venous access port

majority of cases in our hospital. However, some issues remained, such as the occurrence of complications associated with the CV-port system, which led to changes to either another treatment regimen or to emergency hospital admission. These complications associated with the port and catheter included three instances of catheter rupture and embolization, venous thrombosis, and infection. We herein discuss the placement methods, the appropriate maintenance of CV-ports, and the measures taken to address these complications when they occur.

Catheter rupture and embolization

Pinch-off syndrome occurs when the CV access devices placed via the subclavian vein become obstructed due to thrombosis, impingement against a vein wall, or compression between the clavicle and the first rib. Luminal narrowing and complete catheter fracture occur in approximately 1% of catheter placements [11]. One case of catheter pinch-off was experienced at our institution during the study period. The patient did not report an active exercise history, but the subject had a small physique, weighed 45 kg, and was 145 cm in height. A catheter tip measuring 5 cm in length caused an embolus to a pulmonary artery. The catheter was withdrawn with a snare from the right inguinal vein by a radiologist. A puncture point is important to avoid pinch-off points. The catheter should be preferentially placed on the lateral side of the subclavian vein or in the internal jugular vein to avoid a pinch-off point [12]. Peripheral arm ports have been implanted in some CRC patients with no incidences of catheter POS [13]. The supraclavicular technique provides the best results with regard to the percutaneous introduction of large-bore central venous catheters [14]. At our institution, the most general approach from the right subclavian vein is the first choice of a puncture. There are no reports of cases that have an increased tendency to have pinch-offs, but we perform a puncture from another portion; namely, the right supraclavian vein or left subclavian vein, not the right subclavian vein, due to the fact that patients who actively exercise or have a small physique may experience POS.

Port connector rupture is usually caused by the method used to place the CV-port device. The method for connecting a port and catheter varies with the CV-port device, and the surgeon must confirm the type of CV-port device and the method used to ensure a proper connection.

Venous thrombosis

Catheter-related central venous thrombosis (CRCVT) occurs at a rate of 12–66% [15, 16]. In a prospective study, CRCVT was observed in 63 of 95 (66%) patients; however, it was symptomatic in only 4 of 63 (6%) of these patients [15]. There is no prognostic marker for venous thrombotic complications [16]. Three recent clinical trials investigated the effects of prophylactic anticoagulation with either low molecular weight heparin or low dose warfarin in cancer patients who had central venous devices [17–19]. However, these studies did not support the routine use of prophylactic anticoagulation in cancer patients with venous catheters to prevent catheter-induced thrombosis. Based on these results, routine anticoagulation is not recommended [20]. Anticoagulant administration just after the placement of the CV-port system is not used in our hospital. Two thrombosis cases were detected at our institution during the study period. These patients were diagnosed by injecting contrast media from the port and median vein on the port insertion side. The IRIS regimen (a combination therapy of the oral anticancer drug S-1 and irinotecan) was administered for the current patient series when the CV-port could not be replaced due to thrombosis. In the present study, thrombosis improved after the administration of anticoagulant therapy. Both patients had the CV-port system put in place again, and the FOLFOX regimen was restarted.

Infection

A diagnosis of a catheter-related infection might be difficult in the absence of local signs of inflammation [21]. Routine device removal is not recommended for most patients. Empirical antibiotics are administered when the patient presents with sepsis or septic shock. Port systems must be removed in case of a persistent relapse of infection after antibiotic treatment, at signs of port or catheter tunnel infection, for unstable patients, or after the development of systemic complications [22, 23]. However, CRC patients undergoing perioperative chemotherapy have had highly

invasive surgery, and the general opinion is that these guidelines do not apply to most of these patients. A high fever after CRC resection is usually attributable to an infection at the surgical site or an infection of the CV-port system. In our hospital we experienced a patient who demonstrated complications associated with a biliary fistula after hepatectomy, who continued to have a high fever after antibiotic treatment. The CV-port system was withdrawn, but no bacteria were detected on the catheter. However, we thought that the CV-port system should be withdrawn in such a case, contrary to popular opinion.

In conclusion, the management of the CV-port system is an important factor in the administration of chemotherapy to outpatients with CRC. We have described proper CV-port system placement and have summarized a recent report about the tendencies of port complications. We have also explained measures that were used to treat the complications in our experimental cases. The chemotherapeutic treatment of outpatients with the CV-port system is therefore best performed when the physicians are aware of these complications and how to best treat patients for CV-port complications without compromising their anticancer treatment.

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ORIGINAL ARTICLE

A randomized phase-II trial comparing sequential and concurrent paclitaxel with oral or parenteral fluorinated pyrimidines for advanced or metastatic gastric cancer

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Abstract
Background The purpose of this study was twofold: (1) to compare S-1 with infusional 5-fluorouracil (FU) to determine which would be a better partner of paclitaxel (PTX), and (2) to compare a concurrent strategy with a sequential one, the latter strategy being the one that is widely used in Japanese general practice.
Methods The 161 eligible patients were randomized into four arms to receive the following regimens: A (sequential), intravenous 5-FU at 800 mg/m^2 for 5 days every 4 weeks followed by weekly PTX at 80 mg/m^2; B (sequential), S-1 at 80 mg/m^2 for 4 weeks and 2-week rest followed by PTX; C (concurrent), intravenous 5-FU at 600 mg/m^2 for 5 days and weekly PTX at 80 mg/m^2 every 4 weeks; and D (concurrent), S-1 for 14 days and PTX at 50 mg/m^2 on days 1 and 8 every 3 weeks. The primary endpoint was the overall survival (OS) rate at 10 months.
Results The ten-month OS rates in arms A, B, C, and D were 63, 65, 61, and 73%, respectively. The OS was best in the concurrent S-1/PTX arm, with a mean survival time of

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15.4 months, but no significant difference was observed between the four arms. Response rates were higher in the concurrent arms than in the sequential arms.

Conclusion Our study did not show sufficient prolongation of survival with the concurrent strategy to proceed to a phase-III trial; however, the sequential arms showed survival comparable to that in the concurrent arms, with less toxicity. In patients who are ineligible for cisplatin (CDDP), sequential treatment starting with S-1 and proceeding to PTX would be a good alternative strategy, considering quality of life (QOL) and the cost-benefits of an oral agent as first-line treatment.

Keywords Advanced gastric cancer · Paclitaxel · S-1 · Sequential chemotherapy · Concurrent combination chemotherapy · Randomized phase-II trial

Introduction

Gastric cancer is the second most common cause of cancer-related death worldwide [1]. Most patients (except those from northeast Asian countries) present with advanced, inoperable, or metastatic disease, and the 5-year survival rate is approximately 10–15%. Palliative chemotherapy for advanced disease improves survival as compared with the best supportive care [2–4]. Despite the innumerable efforts of investigators in various countries to test various chemotherapeutic and immunotherapeutic agents and combination regimens, there has been little progress in the therapy for patients with advanced gastric cancer.

Probably because there is less evidence regarding the treatment of gastric cancer compared to that of other malignancies, the standard treatment for gastric cancer differs from country to country, although most of the "standard" regimens do not have sufficient evidence. Moreover, the insurance systems in most western countries approve only first-line treatment, and in these countries, doublet or triplet therapies could be the standard choice, while some countries, including Japan, approve second- and greater-line strategies, where we can choose not only concurrent but also sequential strategies. Reflecting these historical and social circumstances, "standard" treatment for gastric cancer shows wide variety, with some confusion. In Japan, the evidence-based standard regimen involved continuous infusion of 5-fluorouracil (5-FU) only (JCOG9205) before the results of the Japan Clinical Oncology Group (JCOG) 9912 and SPIRITS trials had been obtained [5–7]. After the results of SPIRITS trial were shown, S-1 plus cisplatin (CDDP) has been accepted as the standard first-line treatment for patients with good condition, but S-1 without CDDP was also widely used in general practice. This means we still need an alternative strategy, whose sequence starts from a fluoropyrimidine (infusional 5-FU or oral S-1) with or without other agents.

As for candidates as the fluoropyrimidine partner, some potent agents have been approved for gastric cancer in the past two decades. One of the promising agents was paclitaxel (PTX) [8], which had shown beneficial results in single use or concurrent use with a fluoropyrimidine [9–12]. However, these studies were conducted as single-arm phase I–II trials. Hence, the choice between sequential and concurrent strategies for fluoropyrimidine and PTX remains unclear.

We therefore planned a randomized phase-II trial to compare the following four treatment regimens: A, sequential 5-FU monotherapy followed by PTX monotherapy; B, sequential S-1 monotherapy followed by PTX monotherapy; C, concurrent 5-FU plus PTX [11]; and D, concurrent S-1 plus PTX [12]. The purpose of the study was twofold: (1) to compare S-1 with infusional 5-FU to determine which was the better partner of PTX, and (2) to compare a concurrent strategy with a sequential one, the latter strategy being the one that is widely used in Japanese general practice.

Patients and methods

The detailed study design and protocol treatment of this study has already been described by Morita et al. [13]. Below we outline a summary of the methodological issues in this study with the protocol (informed consent form) that was amended after the SPIRITS trial.

Eligibility criteria

Patients more than 20 years of age with histologically confirmed non-resectable advanced or recurrent gastric cancer were eligible. Patients who had undergone prior anti-tumor therapy (except for surgery and postoperative adjuvant chemotherapy) were excluded. Patients had to have adequate renal, hepatic, hematologic, and cardiac function, with an Eastern Cooperative Oncology Group performance status (PS) of 0–1. Patients had to be able to take food via the oral route to be considered for enrolment in the study.

The protocol was approved by the Institutional Review Board (IRB) of each institution, and written informed consent was obtained before treatment. Participating investigators were instructed to send an eligibility criteria report to the data center operated by the non-profit organization Epidemiological and Clinical Research Information Network (ECRIN). Eligible patients were registered and then randomized to receive either of the four treatment regimens (A, B, C, and D), using a centralized dynamic

randomization method with the following balancing factors: measurable disease according to criteria set by Response Evaluation Criteria in Solid Tumours (yes/no); disease type [inoperable advanced/postoperative recurrent (with postoperative chemotherapy)/postoperative recurrent (with no postoperative chemotherapy)]; PS (0/1); peritoneal metastasis based on diagnosis with images (yes/no); age (<75 years/≥75 years), and institution. Information regarding the necessary follow-up examinations and chemotherapy schedule was then sent from the ECRIN data center. The accrual started in December 2005 and was continued for 3 years.

Projected treatments

Based on previous trials, we adapted four promising regimens for this selection design trial [13]. Patients in arm A received sequential therapy with intravenous (i.v.) 800 mg/m^2 5-FU daily for 5 days every 4 weeks until progression, followed by PTX 80 mg/m^2 on days 1, 8, and 15 every 4 weeks. Patients in arm B received sequential therapy with 80 mg/m^2 of oral S-1 daily for 4 weeks and 2-week rest after the administration (total of 6 weeks per single course) until progression. This was followed by PTX, utilizing the same administration dose and schedule as that in arm A's second-line PTX. Patients in arm C received a combination therapy with 600 mg/m^2 5-FU (i.v.) daily for 5 days from day 1 and infusion of 80 mg/m^2 PTX on days 8, 15, and 22 every 4 weeks. Patients in arm D received a combination therapy with 80 mg/m^2 oral S-1 for 14 days from day 1 and infusion of 50 mg/m^2 PTX on days 1 and 8 every 3 weeks. In the sequential treatment arms A and B, the administration of 5-FU or S-1 monotherapy was discontinued if the following were observed: (1) disease progression or occurrence of new disease; (2) grade-4 nonhematological toxicities evaluated according to the Common Terminology Criteria for Adverse Events version 3.0; (3) adverse events causing patients to refuse treatment or causing a clinician to discontinue treatment; (4) increase in the tumor markers carcinoembryonic antigen (CEA) and/or cancer antigen (CA) 19-9 in two or more consecutive measurements or symptomatic progression (e.g., cancer pain and dysphagia). An irinotecan-containing regimen was recommended for use in case further lines of treatment were to be given.

Follow-up

Disease progression and occurrence of new disease were examined using radiographs, computed tomography (CT) or magnetic resonance imaging (MRI) of the abdomen, and thoracic CT and measurements of the tumor markers CEA and CA19-9. These examinations were performed at baseline and at least every 4–5 weeks during treatment. Blood tests and symptom checks were performed before treatment and at least every 2 weeks during treatment. In cases where therapy was discontinued owing to toxicity, clinicians followed up patients until they recovered from the effects of toxicity.

Study design and statistical methods

The primary aim of this study was to compare treatment regimens A–D in terms of the primary endpoint of the 10-month overall survival (OS) rate. In addition, OS and treatment failure curves were constructed as time-to-event plots using the Kaplan–Meier method [14]. Time-to-event curves were compared using log-rank tests and the hazard ratio (HR) estimated by Cox regression models [15]. The prevalence of grade-3 or grade-4 adverse events was compared between the treatment arms. Calculation of the sample size required 40 patients in each arm to assure 80% probability in order to select the best treatment arm [16] as long as the true expected 10-month OS rate exceeded that of any other arm by at least 15%. The total number of patients to be accrued was set at 160.

Protocol amendment after SPIRITS trial

After the results of the SPIRITS trial were publicized, standard first-line therapy in Japan shifted from monotherapies with 5-FU or S-1 to an S-1/CDDP combination. The protocol committee of the present trial discussed this issue and decided not to change the protocol treatments, because none of the treatment arms has actually been shown to be inferior to the S-1/CDDP combination. Instead, all patients who became candidates for accrual in the trial after the results of the SPIRITS trial were publicized were to be informed of the novel standard treatment in Japan, using a newly compiled explanatory note, and they were to be offered the alternative of receiving the combination therapy instead of participating in the trial. Each participating institution agreed on the use of the newly compiled explanatory note without correction in the study protocol itself, and case recruitment was re-started after the IRB approval of the amendment was obtained.

Results

A total of 161 patients were enrolled in the trial from December 2005 to November 2008. The numbers of patients in arms A, B, C, and D were 40, 40, 41, and 40, respectively. Two patients in arm A and two in arm C declined therapies before the start of the assigned treatment. Therefore, 38, 40, 39, and 40 patients in arms A, B,

C, and D, respectively, were considered to be eligible for evaluation (Fig. 1). Initial patient characteristics in the four arms were well matched (Table 1). The median age was 67 years (range 40–90 years).

Survival

The ten-month OS rates predetermined as the primary endpoint were 63, 65, 61, and 73% in arms A, B, C, and D, respectively. Although concurrent therapy with S-1 plus PTX demonstrated the best survival benefit among the four arms, the difference in OS rates between the arms with highest (D) and lowest (C) rates was less than the predetermined criterion (i.e., 15%). Kaplan–Meier survival curves did not show a significant difference between the four arms (Fig. 2). The survival rates in the sequential (A, B) and concurrent (C, D) arms were almost identical ($p = 0.93$) (Fig. 3a). In addition, no difference in survival was observed between the 5-FU-containing regimens (arms A and C) and the S-1-containing regimens (arms B and D) ($p = 0.83$) (Fig. 3b).

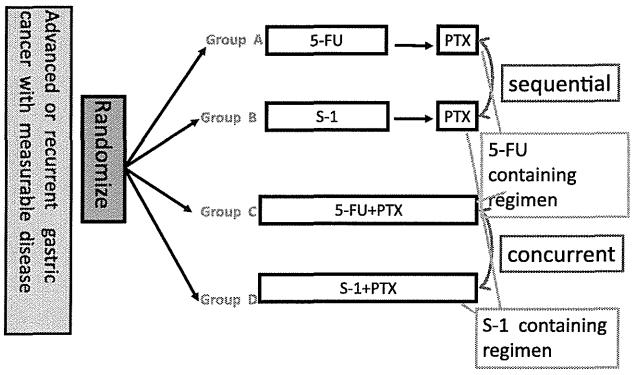

Fig. 1 CONSORT diagram that accounts for all patients. *5-FU* 5-fluorouracil, *PTX* paclitaxel

Time to treatment failure (TTF)

In arms A and B, TTF was calculated by the addition of the prior 5-FU or S-1 treatment period and the sequential PTX period. Median TTF values were 213, 222, 177, and 189 days in arms A, B, C, and D, respectively. No difference was observed between the four arms. However, Kaplan–Meier TTF curves for sequential and concurrent regimens showed better TTF in favor of sequential treatment compared with concurrent treatment (HR 0.71, 95%

Table 1 Patient characteristics

Treatment arm	Arm A 5-FU→PTX $n = 38$	Arm B S-1→PTX $n = 40$	Arm C 5-FU+PTX $n = 39$	Arm D S-1+PTX $n = 40$
Gender				
Male	25 (65.8%)	28 (70.0%)	28 (71.8%)	32 (80.0%)
Female	13 (34.2%)	12 (30.0%)	11 (28.2%)	8 (20.0%)
Age (years)				
Median	67.0	68.0	67.3	66.6
Range	48–79	51–81	40–82	47–90
74≤	31 (81.6%)	33 (82.5%)	31 (79.5%)	31 (77.5%)
≤75	7 (18.4%)	7 (17.5%)	8 (20.5%)	9 (22.5%)
Performance status				
0	29 (76.3%)	27 (67.5%)	25 (64.1%)	28 (70.0%)
1	9 (23.7%)	13 (32.5%)	14 (35.9%)	12 (30.0%)
Stage				
Non-resectable, no previous chemotherapy	31 (81.6%)	33 (82.5%)	32 (82.1%)	32 (80.0%)
Recurrent after curative surgery, adjuvant chemotherapy (+)	2 (5.3%)	1 (2.5%)	3 (7.7%)	3 (7.5%)
Recurrent after curative surgery, adjuvant chemotherapy (–)	5 (13.2%)	6 (15.0%)	4 (10.3%)	5 (12.5%)
Peritoneal metastasis				
Yes	9 (23.7%)	13 (32.5%)	5 (12.8%)	10 (25.0%)
No	29 (76.3%)	27 (67.5%)	34 (87.2%)	30 (75.0%)
Measurable disease				
Yes	19 (50.0%)	23 (57.5%)	17 (43.6%)	20 (50.0%)
No	19 (50.0%)	17 (42.5%)	22 (56.4%)	20 (50.0%)

5-FU 5-fluorouracil, *PTX* paclitaxel