Table 1. Patient characteristics.

| Characteristic | |
|---|---|
| Median age in years (range) | 64 (47–78) |
| Sex, *n* (%) | |
| Males | 10 (55.6) |
| Females | 8 (44.4) |
| Eastern Cooperative Oncology Group performance status, *n* (%) | |
| 0 | 13 (72.2) |
| 1 | 4 (22.2) |
| 2 | 1 (5.6) |
| Pancreatic tumour location, *n* (%) | |
| Head | 11 (61.1) |
| Body/tail | 5 (27.8) |
| Tail | 1 (5.6) |
| Multicentric | 1 (5.6) |
| Biliary stent, *n* (%) | |
| Yes | 8 (44.4) |
| No | 10 (55.6) |
| Stage, *n* (%) | |
| Locally advanced | 6 (33.3) |
| Metastatic | 12 (66.7) |
| Sites of metastatic disease, *n* (%) | |
| Liver | 5 (27.8) |

Table 2. Clinical data for patients treated with gemcitabine plus regional hyperthermia.

| | |
|---|---|
| No. of patients | 18 |
| No. of cycles of gemcitabine | |
| Median | 6 |
| Range | 1–20 |
| Relative dose intensity of gemcitabine | |
| Average | 0.86 |
| Median | 0.87 |
| Range | 0.64–1.0 |
| Frequency of hyperthermia | |
| Median | 21.5 |
| Range | 2–77 |

Of the 18 patients, five had liver metastases, two presented with peritoneal carcinomatosis, and six presented with distant lymph node metastases. After discontinuation of treatment, 11 patients (61.1%) received anticancer treatments, whereas the remaining seven received best supportive care (38.9%). Of the 11 treated with the second-line therapy, five received S-1 monotherapy and six received S-1 plus gemcitabine combination therapy. S-1, an oral fluoropyrimidine, is one of the key drugs used to treat pancreatic cancer in Japan, and a randomised phase III study revealed that the efficacy of S-1 as a first-line treatment for pancreatic cancer was similar to that of gemcitabine [16].The median number of gemcitabine treatment cycles was 6 (range, 1–20 cycles), and the median relative gemcitabine dose intensity was 0.87. The frequency of hyperthermia ranged from 2 to 77 sessions (median, 21.5 sessions) (Table 2).

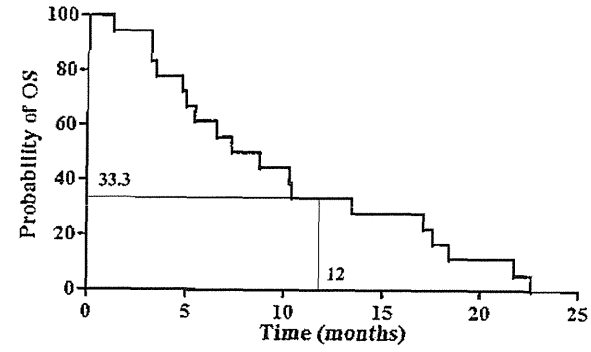


Figure 2. Kaplan–Meier estimates of overall survival (OS) in the study population ($n=18$).

*Efficacy*

Median OS was 8 months, and the 1-year survival rate was 33.3% (Figure 2); the null hypothesis (1-year survival ≤10%) was therefore rejected. Survival curves were significantly different between patients with locally advanced pancreatic cancer and those with metastatic lesions when analysed by the log rank test ($P=0.0067$, Figure 3). Median OS was 17.74 months for patients with locally advanced cancer and 5.22 months for patients with metastases. One-year survival also differed between the two groups; however, the difference was not significant (66.7% versus 16.7%; P = 0.107). Of the 18 patients, two (11.1%) exhibited a partial response (PR), nine(50%) had stable disease (SD), and seven (38.9%) had progressive disease (Table 3). The response rate was 11.1% and the disease control rate (DCR, complete response + PR + SD) was 61.1%. There was no correlation between the RF output power and treatment response (data not shown).

*Toxicity*

The adverse events reported in this study are shown in Table 4. The major grade 3–4 adverse events were neutropenia (33.3%) and anaemia (16.7%). Haematological toxicity was mostly transient, and there were no episodes of infection with ≥grade 3 neutropenia. The most common non-haematological events were anorexia (61.1%) and fatigue (50%), and most of these were mild. Grade 3 or higher non-haematological adverse events included anorexia ($n=1$) and gastrointestinal bleeding ($n=1$). The episode of gastrointestinal bleeding was considered unrelated to treatment. All hyperthermia-related adverse events were mild and included pain and a skin rash. No other severe or unexpected toxicities were observed.

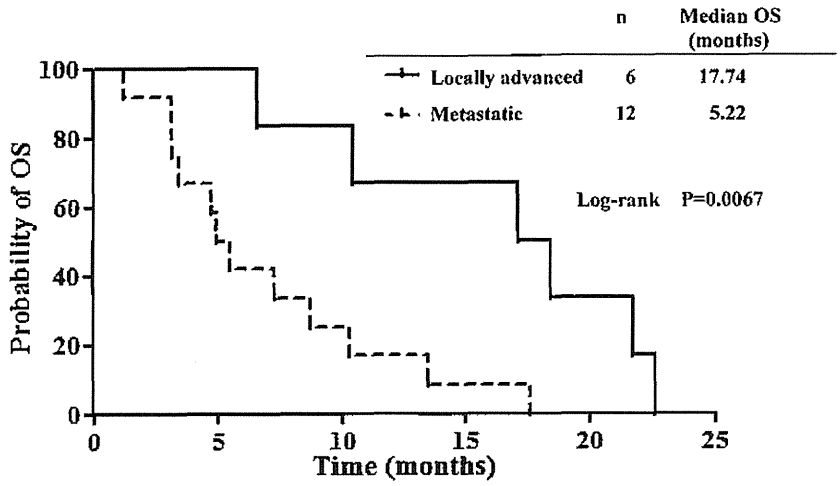


Figure 3. Kaplan–Meier estimates of overall survival (OS) in patients with locally advanced pancreatic cancer ($n=6$) and patients with metastatic pancreatic cancer ($n=12$). The P value was calculated using a two-sided log rank test.

Table 3. Tumour response.

| No. of patients | Response | | | | Response rate (%) |
|---|---|---|---|---|---|
| | CR | PR | SD | PD | |
| 18 | 0 | 2 | 9 | 7 | 11.1 |

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 4. Maximum toxicities per patient during all cycles.

| | Any grade, *n* (%) | Grade 3–4, *n* (%) |
|---|---|---|
| Non-haematological | | |
| Fatigue | 9 (50) | 0 |
| Anorexia | 11 (61.1) | 1 (5.6) |
| Nausea | 4 (22.2) | 0 |
| Diarrhoea | 2 (11.1) | 0 |
| Mucositis | 4 (22.2) | 0 |
| Fever (in the absence of neutropenia) | 4 (22.2) | 0 |
| Skin rash (associated with hyperthermia) | 5 (27.7) | 0 |
| Haemorrhage, GI-duodenum | 1 (5.6) | 1 (5.6) |
| Haematological | | |
| Leucopenia | 13 (72.2) | 1 (5.6) |
| Neutropenia | 8 (44.4) | 6 (33.3) |
| Thrombocytopenia | 10 (55.6) | 0 |
| Anaemia | 10 (55.6) | 3 (16.7) |
| Increased aspartate aminotransferase | 4 (22.2) | 0 |
| Increased alanine aminotransferase | 4 (22.2) | 0 |

GI, gastrointestinal.

## Discussion

Although single-agent gemcitabine is currently the standard treatment for patients with advanced pancreatic cancer, objective responses are low and median survival benefit is modest relative to 5-FU monotherapy [1, 2]. To improve treatment efficacy, many phase III trials comparing gemcitabine monotherapy with gemcitabine combination therapy for advanced pancreatic cancer have been undertaken [2].Unfortunately, almost all combinations of gemcitabine with cytotoxic and target agents failed to yield any additional benefits over gemcitabine monotherapy [2]. Only two reports exist on combined regional hyperthermia and chemotherapy for advanced pancreatic cancer [10, 11]. In a retrospective study we reported that seven patients treated with this combination achieved a median OS of 10.9 months [10]. Tschoep et al. [11] reported that 22 patients with disease progression after gemcitabine-based first-line chemotherapy were treated with gemcitabine, cisplatin, and regional hyperthermia as second-line treatment. The median time to treatment failure was 4.2 months and median OS was 16.9 months with this regimen. To the best of our knowledge, this is the first prospective study of regional hyperthermia combined with chemotherapy as the first line of treatment in patients with advanced pancreatic cancer.

In this study, regional hyperthermia combined with gemcitabine resulted in a median OS of 8 months, which was longer than that reported for gemcitabine monotherapy [2].The response rate was 11.1% and the DCR was 61.1%. Furthermore, the 1-year survival rate was 33.3%, which was above the pre-established threshold required for the regimen to be considered effective. Patients with locally advanced pancreatic cancer had a better outcome than those with metastatic pancreatic cancer (median OS, 17.7 months versus 5.2 months, respectively).The results in patients with locally advanced, non-metastatic pancreatic cancer compare favourably with previous studies of 5-FU or gemcitabine with radiation, which reported a median OS of 8–10 months [17–19].

Combinations of regional hyperthermia with gemcitabine did not yield any additional toxicities over those yielded by gemcitabine monotherapy, except for mild pain and skin rash. Of the 18 patients in this study, 8 (44.4%) had biliary stents. Although there was a possibility of the metallic stents producing high temperatures, biliary stent-related complications did not occur. Thermal profiles during heating with the Thermotron RF-8 in a static phantom embedded with a metallic stent have revealed that temperatures around the stent do not rise too high in the absence of stent obstruction [20]. Moreover, severe complications associated with RF capacitive hyperthermia therapy in patients with metallic biliary stents have not been reported [10, 20].

The biological rationale for using hyperthermia against malignant tumours is that malignant tumour cells may have a lower thermal death threshold than do normal cells [21]. Hypovascular tumours, such as pancreatic cancer, retain more heat than surrounding tissues, and consequently, tumour temperature rises above that of normal tissues. Pancreatic cancer is considered a good target for hyperthermic treatment. Hyperthermia was shown to enhance the cytotoxicity of several chemotherapeutic agents [7, 21], although the timing of hyperthermia plays a critical role in its efficacy [22].Simultaneous treatment with gemcitabine and hyperthermia was reported to decrease cytotoxicity in a mouse model [23], whereas an interval of 20–24 h between gemcitabine administration and hyperthermia led to enhanced cell death [8]. Decreased cytotoxicity after simultaneous hyperthermia and gemcitabine treatment may result from the inhibition of gemcitabine-activated triphosphate metabolism [8]. We showed in an *in vitro* study that hyperthermia enhanced gemcitabine cytotoxicity, particularly when it was performed 24 h before or after gemcitabine administration [9]. Our treatment schedule was based on these results.

Reportedly, NF-$\kappa$B is activated by anticancer agents, including gemcitabine, in tumour cells, and NF-$\kappa$B activation mediates the amplification of metastatic potential and resistance to chemotherapy [24–26]. Transforming growth factor-beta (TGF-$\beta$) is known to be produced by tumour and/or stromal cells and promotes epithelial-to-mesenchymal transition (EMT), which is crucial in cancer invasion and metastasis. We recently demonstrated that hyperthermia inhibits gemcitabine-induced activation of NF-$\kappa$B in pancreatic cancer cell lines [9] and the production of TGF-$\beta$ in tumour cell lines and in a mouse tumour model [27]. Moreover, we found that heat treatment suppressed TGF-$\beta$-induced EMT *in vitro* (manuscript in submission). Since regional hyperthermia combined with gemcitabine improved the prognosis of patients with locally advanced pancreatic cancer in this study, EMT inhibition may be the principal mechanism by which hyperthermia influences the progression of pancreatic cancer.

The present study has a major limitation in that thermometry could not be assessed. Intratumoural temperatures reportedly correlate with objective tumour response during deep regional hyperthermia using an 8-MHz RF-capacitive heating device [28, 29]. The correlation between intratumoural temperature and treatment response should be assessed to evaluate the potential contribution of regional hyperthermia to clinical outcomes in patients with pancreatic cancer. Thermometry has also been investigated in patients administered deep regional hyperthermia, especially those with pelvic tumours and soft-tissue sarcomas [30, 31]. However, direct intratumoural measurement in patients with pancreatic cancer is more invasive and clinically difficult to manipulate. Direct intratumoural measurements for deep-seated tumours also offer the possibility of severe complications (e.g. subcutaneous or deep infection, intolerable pain, bleeding, and a possibility of cancer spread) [32].It has been reported recently that intraluminal thermometry (e.g. intra-oesophageal, intrarectal, and intravesical) provides sufficient information required for deep regional hyperthermia therapy inpatients with thoracic and pelvic tumours [32–34]. Fatehi et al. reported that intratumoural and intraluminal temperatures during individual treatments were highly correlated, and the average intratumoural and intraluminal temperatures were similar [34]. Therefore, intraduodenal temperature may be used as a promising parameter for the assessment of deep regional hyperthermia therapy in patients with pancreatic cancer. Other limitations of this study include its small sample size and the inclusion of patients with both locally advanced and metastatic disease. Because the prognosis of locally advanced and metastatic disease was distinctly different in this study, the efficacy of regional hyperthermia in patients with locally advanced pancreatic cancer should be evaluated separately from patients with metastatic disease.

In conclusion, we demonstrated the safety of combined regional hyperthermia and gemcitabine treatment as well as a superior OS relative to published rates in patients with locally advanced pancreatic cancer. This study succeeded in improving the 1-year survival rate to beyond 30%; however, the difference in median OS between patients with locally advanced and metastatic pancreatic cancer is relatively large. Although the reason for this difference is unknown, EMT inhibition by regional hyperthermia may explain these findings. Based on the results of this study, a randomised trial focusing on locally advanced pancreatic cancer will be

necessary in the near future to clarify the efficacy of combined hyperthermia and gemcitabine therapy.

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ORIGINAL ARTICLE

# Phase II trial of combined treatment consisting of preoperative S-1 plus cisplatin followed by gastrectomy and postoperative S-1 for stage IV gastric cancer

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**Abstract**
*Background* To improve the poor prognosis in patients with stage IV (StIV) gastric cancer (GC), we conducted a multicenter phase II study of preoperative S-1 plus cisplatin followed by gastrectomy and postoperative S-1 for StIV GC (the protocol is registered at the clinical trial site of the National Cancer Institute; KYUH-UHA-GC03-01, NCT00088816).
*Methods* Eligibility criteria included histologically proven StIVGC. Patients received S-1 (80 mg/m$^2$/day, days 1–21) plus cisplatin (60 mg/m$^2$ on day 8) for 2 courses. After preoperative chemotherapy (CTx), radical gastrectomy was performed. Postoperative S-1 (80 mg/m$^2$/day, days 1–14) was administered every 3 weeks for 1 year.
*Results* Fifty-one patients were enrolled and all patients were followed for more than 2 years. The 2-year overall survival and progression-free survival rates were 43.1% (95% confidence interval [CI] 29.4–56.1%) and 33.3% (95% CI 20.9–46.2%), respectively. Preoperative chemotherapy was accomplished in 44 patients (86.3%). These 44 patients underwent surgery and R0 resection was achieved in 26. The rate of R0 resection for GC with a single StIV factor ($n = 24$) was 79.2% and that for GC with multiple StIV factors ($n = 27$) was 25.9%. All patients with cancer cells in peritoneal washings (cytology [Cy] 1) alone ($n = 12$) became Cy0 after preoperative chemotherapy. Postoperative chemotherapy was completed in 11 patients, including 8 with Cy1 alone. No treatment-related death was recorded. Recurrences were observed in 14 patients after R0 resection. The most frequent recurrence site was the peritoneum. Patients who underwent R0 resection and those with Cy1 alone had a better survival.
*Conclusions* This perioperative treatment was safe and feasible for StIVGC but failed to show a survival benefit. In patients with StIVGC with Cy1 alone this treatment resulted in a better prognosis.

**Keywords** Gastric cancer · Induction chemotherapy · Surgery · S-1 plus cisplatin

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## Introduction

Gastric cancer (GC) is one of the leading causes of cancer death worldwide [1]. It is often diagnosed at an advanced stage. The prognosis of stage IV GC is poor (e.g., the 1-, 2-, and 5-year survival rates of stage IV GC are approximately 40, 20, and <10%, respectively [2, 3]), even if patients are treated surgically.

Recently, several novel regimens of combined chemotherapy, including S-1 plus cisplatin, have produced improved overall survival (OS) rates in patients with unresectable stage IV GC [4–6]. The median survival time in patients treated with S-1 plus cisplatin was 13.0 months, and the response rate with this regimen was 54%. But its

2-year OS rate, 23.6% (95% confidence interval [CI] 16.8–30.4), was still dismal [6].

Combinations of some highly effective chemotherapeutic regimens and surgery have given favorable results in resectable, locally advanced GC [7–11]. Preoperative chemotherapy for advanced GC possesses theoretical benefits. First, this chemotherapy usually shrinks cancerous tissue, increasing the likelihood of R0 resection with extended surgery. Second, more intensive chemotherapy than that used postoperatively is possible, with a higher compliance rate. Third, distant occult metastases can be treated before local therapy has begun. Furthermore, postoperative S-1 alone has been proven beneficial for treating stage II and stage III GC [12]. Hence, one of the most potentially favorable multimodal treatments for stage IV GC would be a combination of preoperative administration of S-1 plus cisplatin, subsequent gastrectomy with D2 nodal dissection, and postoperative S-1 administration. The present study was conducted to evaluate the efficacy, feasibility, and safety of this novel multimodal treatment for stage IV GC.

## Patients and methods

This was a prospective multicenter phase II study conducted at eight centers of the Kyoto University Surgical Oncology Group in Japan between May 2003 and March 2008. This protocol is registered at the clinical trial site of the National Cancer Institute (KYUH-UHA-GC03-01, NCT00088816).

### Patients

Eligibility criteria included: (1) pretreatment (pre-) histologically proven stage IV GC (pre-N3, pre-T4N2, pre-H1, pre-P1, pre-Cy1, pre-M1) diagnosed by pretreatment helical computed tomography (CT) and staging laparoscopy within 4 weeks before registration, (2) no prior therapy, (3) Eastern Clinical Oncology Group (ECOG) performance status of 0 or 1, and (4) age of 20–80 years. Patients must have had no signs of organ failure, including that of bone marrow, heart, lungs, and kidneys. Acceptable hematologic (white blood cell count $\geq$4,000/μL and $\leq$12,000/μL, neutrophil count $\geq$2,000/μL, platelet count $\geq$100,000/μL, hemoglobin $\geq$9.0 g/μL), renal (creatinine clearance $\geq$ 50 mL/min), hepatic (aspartate aminotransferase and alanine aminotransferase $\leq$2.5 times the upper limit at each institution, serum total bilirubin $\leq$1.5 mg/dL), and respiratory function (arterial partial pressure of oxygen [$PaO_2$] $\geq$ 70 torr in room air) was required for enrollment in the study.

Exclusion criteria included active gastrointestinal bleeding, infection, diarrhea, simultaneous double cancer, pregnancy, interstitial pneumonia, bowel obstruction, past history of myocardial infarction, massive ascites, severe drug allergy, or severe diabetes. The protocol was approved by the ethics review committees of all participating centers, and all patients gave written informed consent. All patients were registered centrally at the Translational Research Informatics Center, Kobe, Japan, where data management and statistical analysis were performed.

### Preoperative chemotherapy

Preoperative chemotherapy was administered for two cycles. Each cycle consisted of daily oral administration of S-1 for 21 days (80 mg/$m^2$/day, days 1–21) and intravenous cisplatin (60 mg/$m^2$), with hydration, on day 8; followed by a 2-week recovery period [6]. The toxicity of the induction chemotherapy was assessed on the basis of the National Cancer Institute's common toxicity criteria version 2.0 (NCI-CTC) [13]. If patients had grade 3/4 hematologic or nonhematologic toxicity, including grade 2 or above diarrhea, liver dysfunction, stomatitis, and other toxicities of grade 3 or above, chemotherapy was postponed until recovery. If recovery did not occur within 4 weeks, the chemotherapy was stopped.

Some 7–13 days after each course, resectability and clinical response were evaluated mainly on the basis of CT findings. If curative resection was possible after the second course, the patient underwent surgery 2–4 weeks after completion of the chemotherapy. If patients experienced disease progression or severe adverse events, the protocol was discontinued. Treatment after the discontinuation of preoperative chemotherapy was at the physician's discretion.

### Surgery

The surgical criteria included possibility of R0 resection, no evidence of infection, no signs of organ failure, and a neutrophil count of more than 1,500/μL. After laparotomy, resectability was again evaluated. The standard surgical procedure was gastrectomy with D2 nodal dissection. The extent of gastrectomy (total or subtotal) depended on the site and size of the primary tumor. For R0 resection, paraaortic nodal dissection (D3), splenectomy and/or distal pancreatectomy, or partial hepatectomy was attempted if cytologic findings were negative. Palliative gastrectomy was attempted in noncurative cases if the tumor was symptomatic and resectable.

## Postoperative chemotherapy

Postoperative S-1 (80 mg/m$^2$/day, days 1–14) was administered every 3 weeks for 1 year. Eligibility criteria for postoperative chemotherapy included resection of the primary lesion and no definite disease progression at surgery. If gastrectomy was not curative, cessation of the protocol was at the physicians' discretion. Other eligibility criteria were the same as those for preoperative chemotherapy. For 1 year, S-1 was repeatedly administered in a 2-week cycle, followed by 1 week of rest. Criteria for discontinuation and cessation of the protocol were the same as those for preoperative chemotherapy. If recurrence or progressive disease was confirmed, the protocol was stopped. After completion of the protocol without recurrence, no further treatment was administered until tumor recurrence was identified. Treatment for recurrence or progressive disease was at the physicians' discretion.

## Endpoints and evaluation

The primary endpoint was the 2-year OS rate, because the prognosis of stage IV GC is poor. The secondary endpoints were the progression-free survival (PFS) rate, objective response rate, pathologic response rate, R0 resection rate, surgical complications, the first recurrence sites, and toxic reactions. R0 resection was defined as pathologically confirmed complete resection of the cancerous lesions with negative peritoneal washing cytology.

The pretreatment stage at registration (pre-stage) was diagnosed according to the Japanese Gastric Cancer Association (JGCA) staging system (13th edition) [14] on the basis of CT and staging laparoscopic findings. If the short-axis diameter of the lymph nodes was greater than 10 mm on a CT scan, the lymph nodes were diagnosed as "metastatic" [15, 16]. The anatomic location of metastatic lymph nodes was also identified on CT scans. According to the JGCA staging system, N stage was defined by the anatomic location of the metastatic lymph nodes. At staging laparoscopy, T stage was diagnosed as pre-T3 (tumor penetration of serosa; SE) if the tumor was definitely exposed on the serosal surface. If cancerous changes on the serosal surface were considered minimal at staging laparoscopy, the T stage was diagnosed as pre-T2 (tumor invasion of muscularis propria; MP, tumor invasion of subserosa; SS). The diagnosis of pre-T4 (tumor invasion of adjacent structures; SI) was assessed by CT and laparoscopic findings. Cytology and peritoneal tumor dissemination were also assessed at pretreatment staging laparoscopy [17–20] and at surgery. The cells collected by peritoneal washing were examined cytopathologically using conventional Papanicolaou and Giemsa staining. When definite cancer cells or clusters were identified, patients were diagnosed as pre-Cy1, according to the *Japanese classification of gastric carcinoma* of the JGCA [14]. Objective tumor response was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 on the basis of the CT findings [21]. Postoperative final tumor status (post-stage) was diagnosed by comprehensive findings based on clinical, surgical, and pathological findings according to the JGCA classification [14]. Surgical specimens were evaluated pathologically and graded according to the proportion of tumor affected by degeneration or necrosis [11]: grade 0, no part of tumor affected; grade 1a, less than one-third affected; grade 1b, between one-third and two-thirds affected; grade 2, between two-thirds and the entire tumor affected; and grade 3, no residual tumor. The toxicity of preoperative and postoperative chemotherapy was assessed on the basis of NCI-CTC version 2, as described earlier.

## Statistical analyses

All data from eligible patients were based on the intention-to-treat principle. This study was designed as a phase II trial with a single stage of accrual. The threshold 2-year survival probability was estimated to be 35%, determined by examining historical data from the Department of Gastroenterological Surgery, Kyoto University, that were collected from 1996 to 2002 ($n = 43$). The expected 2-year survival rate for this therapy was estimated to be 55%. Assuming that two-sided type I errors were 5% and that the power was 80%, the required sample size was calculated to be 46. The planned sample size was set at 50, with the consideration of approximately 5% of patients being ineligible. OS and PFS were estimated using the Kaplan–Meier

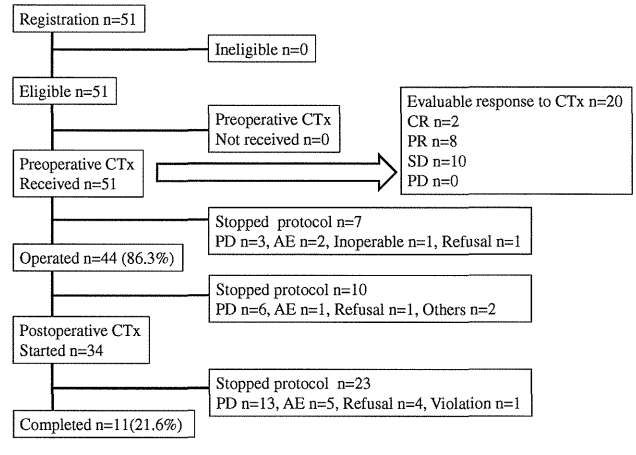


**Fig. 1** Flow diagram of 51 eligible patients. *CTx* chemotherapy, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *AE* adverse effects

**Table 1** Characteristics of patients and tumor status before and after treatment

| | *n* | % |
|---|---|---|
| Age, years, median (range) | 63 (35–79) | |
| Gender | | |
| Male | 29 | 56.9 |
| Female | 22 | 43.1 |
| ECOG performance status | | |
| 0 | 44 | 86.3 |
| 1 | 7 | 13.7 |
| Histology | | |
| Intestinal | 13 | 25.5 |
| Diffuse | 38 | 74.5 |
| | Pre- ($n = 51$) | Post- ($n = 44$) |
| Stage | | |
| IA | 0 | 2 |
| IB | 0 | 6 |
| II | 0 | 5 |
| IIIA | 0 | 3 |
| IIIB | 0 | 3 |
| IV | 51 | 25 |
| T stage | | |
| T1 (M, SM) | 0 | 1 |
| T2 (MP, SS) | 7 | 18 |
| T3 (SE) | 38 | 21 |
| T4 (SI) | 6 | 2 |
| TX | 0 | 2 |
| N stage | | |
| N0 | 13 | 11 |
| N1 | 15 | 13 |
| N2 | 10 | 11 |
| N3 | 11 | 7 |
| NX | 2 | 2 |
| Peritoneal cytology | | |
| Cy0 | 15 | 32 |
| Cy1 | 36 | 10 |
| CyX | 0 | 2 |
| Peritoneal metastasis | | |
| P0 | 26 | 29 |
| P1 | 25 | 15 |
| Liver metastasis | | |
| H0 | 46 | 40 |
| H1 | 5 | 4 |
| Distant metastasis | | |
| M0 | 46 | 43 |
| M1 | 5 | 1 |
| Pathological response (primary lesion) | | |
| Grade 0 | | 6 |
| Grade 1a | | 15 |
| Grade 1b | | 6 |
| Grade 2 | | 14 |
| Grade 3 | | 0 |
| Not resected | | 3 |

*ECOG* Eastern Cooperative Oncology Group, *Pre-* pretreatment tumor status at registration, *Post*-postoperative final tumor status diagnosed by comprehensive findings based on clinical, surgical and pathological findings according to the Japanese Gastric Cancer Association (JGCA) classification [14], *M* tumor invasion of mucosa and/or muscularis mucosa, *SM* tumor invasion of submucosa, *MP* tumor invasion of muscularis propria, *SS* tumor invasion of subserosa, *SE* tumor penetration of serosa, *SI* tumor invasion of adjacent structures

method. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

## Results

### Patient characteristics

Between May 2003 and March 2008, 51 patients with stage IV GC were enrolled in this study and underwent preoperative chemotherapy. Disposition of the enrolled patients is shown in Fig. 1. No patients were ineligible, and all were observed for more than 2 years after registration. Table 1 shows the characteristics and tumor status of the 51 eligible patients. The median follow-up period of all patients was 19.2 months (range 4.5–81.5). Patients with a single pre-stage IV factor ($n = 24$) included 12 cases of pre-Cy1, 5 cases of pre-P1, 5 cases of pre-N3, and 2 cases of pre-H1. Simultaneous multiple pre-stage IV factors were observed in the remaining 27 patients. Patients with multiple pre-stage IV factors ($n = 27$) included 1 case of pre-(Cy1, P1, N3, M1), 1 case of pre-(Cy1, P1, N3), 2 cases of pre-(Cy1, P1, T4N2), 1 case of pre-(Cy1, P1, H1), 1 case of pre-(Cy1, H1, M1), 15 cases of pre-(Cy1, P1), 1 case of pre-(Cy1, N3), 1 case of pre-(Cy1, T4N2), 1 case of pre-(Cy1, H1), and 3 cases of pre-(N3, M1).

### Survival

The 2-year OS and PFS rates were 43.1% (95% CI 29.4–56.1) and 33.3% (95% CI 20.9–46.2), respectively (Fig. 2a, b). The median OS and PFS were 19.2 and 9.3 months, respectively. There were significant differences in OS in favor of both GC with R0 resection ($p < 0.0001$) and GC with pre-Cy1 alone without any other stage IV factors ($p = 0.0041$), as shown in Fig. 2c, d. The 2-year OS of GC with pre-Cy1 alone was 75.0% (95% CI 40.8–91.2) and that of others was 33.3% (95% CI 19.3–48.0).

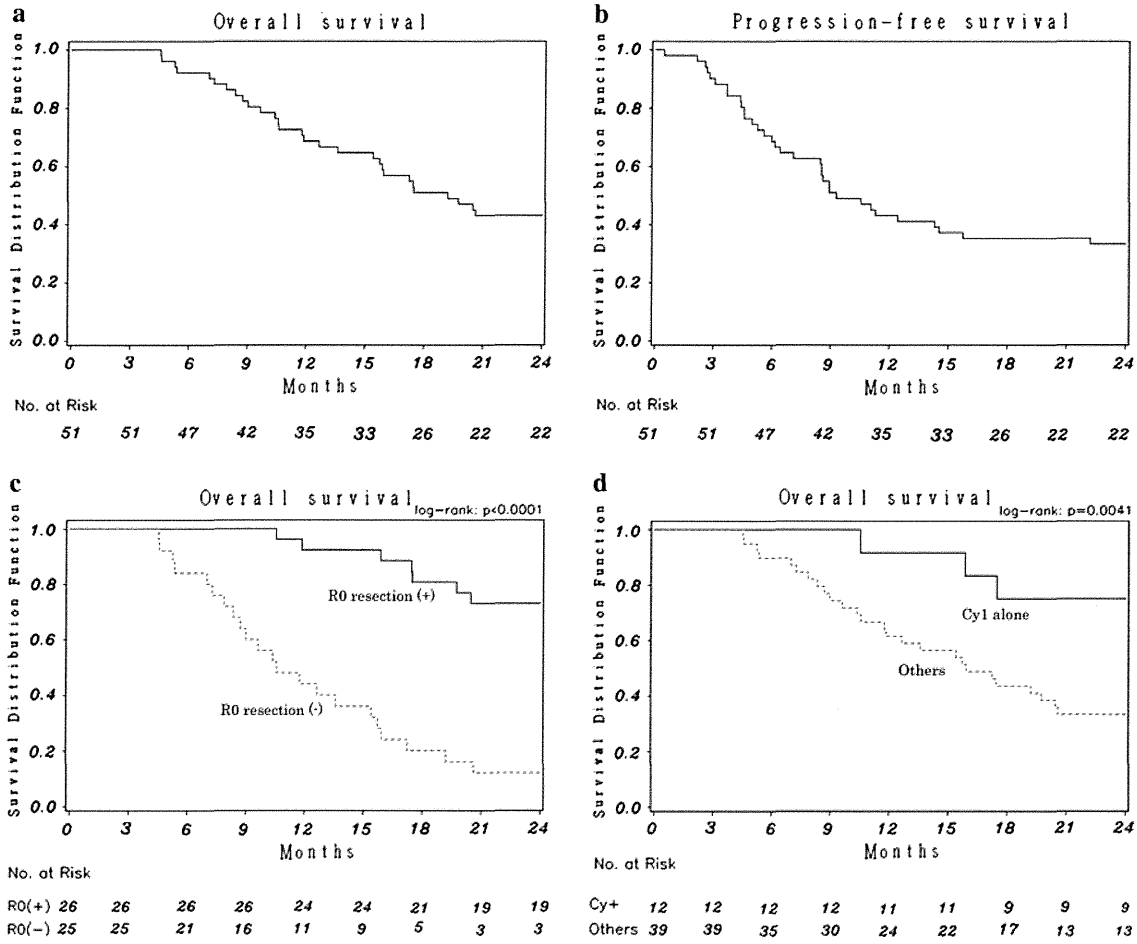


**Fig. 2** Kaplan-Meier survival curves for the 51 eligible patients. **a** Overall survival, **b** progression-free survival. **c** Overall survival in patients grouped according to R0 resection or not. **d** Overall survival in patients grouped according to pretreatment Cy1 alone and others

## Objective response to preoperative chemotherapy and adverse events

Measurable lesions were confirmed in 20 patients. The objective response rate for these lesions, according to the RECIST, was 50.0% (95% CI 27.2–72.8). Preoperative chemotherapy was completed in 44 patients (86.3%). Reasons for stopping treatment are noted in Fig. 1.

Toxic reactions of grade 3 or above are listed in Table 2. Severe adverse events occurring during preoperative chemotherapy were noted in two patients (3.9%). Grade 4 toxic reactions of leukocytopenia, neutropenia, and anemia were observed in one patient. Cardiopulmonary arrest after diarrhea was observed in another patient, who was resuscitated successfully.

## Surgical outcome

Surgery was performed in 44 patients, including total gastrectomy in 30, distal gastrectomy in 11, simple laparotomy in two, and gastrojejunostomy in one patient. The median (range) blood loss and surgery duration were 669 (0–3,228) mL and 286 (50–447) min, respectively. Extended nodal dissection was performed in 36 patients (D2, 32; D3, 4). Palliative gastrectomy with D0/D1 was performed in 5 patients to maintain oral intake. Combined resection was performed in 35 patients, including additional resection of 62 organs; namely, the spleen ($n = 25$), gallbladder ($n = 21$), distal pancreas ($n = 7$), colon ($n = 3$), liver ($n = 2$), mesocolon ($n = 2$), diaphragm ($n = 1$), and adrenal gland ($n = 1$). The surgical complication rate was 18.2%, including three cases of pancreatic fistula and abdominal abscess, and one case of anastomotic leakage.

The postoperative final tumor status (post-stage) of the 44 patients who underwent surgery was determined with comprehensive findings based on clinical, surgical, and pathologic findings according to the JGCA classification (Table 1).

## R0 resection rate

According to the pretreatment evaluation, 31 patients were diagnosed as "unresectable". After induction chemotherapy, 44 patients were diagnosed as "resectable" by CT and

**Table 2** Adverse events of chemotherapy according to NCI-CTC version 2

| | Preoperative S-1 + CDDP ($n$ = 51) | | Postoperative S-1 ($n$ = 34) | |
|---|---|---|---|---|
| | Grade 3/4 | % | Grade 3/4 | % |
| Leukocytopenia | 6 | 12 | 1 | 3 |
| Neutropenia | 13 | 25 | 4 | 12 |
| Thrombocytopenia | 2 | 4 | 0 | 0 |
| Anemia | 6 | 12 | 1 | 3 |
| Fatigue | 5 | 10 | 2 | 6 |
| Anorexia | 11 | 22 | 3 | 9 |
| Nausea | 6 | 12 | 2 | 6 |
| Vomiting | 4 | 8 | 0 | 0 |
| Diarrhea | 1 | 2 | 2 | 6 |
| DIC | 1 | 2 | 0 | 0 |
| Dehydration | 1 | 2 | 0 | 0 |
| Dizziness | 0 | 0 | 1 | 3 |

*NCI-CTC* National Cancer Institute common toxicity criteria, *CDDP* cisplatin, *DIC* disseminated intravascular coagulation

**Table 3** Relationship between pre-stage IV factors and R0 resection

| | No. | R0 | %R0 | MST (95% CI) (months) |
|---|---|---|---|---|
| Total | 51 | 26 | 51.0 | 19.2 (15.4–43.5) |
| Multiple pre-stage IV factors | 27 | 7 | 25.9 | 11.9 (9.0–17.5) |
| Single pre-stage IV factor | 24 | 19 | 79.2 | 56.3 (19.8–NE) |
| Pre-Cy1 | 12 | 12 | 100 | NE (56.3–NE) |
| Pre-P1 | 5 | 2 | 40 | 43.5 (7.9–NE) |
| Pre-N3 | 5 | 4 | 80 | 31.6 (17.3–NE) |
| Pre-H1 | 2 | 1 | 50 | NE (15.9–NE) |

*Pre-* pretreatment clinical tumor status at registration, *MST* median survival time, *CI* confidence interval, *NE* not estimated

they underwent surgery. R0 resection after induction chemotherapy was accomplished in 26 patients. Noncurative factors in R1/2 cases ($n$ = 18) included 3 cases of unresectable primary tumor, 13 cases of post-P1 and/or Cy1, 1 case of residual nodal metastasis, and 1 case of multiple liver metastasis. The relationship between stage IV status at registration (pre-stage IV) and R0 resection is summarized in Table 3. Twelve patients showed pre-Cy1 without other c-stage IV factors (Cy1 alone). In these 12 patients, eradication of free peritoneal gastric cancer cells (FPGCs) with preoperative chemotherapy was observed and this led to R0 resection. Of 15 patients with pre-Cy0, two patients became post-Cy1 after preoperative chemotherapy.

## Postoperative chemotherapy and recurrence

Of the 26 patients that underwent R0 resection, three patients showed no recurrence without postoperative chemotherapy. Reasons for not performing postoperative chemotherapy in these three patients were that 1 patient was ineligible for the starting criteria of the postoperative chemotherapy, 1 patient had prolonged pneumonia, and 1 patient refused. Postoperative chemotherapy was started in 34 patients, including 23 patients with R0 resection, and

**Table 4** Postoperative chemotherapy and recurrence in patients with R0 resection ($n$ = 23)

| | Start | Completion | Rec.-free (%) |
|---|---|---|---|
| Total | 23 | 11 | 9 (39) |
| Multiple pre-stage IV factors | 7 | 2 | 0 (0) |
| Single pre-stage IV factor | 16 | 9 | 9 (56) |
| Pre-Cy1 | 11 | 8 | 7 |
| Pre-P1 | 1 | 0 | 0 |
| Pre-N3 | 3 | 0 | 1 |
| Pre-H1 | 1 | 1 | 1 |

*Pre-* pretreatment clinical tumor status at registration, *Rec.-free* recurrence free

was completed in 11. No patients with noncurative resection completed 1 year of postoperative chemotherapy ($n$ = 11). The relationship between postoperative chemotherapy and recurrence in R0 resection patients ($n$ = 23) is summarized in Table 4. The adverse effects of the postoperative chemotherapy are presented in Table 2. Grade 4 toxic effects of neutropenia, fatigue, and dizziness were observed in one patient. Throughout the treatment period, severe adverse events were recorded in three patients (5.9%). No treatment-related death occurred. Of the 26 patients with R0 resection, 14 experienced recurrence. The

most frequent recurrence site was the peritoneum ($n = 8$), followed by the lymph nodes ($n = 3$).

## Discussion

The results of the present study indicate that the multimodal treatment used was safe and well tolerated by patients with stage IV GC. The clinical response rate for the preoperative chemotherapy was compatible with that reported in a previous study [6]. Surgical morbidity was acceptable. No treatment-related death occurred. With a mature follow-up, the 2-year OS rate of this treatment was 43% (95% CI 29.4–56.1). This appeared to be better than the 2-year OS of 20–25% that was estimated from previous studies of stage IV GC patients treated with chemotherapy alone or with surgery and postoperative chemotherapy [2, 3, 6, 22]. However, our study failed to show a survival benefit, because our threshold 2-year survival probability was estimated to be 35%.

Although most of the eligible patients in our study had microscopic or macroscopic peritoneal dissemination, R0 resection was achieved in 51% of the patients after preoperative chemotherapy with S-1 plus cisplatin. R0 resection is reported to be one of the most reliable prognostic indicators for patients after preoperative chemotherapy [10, 17]. In line with these reports, the R0 resection group in the present study exhibited a significantly better prognosis. It is well known that the prognosis in patients with GC with multiple stage IV factors is miserable [23–25]. By virtue of staging laparoscopy, we frequently confirmed overt peritoneal carcinomatosis (pre-P1) or FPGCs (pre-Cy1). Other synchronous pre-stage IV factors were identified in 80% of pre-P1 patients and 66% of pre-Cy1 patients. The R0 resection rate was low in these patients, mainly due to remaining peritoneal carcinomatosis or FPGCs. Preoperative S-1 plus cisplatin might be inadequate for treating GC with multiple c-stage IV factors. In this situation, preoperative staging laparoscopy after the induction chemotherapy could have been useful to prevent futile laparotomy.

As for GC with a single pre-stage IV factor, the R0 resection rate in our study was as high as 79%. R0 resection due to eradication of FPGCs with preoperative chemotherapy was recorded in all patients with pre-Cy1 alone, leading to an improved prognosis. Generally, the 2-year overall survival (OS) rate of GC with pre-Cy1 alone (around 25–30%) is poor, even when conventional postoperative chemotherapy is administered [22, 26, 27]. In general, preoperative chemotherapy using a conventional regimen is insufficient for GC with pre-Cy1 [28]. Recently, improved prognosis was reported in patients with pre-Cy1 alone as a result of the eradication of FPGCs with preoperative cisplatin and folic acid plus fluorouracil. However, the eradication rate was low (37%), and a shift from negative to positive cytology findings during preoperative chemotherapy was observed in 24% of patients [29]. As for adjuvant chemotherapy for GC with post-Cy1 alone, the 2-year OS rate of these patients who underwent radical surgery followed by S-1 alone was reported to be 47% [30]. In contrast, in the present study, the eradication rate of FPGCs was 100%, leading to a 2-year OS rate of 75.0% (95% CI 40.8–91.2) in the patients with pre-Cy1 alone, and two patients (3.9%) had a shift from negative to positive cytology findings. This result was compatible with findings in our preliminary reports [7, 31]. S-1 shows a high rate of transfer into the peritoneal cavity [32]. Compared with S-1 alone, S-1 plus cisplatin improves the prognosis of patients with peritoneal metastasis [6]. These features of S-1 plus cisplatin might have contributed to the high eradication rate of FPGCs in the present study. Also, due to the high eradication rate of FPGCs, pretreatment staging laparoscopy is strongly recommended to distinguish advanced GC with pre-Cy1 from that with pre-Cy0.

Low compliance is often reported for postoperative chemotherapy, because the toxicity of combination regimens cannot be tolerated by patients after gastrectomy [8, 9]. Postoperative S-1 plus cisplatin has also been reported to be too toxic after gastrectomy [33]. To improve compliance with postoperative chemotherapy, S-1 alone was adopted as a postoperative regimen [12]. Consequently, the toxic profile of postoperative S-1 alone was milder than that of preoperative S-1 plus cisplatin. In the present study, the low completion rate of the postoperative chemotherapy was due to tumor progression in patients with multiple stage IV factors. Despite the postoperative chemotherapy, all patients with multiple pre-stage IV factors experienced recurrence after R0 resection. On the other hand, the recurrence-free rate of patients with a single pre-stage IV factor was 56% after postoperative chemotherapy. In particular, due to the low frequency of recurrence, patients with pre-Cy1 alone showed a high postoperative chemotherapy completion rate. Postoperative chemotherapy with S-1 alone might be effective in treating stage IV GC if the latent tumor burden is minimal after R0 resection.

In conclusion, the administration of preoperative S-1 plus cisplatin, followed by surgery and postoperative S-1, is safe and feasible for stage IV GC. However, this multimodal approach failed to show a beneficial effect for GC with multiple stage IV factors. Although the sample size in our study was small and 2 of 15 pre-Cy0 patients became post-Cy1, it seems that patients with pre-Cy1 with no additional non-curative factors might be good candidates for the present approach. Further clinical investigations for this subset should be undertaken using promising perioperative chemotherapy.

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**Conflict of interest** The authors indicated no potential conflicts of interest.

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CASE REPORT

# Adrenal cavernous hemangioma with subclinical Cushing's syndrome: report of a case

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**Abstract** Cavernous hemangioma of the adrenal gland is a rare tumor, which does not usually have endocrinological function. We report to our knowledge, the third documented case of a functioning adrenal hemangioma. Interestingly, this tumor indicated glucocorticoid hypersecretion, whereas the two previous cases showed mineralocorticoid hypersecretion. The tumor was 5 cm in diameter with typical computed tomography and magnetic resonance imaging findings. Subclinical Cushing's syndrome was diagnosed preoperatively, as there was insufficient suppression of cortisol by low-dose dexamethasone, a low adrenocorticotropic hormone (ACTH) concentration, and diminished ACTH and cortisol circadian rhythms without the typical clinical manifestation and symptoms of hypercortisolism. Intraoperative hypotension occurred immediately after tumor removal and following postoperative adrenal insufficiency, which support that the tumor was hyperfunctioning. The postoperative adrenal insufficiency had recovered completely by 12 months after the operation.

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**Keywords** Adrenal cavernous hemangioma · Adrenocortical hyperfunction · Subclinical Cushing's syndrome · Postoperative adrenal insufficiency

## Introduction

The universal popularity of radiological imaging has resulted in frequent detection of adrenal incidentalomas. However, cavernous hemangioma of the adrenal gland is still rare. We report an interesting case of adrenal function being affected by a hemangioma because of its highly unusual endocrinological characteristics of subclinical Cushing's syndrome.

## Case report

A 75-year-old woman was referred to our clinic for investigation of a left upper abdominal mass, detected incidentally on a computed tomography (CT) scan done as preoperative screening for metastasis of left breast cancer. She had undergone left mastectomy 3 months before the referral and the pathological diagnosis was stage IIA breast cancer without distant metastasis. On physical examination, the patient was 139 cm tall and weighed 38 kg. There were no signs suggestive of Cushing's syndrome, such as central obesity, a moon face or fat pads along the collar bone and neck. She was taking nisoldipine 5 mg per day for hypertension and her arterial blood pressure was 130/80 mmHg. The results of laboratory tests were all normal except for a low serum potassium level of 3.1 mEq/L.

Abdominal enhanced CT showed a round and homogenous mass, 5 cm in diameter, superior to the left kidney

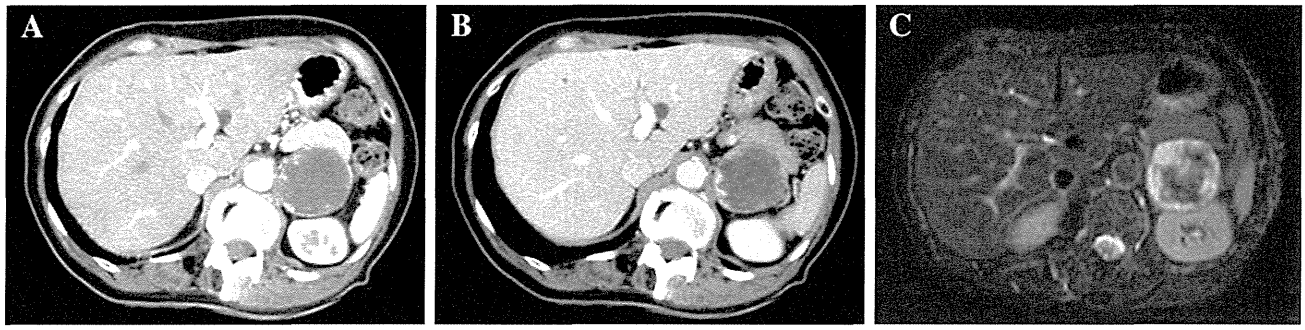


**Fig. 1** **a** Arterial phase of enhanced computed tomography (CT) showed a well-circumscribed left adrenal tumor with low-density central lesion and peripheral enhancement. **b** Venous phase of the enhanced CT showed centripetal enhancement of the mass. **c** T2-weighted magnetic resonance imaging (MRI) with gadolinium enhancement also showed peripheral enhancement. A stellate hypointense area with a heterogeneous signal was seen in the inner portion of the tumor

and immediately adjacent to the distal pancreas. The low-density area was surrounded by a thin enhanced ring (Fig. 1a, b). On T1- and T2-weighted magnetic resonance imaging (MRI), the tumor had a homogenous and hyperintense area with a hypointense ring in the peripheral region, which was enhanced by gadolinium (Fig. 1c). Abdominal angiography indicated a faint tumor stain, which was fed by a branch from the left renal artery. On metaiodobenzylguanidine (MIBG) imaging, the tumor had faint ringed uptake in the early phase, which disappeared 24 h later.

Endocrinological examinations showed a low plasma concentration of adrenocorticotropic hormone (ACTH) of 5.2 pg/mL (normal range 7.4–55.7 pg/mL) and a normal serum concentration of cortisol of 16.5 μg/dL (normal range 4.5–21.1 μg/dL), but the circadian rhythms of both ACTH and cortisol were lost. After suppressing dexamethasone, the cortisol concentration was not suppressed (being 6.1 μg/dL after 1 mg orally and 7.4 μg/dL after 2 mg orally), which suggested that the tumor was associated with subclinical Cushing's syndrome.

At surgery, the tumor was found localized under the layer of Gerota's fascia, without invasion of other organs such as the pancreas. It was finally removed after dissecting it from the surrounding connective tissue containing the left superior adrenal arteries and ligating the left middle and inferior adrenal arteries arising from the abdominal aorta and left renal artery, respectively, and the left adrenal vein draining into left renal vein. Although the patient's preoperative blood pressure was normal, at 120/70 mmHg, her systolic blood pressure dropped from 120 to 80 mmHg only 10 min after the tumor was resected. The intravenous infusion of dopamine, 7 μg/kg/min, was ineffective, but it increased to over 100 mmHg with the bolus administration of hydrocortisone 100 mg.

Pathological examination revealed a 5 × 5 × 3 cm oval, smoothly encapsulated and moderately firm mass, with cross-section of reddish-brown and ash-gray organized hematoma covered with yellowish thin cortical tissue (Fig. 2a, b). Microscopically, the hemorrhagic lesion consisted of spotty cavernous dilated vessels with clustered erythrocytes (Fig. 2c). Immunohistochemical examination revealed vessels lined with vascular endothelial cells specifically positive for blood coagulation factor VIII (Fig. 2d). The peripheral cortical tissue consisted of normal cells without apparent hyperplasia (Fig. 2e).

After the operation, we administered hydrocortisone 100 mg intravenously for 2 days, which was then tapered. An ACTH stimulation test done 1 week postoperatively showed almost no increase in the serum cortisol level: from 4.1 to 5.9 μg/dl, 60 min after the administration of ACTH 250 μg. These results suggested a slight degree of hypofunction of the adrenal gland after the operation. The patient was commenced on oral hydrocortisone, 20 mg/day, which was tapered and withdrawn 12 months after the operation, when the serum concentrations of ACTH and cortisol had normalized (Fig. 3).

## Discussion

The widespread use of radiological imaging has resulted in more and more adrenal incidentalomas being detected [1]; however, adrenal hemangioma is still rare. Our search of the literature found only 57 cases reported in English since Johnson and Jeppesen [2] described the first surgical case. The risk of primary adrenal malignancy is related to the mass size, with a diameter greater than 4 cm having 90 % sensitivity but low specificity. Adrenal incidentalomas greater than 4 cm are an indication for surgery, because the smaller the adenocortical carcinoma, the better the overall prognosis [3, 4].

The tumor in the present case was seen as a typical enhanced CT image, which showed its shape and irregular peripheral enhancement. However, there was no calcification, indicative of multiple phleboliths, which are

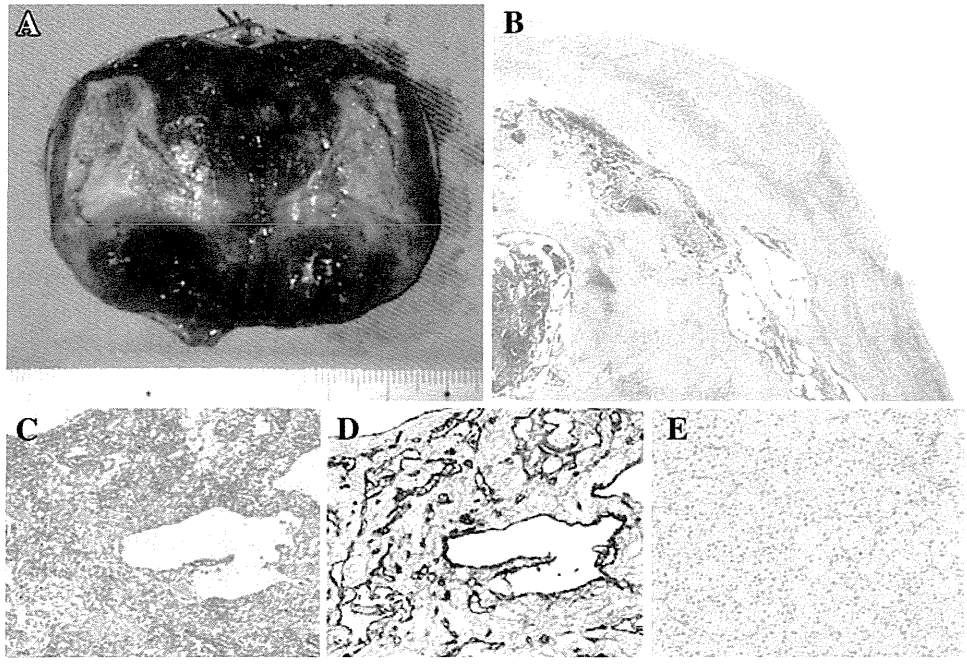


**Fig. 2** **a** Gross specimen of the adrenal hemangioma: a round, well circumscribed and encapsulated, moderately firm mass measuring 5 × 5 × 3 cm. The cut surface consisted of a *reddish-brown and ash-gray structure* with a thin *yellowish* rim. **b** Histopathological examination revealed a tumor composed of adrenal tissue at its periphery compressed by the hematoma, organized with cavernous vessels that occupied two-thirds of it (H&E, ×4). **c** In the hemorrhagic lesion, the spotty, cavernous, and dilated blood vessels mixed with clustered erythrocytes were confirmed, but adrenal medullary tissue was not found (H&E, ×40). **d** Immunohistochemical examination revealed that the cavernous vessels were lined with benign vascular endothelial cells positive for blood coagulation factor VIII (×40). **e** The peripheral, thinned *yellowish* layer consisted of normal cortical cells without obvious hyperplasia (H&E, ×40)

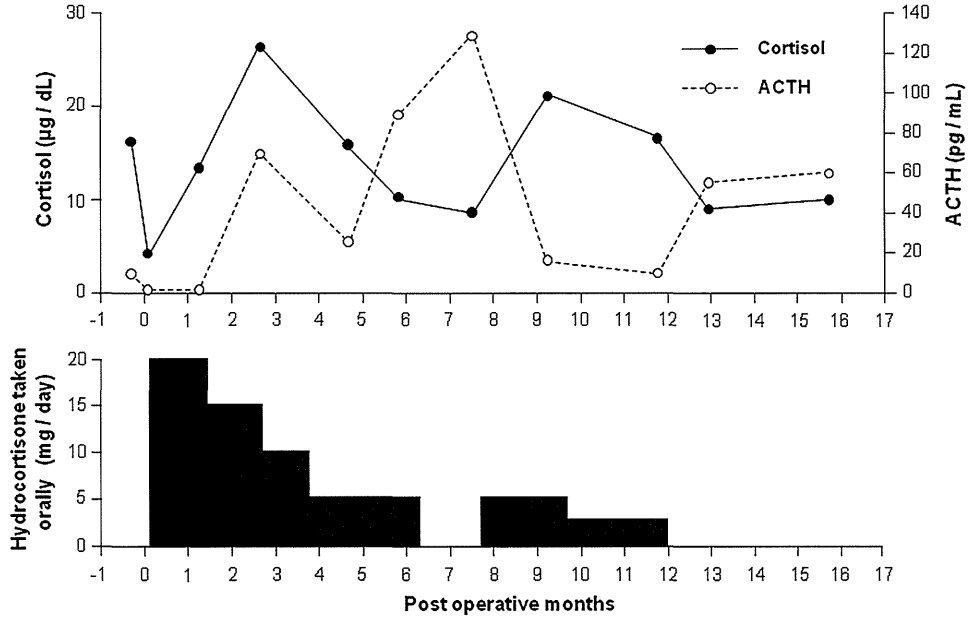


**Fig. 3** Postoperative changes of serum adrenocorticotropic hormone (ACTH) and cortisol levels and the doses of hydrocortisone administered orally

commonly located in the dilated vascular spaces of the hemangioma [5]. The MRI imaging was typical of adrenal hemangioma; namely, a hyperintense tumor on the T1-weighted image with variable signals on the T2-weighted image [6].

Autonomous cortisol secretion was found in 6.4 % of patients with adrenal incidentaloma [3], but adrenal hemangiomas are generally non-functioning. We found only two other case reports of adrenal function being affected by a hemangioma. Table 1 summarizes the data of these two

**Table 1** Clinical data of the three reported cases of functional adrenal hemangioma

| Reference | Sex | Age (yr) | Size (cm) | PMH | Aldosterone (pg/mL) [35.7–240][a] | PRA (ng/mL/H) [0.3–2.9][a] | Urinary excretion of normetanephrine (mg/day) [0.09–0.33][a] | ACTH (pg/L) [9–52][a] | Cortisol (μg/dL) [4.5–21.1][a] | Cortisol after low-dose dexamethasone suppression test (μg/dL) [<5][a] |
|---|---|---|---|---|---|---|---|---|---|---|
| Stumvoll et al. [7] | F | 60 | 8 | HTN | 127 | 0.08 | ND | <4.5 | 16 (8 a.m.)<br>18 (4 p.m.) | ND |
| Ng et al. [8] | M | 59 | 3 | HTN<br>HEP B | 193 | 0.05 | 1.3 times the upper limit of the reference range | ND | 10.0 (8 a.m.)<br>8.3 (12 p.m.) | 0.36 |
| The present case | F | 75 | 5 × 5 × 3 | HTN | 65 | 0.2 | 0.16 | 5.2 | 16.5 (7 a.m.)<br>8.2 (11 p.m.) | 6.1 |

*M* male, *F* female, *yr* year-old, *PMH* past medical history, *HTN* hypertension, *HEP B* hepatitis B, *PRA* plasma renin activity, *ACTH* adrenocorticotropic hormone, *ND* not described

[a] Normal range

cases and the present case. The first case of a functioning hemangioma [7], which was 8 cm in diameter, was associated with potassium wasting syndrome (hypernatremia, hypokalemia, increased urinary potassium excretion), hypercortisolism, and decreased plasma renin activity, all of which resolved 4 weeks after surgery. The second case of functioning hemangioma [8], which was 3 cm in diameter, was associated with clinical evidence of aldosterone excess and elevated levels of urinary catecholamines and metanephrines, both of which normalized after laparoscopic resection. Our case represents the third report of a functioning adrenal hemangioma, indicative of glucocorticoid hypersecretion, whereas the two previous cases showed mineralocorticoid hypersecretion. Our case demonstrated insufficient suppression of cortisol by low-dose dexamethasone, low ACTH concentration and diminished ACTH and cortisol circadian rhythms. The term "subclinical Cushing's syndrome" refers to autonomous cortisol secretion in patients who lack the typical clinical manifestation and symptoms of hypercortisolism [1]. This syndrome is being reported more frequently with the increasing incidence of adrenal incidentaloma detection. The gold standard for diagnosis has not been defined, but blunt circadian rhythms of cortisol and suppressed ACTH level are highly indicative of the disease [9, 10]. The biochemical findings in this case are consistent with subclinical Cushing's syndrome. Plasma aldosterone concentration (PAC), plasma rennin activity (PRA) and their ratio (PAC/PRA) were all normal. Moreover, the occurrence of intraoperative hypotension immediately after tumor removal and the ensuing postoperative adrenal insufficiency support the fact that the tumor was hyperfunctioning.

In summary, we reported this adrenal cavernous hemangioma because of its highly unusual endocrinological characteristics of subclinical Cushing's syndrome and postoperative adrenal insufficiency, which recovered 12 months after the operation.

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# Phenotypic Analysis of Monocyte-derived Dendritic Cells Loaded with Tumor Antigen with Heat-shock Cognate Protein-70

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**Abstract.** *Background/Aim: The cross-presentation system of tumor antigen by monocyte-derived dendritic cells (mo-DCs) has been observed under appropriate conditions. Both CD14-negative and CD1a-positive phenotypes were critical in our previous study. This study compared the phenotype of mo-DCs and identified the conditions that favored T helper-1 (Th1) cytokine production after stimulation with the hsc70 and NY-ESO-1 p157-165 epitope fusion protein (hsc70/ESO p157-165). Materials and Methods: The mo-DCs were induced from healthy donors. Their surface markers and cytokine production were examined after stimulation with hsc70/ESO p157-165. Results: $CD1a^+$ and $CD1a^-$ mo-DCs were generated in half of the healthy donors. The concentration of fetal calf serum in the culture medium was critical for the induction of $CD1a^+$ DCs, which were able to produce interleukin-12 (IL-12), but not IL-10. Neutralizing IL-6 and IL-6R antibodies affected the expression of CD1a. Conclusion: Anti IL-6 analogs may be effective adjuvants for the development of mo-DC-based cancer vaccine.*

NY-ESO-1 is a promising target antigen for specific immune recognition of cancer because it has restricted expression in normal tissue but frequently occurs on human tumors (1-4). Clinical trials with this antigen have been conducted using the NY-ESO-1 peptide, full-length protein, and DNA (5, 6). These cancer vaccines are designed to enhance effector T-cell responses to tumor antigens. An appropriate antigen-presenting cell is required to induce favorable T-cell responses (7).

Dendritic cells (DCs) are the most potent antigen-presenting cells and they have been shown to play a critical role in the generation of immune responses. The unique features of antigen presentation by DCs have generated considerable interest in their use as therapeutic vehicles, especially for vaccination (8, 9). DCs-alone or in complexes with tumor antigens are expected to be a powerful tool in the development of cancer vaccines (10, 11). However, no consensus has yet been reached on the most appropriate DC population to be employed for immunization.

A fusion protein containing the human heat-shock cognate protein-70 (hsc70) and ESO p157-165, epitope of NY-ESO-1 was constructed, as part of the development of a new strategy to vaccinate cancer patients with tumor antigens (12-14). A previous study demonstrated that monocyte-derived (mo)-DCs capture and endogenously process the hsc70/ESO p157-165 fusion protein to major histocompatibility complex (MHC) class I molecules through the cross-presentation pathway (15, 16). However, this cross-presentation system could not always work. This study was conducted to define the appropriate conditions in order to use mo-DCs for vaccination after loading with the hsc70/ESO p157-165 fusion protein.

## Materials and Methods

*Expression and purification of the hsc70 and NY-ESO-1 p157-165 epitope fusion protein.* The hsc70/ESO p157-165 fusion protein was manufactured as previously described (15). Briefly, human cDNA of

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*Key Words:* Dendritic cell, NY-ESO-1, IL-6, hsc-70, phenotypic analysis.

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