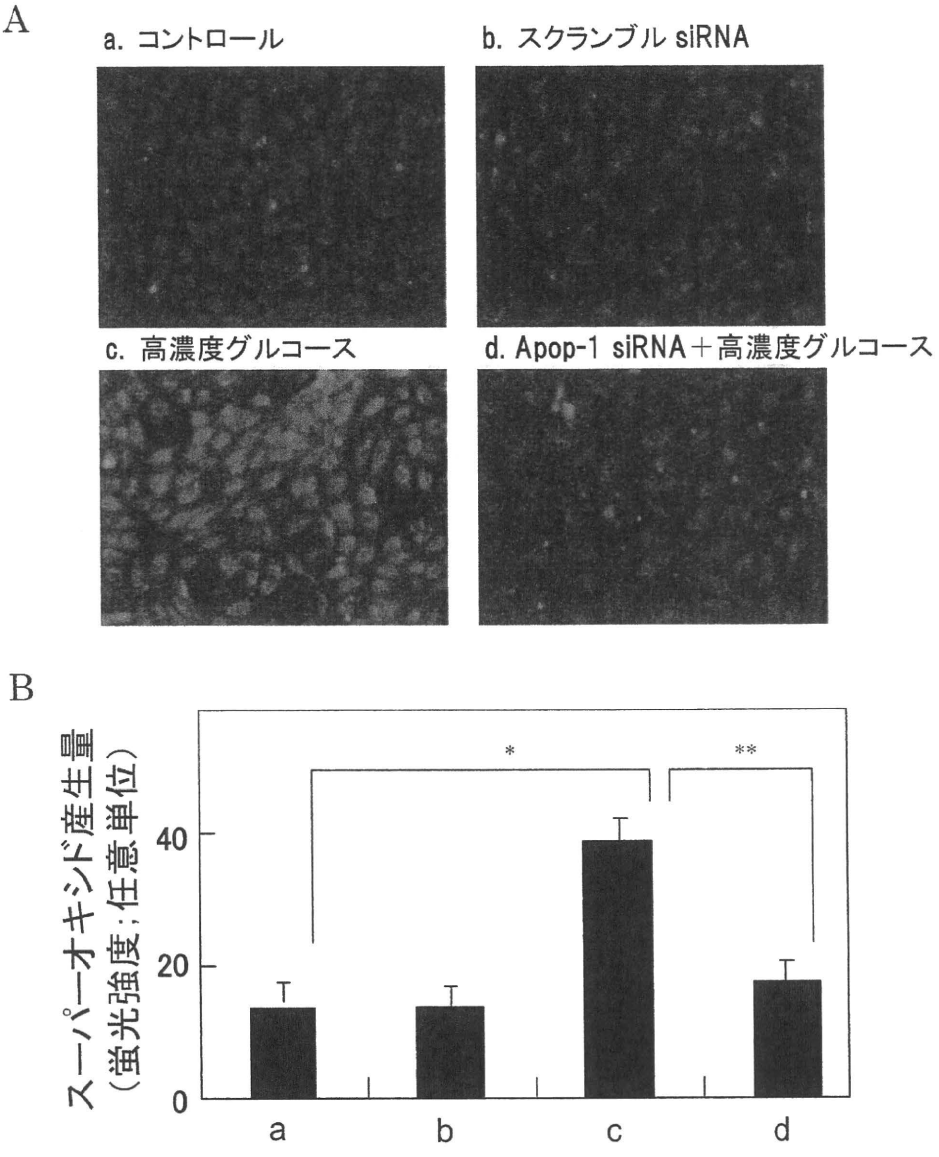


Fig. 5　Apop-1 siRNA 導入によるミトコンドリアのスーパーオキシド産生抑制
A：無処理のコントロール群（a），スクランブル siRNA 導入群（b），高濃度グルコース（33 mM）処理群（c），Apop-1 siRNA 導入 48 時間後，高濃度グルコース処理群（d），それぞれにおいて，MitoSOX を用いて，ミトコンドリアのスーパーオキシド産生量を測定した．B：蛍光強度を定量化し，スーパーオキシド産生量を数値化し，平均値 ± 標準偏差（n = 3）で表した．
（* $p < 0.01$；コントロール群との比較，** $p < 0.01$；高濃度グルコース群との比較）

で処理し，浸透圧のみを上昇させた内皮細胞では，ミトコンドリア障害は認められなかった（Fig. 2）．また，ミトコンドリアのスーパーオキシド産生を促進する DMNQ[11]は，ミトコンドリア障害を誘導した．さらに，活性酸素種消去剤 NAC の添加は，高濃度グルコース処理によるミトコンドリアのスーパーオキシド産生やミトコンドリア障害の誘導を阻害した（Fig. 3A，3B，3C）．

### 3）高濃度グルコース処理による Apop-1 発現増大と siRNA による Apop-1 発現抑制

次に，高濃度グルコース処理が，内皮細胞の Apop-1 発現を誘導する可能性を検討した．内皮細胞を高濃度グルコース溶液（最終濃度 33 mM）で 48 時間処理すると，コントロール群に比し，Apop-1 たん白質発現量が増大した．また，Apop-1 siRNA を導入して，48 時間後の内

皮細胞では，高濃度グルコース処理による Apop-1 たん白質発現増大が有意に抑制された（Fig. 4A，4B）．

**4）高濃度グルコース処理によるミトコンドリアのスーパーオキシド産生増大に対する Apop-1 の関与**

次に，高濃度グルコース処理による内皮細胞のミトコンドリアスーパーオキシド産生を Apop-1 が増強する可能性を検討した．

Apop-1 siRNA を導入して 48 時間後の，Apop-1 発現抑制した内皮細胞では，高濃度グルコース処理（最終濃度 33 mM）によるミトコンドリアのスーパーオキシド産生が有意に抑制された（Fig. 5A，5B）．

## 考　察

本研究は，ミトコンドリア局在たん白質 Apop-1 が，グルコースによる内皮細胞のミトコンドリアの活性酸素種産生増大の増強作用をもつことを初めて示唆したものである．

糖尿病において内皮細胞内に増大する活性酸素種は，NO の防御作用を上回るとともに，NO と反応して強力な細胞障害因子であるパーオキシナイトライト（$ONOO^-$）を産生し，細胞傷害や細胞死を誘導する[12]．内皮細胞の傷害や細胞死は，「傷害反応説」に示されているように，動脈硬化進行を促進する[13]．また，活性酸素種による内皮細胞の傷害は，細小血管障害である腎症や網膜症，神経障害など糖尿病の合併症を発症させる．

ミトコンドリア活性酸素種の産生過程には，スーパーオキシド産生系である Complex I や Complex III と，その消去系である MnSOD のバランスが関係している可能性がある．最近，ミトコンドリア活性酸素種の産生にミトコンドリア局在タンパク質 monoamine oxidase（MAO）や $p66^{Shc}$ が関与すると報告された．すなわち，MAO 作用を阻害する薬剤が活性酸素種の産生や虚血性心筋障害を抑制すること[14]，また，$p66^{Shc}$ 欠損マウスでは，ミトコンドリア活性酸素種の産生が低下し，高脂質食による動脈硬化病変が軽減した[15]．特に，$p66^{Shc}$ は，Apop-1 と同様にアポトーシス誘導作用を有するミトコンドリア局在たん白質であるため，両者がどのように関連するかを検討する必要がある．また，心筋細胞においては，ミトコンドリアの活性酸素種や細胞膜の NAD(P)H オキシダーゼを介して，初期に産生された活性酸素種が，その後のミトコンドリア活性酸素種の産生をさらに増大するポジティブフィードバック（活性酸素種による活性酸素種産生誘導）機構が存在する[16]．内皮細胞にも，このような機構が存在するか検討する必要がある．

一方，ミトコンドリアによる活性酸素種の産生増大は，ミトコンドリア機能障害を誘導する．例えば，ミトコンドリアの酵素である aconitase，α-ketoglutarate dehydrogenase，pyruvate dehydrogenase，および complexes I，II，III などが活性酸素種に強い感受性を持ち，酵素活性低下をおこす[17]．また，adenine nucleotide translocase（ANT）は，活性酸素種により酵素活性を低下させるため，細胞の ATP 産生を低下させると報告されている[18]．このような，内皮細胞のミトコンドリア機能障害は，内皮機能低下やアポトーシスを誘導し，動脈硬化の発症を促進する可能性がある[19]．

以上，本研究は，グルコースによる内皮細胞のミトコンドリア活性酸素種の産生をミトコンドリア局在たん白質 Apop-1 が増強することを示唆したものであり，この結果は，今後，糖尿病による動脈硬化の進行に対する Apop-1 をターゲットにした新しい治療法の開発に貢献する可能性を示している．

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## Apop-1, a Novel Mitochondrial Protein, May Enhance the Production of Mitochondrial Reactive Oxygen Species by Glucose Treatment in Endothelial cells

Mami KAWABATA[1], Yukihiro TAKEMURA[3], Rumi FUKADA[2],
Haruna YAMAMOTO[2], Osamu YASUDA[4] and Keisuke FUKUO[1,2]

[1]Administ Food Siences and Nutrition Major, Graduate School of Human Environmental Sciences, Mukogawa Women's University
[2]Department of Food Sciences and Nutrition, School of Human Environmental Sciences, Mukogawa Women's University
[3]Department of Geriatric Medicine, Osaka University Graduate School of Medicine
[4]Department of Cardiovascular Clinical and Translational Research, Kumamoto University Hospital

Accumulating evidence shows that mitochondria-derived reactive oxygen species (ROS) play an important role in the genesis of diabetes mellitus as well as apoptosis and ageing. However, the molecular mechanism of ROS production from the mitochondria remains unclear. Here, we examined whether Apop-1, a novel mitochondrial protein, participates in the induction of mitochondria-derived ROS production by glucose treatment in endothelial cells. Glucose treatment induced an increase in the levels of mitochondria-derived ROS and mitochondrial dysfunction in these cells. N-acetylcysteine (NAC), however, inhibited glucose-induced mitochondrial dysfunction. Apop-1 protein expression was up-regulated by glucose treatment and siRNA-mediated knockdown of the expression of Apop-1 protein significantly reduced glucose-treated mitochondria-derived ROS levels in endothelial cells. These results suggest that Apop-1 may be involved in the pathogenesis of diabetes mellitus by up-regulating ROS production from the mitochondria in endothelial cells.

**Key words:** Apop-1, mitochondria, reactive oxygen species, endothelium

# Survival Analysis of Patients With Duodenal Gastrointestinal Stromal Tumors

*Yuichiro Miki, MD,* Yukinori Kurokawa, MD, PhD,* Motohiro Hirao, MD,* Kazumasa Fujitani, MD,* Yoko Iwasa, MD,† Masayuki Mano, MD,† Shoji Nakamori, MD,* and Toshimasa Tsujinaka, MD**

**Goals:** To evaluate the survival characteristics of patients with duodenal gastrointestinal stromal tumors (GISTs).

**Background:** GISTs represent the most common mesenchymal neoplasms. However, duodenal GISTs are relatively rare, and few studies have been performed with a focus on duodenal GISTs.

**Study:** We collected the data of 41 GIST patients including 7 duodenal cases. Clinicopathologic findings and recurrence-free survival (RFS) of duodenal GIST patients were analyzed.

**Results:** The proportion of having any symptoms was 86% in duodenum, 32% in stomach, and 56% in other GISTs ($P = 0.034$), and the most common symptoms of duodenal GISTs were melena and anemia. The 2-year RFS rates were 51.4% in duodenal GISTs, 78.4% in stomach GISTs, and 100% in other GISTs, and duodenal GISTs showed poorer RFS than nonduodenal GISTs (hazard ratio, 5.1; log-rank $P = 0.019$). Particularly, in low-risk and intermediate-risk group, the hazard ratio of recurrence was 12.3 (log-rank $P = 0.010$). Multivariate Cox analysis showed symptom ($P = 0.007$), mitotic index ($P = 0.011$), and tumor location ($P = 0.043$) were significant prognostic factors of recurrence.

**Conclusions:** RFS of duodenal GISTs was worse than nonduodenal GISTs.

**Key Words:** duodenum, GIST, RFS, survival

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Gastrointestinal stromal tumors (GISTs) represent the most common mesenchymal neoplasms arising within the gastrointestinal tract. These tumors are thought to share a common progenitor cell with the interstitial cells of Cajal, and usually have activating mutations in either c-kit (75% to 80%) or platelet-derived growth factor receptor α (PDGFRA) (5% to 10%), 2 closely related receptor tyrosine kinases.[1] These mutations lead to ligand-independent activation and signal transduction mediated by constitutively activated KIT or PDGFRA. This theory was first proposed by Kindblom et al[2] and Hirota et al[3] revealed an association between the presence of c-kit mutation and tumor development.

GISTs can arise anywhere in the gastrointestinal tract, but their most frequent locations are the stomach (60%) and the small intestine (25%). Duodenal GISTs are relatively rare and comprise about 5% of surgically resected GIST cases.[4,5] Earlier studies have reported that duodenal GISTs were larger than stomach GISTs, and that their most frequent locations were the second and third portions of the duodenum.[4] Owing to the unique and complex anatomy of the duodenum, complete resection of duodenal GISTs sometimes requires wide resection methods such as pancreaticoduodenectomy,[6] which is rarely the case for GISTs in other locations. Only few reports about the characteristics of duodenal GISTs have been published earlier,[7–9] and few studies have been performed a survival analysis of patients with duodenal GISTs. From August 1993 to January 2008, we encountered 41 GIST cases of which 7 were duodenal GISTs. Here we conduct a retrospective cohort study to evaluate the survival characteristics of duodenal GISTs.

## PATIENTS AND METHODS

### Patients

We retrospectively reviewed the records of all patients with GISTs treated at the Osaka National Hospital between August 1993 and January 2008. The diagnosis of GISTs was conducted by histologic examination, immunohistochemical staining for KIT and CD34, and detection of c-kit or PDGFRA mutations.

Data on patients' age, sex, tumor location, symptoms, pathologic findings, c-kit and PDGFRA mutations, treatment, and survival outcome were collected. Tumor size was defined as the largest diameter of the primary tumor in any dimension. Pathologic data included mitotic index and results of immunohistochemical staining for KIT and CD34. Treatment data included type of resection and adjuvant treatment. Tumor size and mitotic index were used for risk classification according to the Fletcher score.[10] However, in this study, we combined low-risk and intermediate-risk patients in the survival analysis because "low-risk" has not been defined for duodenal GISTs.

### Statistical Analysis

Associations between tumor location and clinicopathologic variables were analyzed using the $\chi^2$ test. Recurrence-free survival (RFS) was defined as the time

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From the Departments of *Surgery; and †Pathology, Osaka National Hospital, Osaka, Japan.
The authors declare no conflict of interest, and no grant support.
Reprints: Yukinori Kurokawa, MD, PhD, Department of Surgery, Osaka National Hospital, 2-1-14, Hoenzaka, Chuo-ku, Osaka 540-0006, Japan (e-mail: kurokawa@onh.go.jp).
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**TABLE 1.** Characteristics of 7 Patients With Duodenal GISTs

| Age (y) | Sex | Clinical Symptom | Location of Duodenal GIST | Size (mm) | Operation | Adjuvant Therapy | KIT | CD34 | Mitotic Index (per 50 HPF) | *C-Kit* Mutation | Risk Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 64 | F | Melena | Second part | 60 | Gastrojejunostomy | – | – | – | < 5 | Exon 11 | Intermediate |
| 58 | F | Melena | Second part | 70 | Pancreaticoduodenectomy | – | + | + | < 5 | NA | Intermediate |
| 70 | F | Abdominal mass | Fourth part | 150 | Partial duodenal resection | + | + | + | < 5 | Exon 13 | High |
| 67 | F | Absent | First part | 60 | Partial duodenal resection | – | + | + | < 5 | Exon 11 | Intermediate |
| 39 | F | Melena | First part | 120 | Partial duodenal resection | – | + | + | 5-10 | Exon 11 | High |
| 65 | M | Anemia | Second part | 30 | Partial duodenal resection | – | + | NA | 5-10 | Exon 9 | Intermediate |
| 75 | F | Anemia | Second part | 40 | Pancreaticoduodenectomy | – | + | – | > 10 | Exon 11 | High |

GISTs indicates gastrointestinal stromal tumors; HPF, high-power field; F, female; M, male; NA, not analyzed.

from surgery to either the first recurrence or death from any cause. RFS curves were estimated by the Kaplan-Meier method and compared using the log-rank test. Multivariate Cox regression analyses were performed to adjust for the potential confounding factors whose *P* values were under 0.2 in univariate analyses. All statistical analyses were performed with SPSS software, version 15.0J. *P* values less than 0.05 were considered statistically significant, and all tests were 2-sided.

## RESULTS

### Patient Characteristics

Forty-one patients with GISTs were admitted for treatment to Osaka National Hospital between August 1993 and January 2008, and of these 7 patients (17%) were diagnosed with duodenal GISTs (Table 1). Six of the 7 duodenal GIST patients were female. The second portion of the duodenum was most frequently affected, which is of significance because of the need for pancreaticoduodenectomy if the tumor is located on the same side of intestine as the Papilla Vater. For 1 duodenal GIST patient, we could not perform radical surgery because of severe patient's general condition, whereas the other duodenal GIST patients received complete gross resection. Postoperative complications occurred in 3 of 7 duodenal GIST patients. These complications included pancreatic fistula, and intra-abdominal abscess, but none of the patients died within 1 month after surgery. Only 1 patient received adjuvant chemotherapy after surgery. One patient showed immunohistochemical staining of neither c-kit nor CD34. Six cases had c-kit mutations; 4 for exon 11, 1 for exon 9, and 1 for exon 13. The numbers of intermediate-risk and high-risk patients were 4 (57%) and 3 (43%), respectively.

We compared patients with duodenal GISTs to those with stomach GISTs and other GISTs (Table 2). Among 9 patients with other GISTs, 4 were found in rectum and in small intestine, and 1 in omentum. There were no statistical differences in clinicopathologic factors except for clinical symptoms and CD34 positivity. With regard to immunohistochemical findings, the KIT-positive rate was similar in duodenal and other GISTs, whereas the CD34-positive rate was lower in duodenal GISTs ($P = 0.049$). Although over 30% patients with stomach GISTs were classified as low-risk, there were no low-risk patients among the duodenal and other GIST groups.

Of patients with duodenal GISTs, 86% had symptoms, whereas 32% of patients with stomach GISTs, and 56% of those with other GISTs were affected; this difference was statistically significant ($P = 0.034$). Five of 6 symptomatic patients with duodenal GISTs had melena or anemia, whereas a half of symptomatic patients with stomach GISTs complained of epigastralgia (Table 3).

**TABLE 2.** Comparison of Characteristics Among Duodenal GISTs, Stomach GISTs, and GISTs in Other Locations

| | Duodenum (n = 7) | Stomach (n = 25) | Other (n = 9) | *P* |
|---|---|---|---|---|
| Age (y) | | | | 0.50 |
| Median (range) | 65 (39-75) | 67 (48-82) | 59 (45-86) | |
| Sex | | | | 0.10 |
| Male | 1 (14%) | 13 (52%) | 2 (22%) | |
| Female | 6 (86%) | 12 (48%) | 7 (78%) | |
| Clinical Symptom | | | | 0.034 |
| Absent | 1 (14%) | 17 (68%) | 4 (44%) | |
| Present | 6 (86%) | 8 (32%) | 5 (56%) | |
| Immunohistochemistry | | | | |
| KIT | | | | 0.53 |
| Positive | 6 (86%) | 22 (88%) | 9 (100%) | |
| Negative | 1 (14%) | 3 (12%) | 0 (0%) | |
| CD34* | | | | 0.049 |
| Positive | 4 (67%) | 23 (96%) | 6 (67%) | |
| Negative | 2 (33%) | 1 (4%) | 3 (33%) | |
| Tumor size (cm)† | | | | 0.40 |
| Median (range) | 6.0 (3.0-15) | 5.0 (1.7-24) | 6.0 (2.5-12) | |
| Mitotic Index (per 50 HPF) | | | | 0.59 |
| < 5 | 4 (57%) | 16 (64%) | 3 (33%) | |
| 5-10 | 2 (29%) | 5 (20%) | 3 (33%) | |
| > 10 | 1 (14%) | 4 (16%) | 3 (33%) | |
| Risk Classification | | | | 0.13 |
| Low | 0 (0%) | 8 (32%) | 0 (0%) | |
| Intermediate | 4 (57%) | 7 (28%) | 5 (56%) | |
| High | 3 (43%) | 10 (40%) | 4 (44%) | |
| C-kit mutation‡ | | | | 0.33 |
| Exon 9 | 1 (17%) | 0 (0%) | 1 (50%) | |
| Exon 11 | 4 (66%) | 12 (86%) | 1 (50%) | |
| Exon 13 | 1 (17%) | 1 (7%) | 0 (0%) | |
| Exon 17 | 0 (0%) | 1 (7%) | 0 (0%) | |

*One duodenal GIST case and 1 stomach GIST case were not analyzed.
†One stomach GIST case was not analyzed.
‡One duodenal GIST case, 11 stomach GIST cases, and 7 other GIST cases were not analyzed.
GISTs indicates gastrointestinal stromal tumors; HPF, high-power field.


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**TABLE 3.** Symptoms Among Duodenal GISTs, Stomach GISTs, and GISTs in Other Locations

| | Duodenum (n = 6) | Stomach (n = 8) | Other (n = 5) |
|---|---|---|---|
| Anemia | 2 (33%) | 2 (25%) | 0 |
| Melena | 3 (50%) | 0 | 1 (20%) |
| Epigastralgia | 0 | 4 (50%) | 0 |
| Abdominal mass | 1 (17%) | 1 (13%) | 1 (20%) |
| Nausea | 0 | 1 (13%) | 1 (20%) |
| Others | 0 | 0 | 2 (40%) |

GISTs indicates gastrointestinal stromal tumors.

## Survival

In survival analysis of all GIST patients, the 2-year RFS rates of duodenal, stomach, and other GISTs were 51.4%, 78.4%, and 100%, respectively ($P = 0.058$) (Fig. 1A). As the survival curves of stomach and other GISTs were similar, we combined the stomach GISTs with other GISTs as a nonduodenal group, and compared RFS of duodenal GIST patients with those of nonduodenal GIST patients. As the result, the hazard ratio (HR) of recurrence was 5.1 [95% confidence interval (CI), 1.1-23.2] in the duodenal GIST patients, and the log-rank test showed statistical significance ($P = 0.019$) (Fig. 1B). In the low-risk and intermediate-risk groups specifically, the 2-year RFS rates of patients with duodenal and nonduodenal GISTs were 50% and 100%, respectively, showing a statistical difference (log-rank $P = 0.010$) and the HR of recurrence was 12.3 (95% CI, 1.1-142.9) (Fig. 2A). However, in the high-risk group there was no significant difference in RFS between duodenal GIST patients and nonduodenal GIST patients (log-rank $P = 0.60$) (Fig. 2B), and the HR of recurrence was 1.8 (95% CI, 0.19-17.9).

Univariate analyses revealed that symptom ($P = 0.009$), mitotic index ($P = 0.038$), and tumor location ($P = 0.035$) were the statistically significant prognostic factors of RFS (Table 4). These 3 factors were significantly associated with RFS even in multivariate analysis.

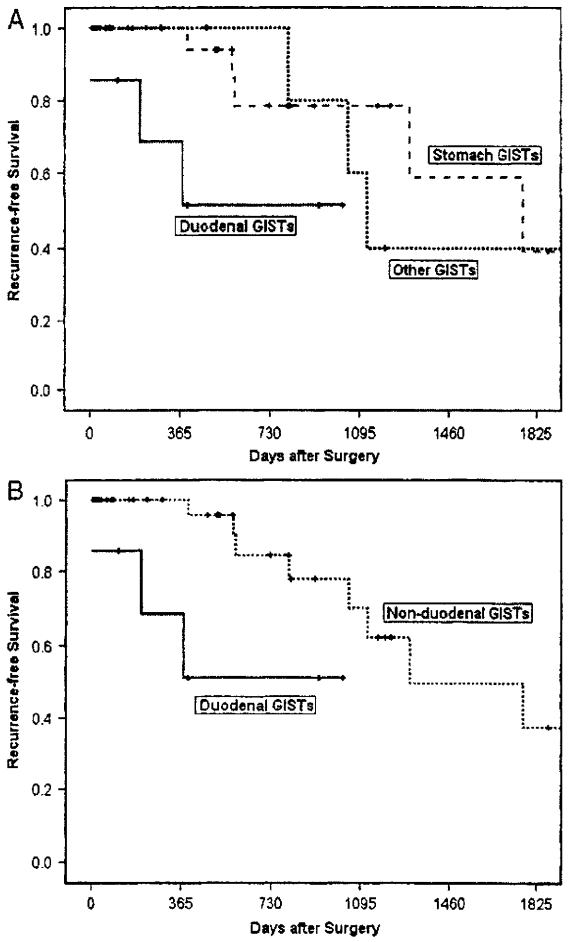


**FIGURE 1.** Recurrence-free survival of patients with gastrointestinal stromal tumors (GISTs) on the basis of tumor location. A, Duodenal versus stomach versus other GISTs. B, Duodenal versus nonduodenal GISTs.

## DISCUSSION

GISTs are often discovered in the stomach and small intestine, but duodenal GISTs comprise only about 5% of these. Although 2 case series have studied duodenal GISTs,[7,8] neither conducted a survival analysis. This study showed that the RFS of duodenal GIST patients was worse than that of patients with stomach GISTs or GISTs in other locations, and the poor prognosis of duodenal GISTs was more remarkable in low-risk and intermediate-risk patient groups. Several earlier studies have reported that patients with GISTs of the small intestine have an unfavorable prognosis, compared with stomach GISTs.[11-13] In this study, we combined small intestine cases with stomach cases, because the survival curves of stomach and other GISTs were similar. Multivariate Cox analyses performed after adjusting for other prognostic factors revealed that tumor location was

**TABLE 4.** Association of Clinicopathological Factors With Recurrence-free Survival

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | Hazard Ratio (95% CI) | P | Hazard Ratio (95% CI) | P |
| Age (> 65 y) | 2.4 (0.64-9.2) | 0.20 | 1.6 (0.34-7.3) | 0.56 |
| Sex (male) | 1.4 (0.43-4.8) | 0.55 | — | — |
| Symptom (present) | 17.4 (2.0-150.1) | 0.009 | 158.1 (3.9-6374.0) | 0.007 |
| Tumor size (> 5 cm) | 1.6 (0.45-5.7) | 0.47 | — | — |
| Mitotic index (≥ 5/50 HPF) | 5.1 (1.1-23.8) | 0.038 | 37.0 (2.3-596.7) | 0.011 |
| Location (duodenum) | 5.1 (1.1-23.3) | 0.035 | 10.9 (1.1-111.1) | 0.043 |

CI indicates confidence interval; HPF, high-power field.

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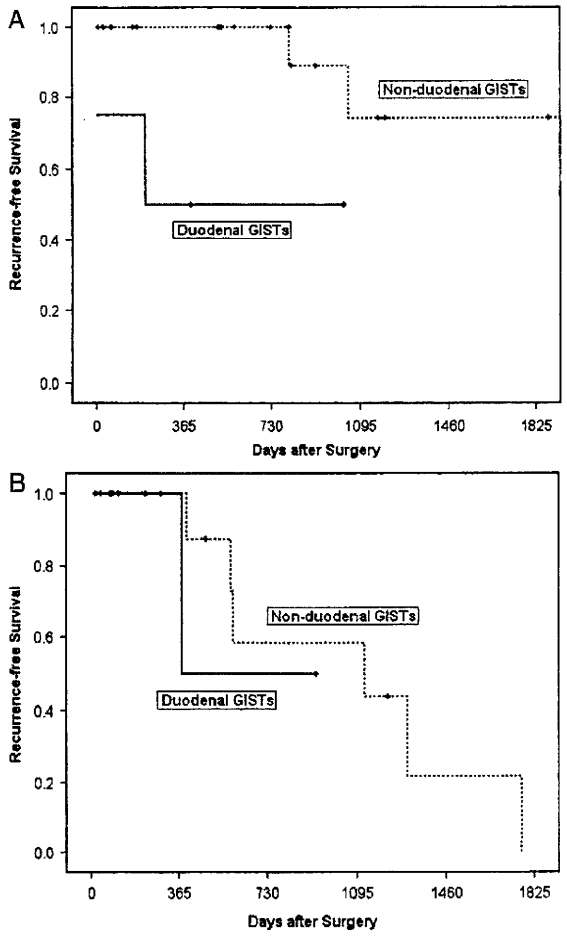


**FIGURE 2.** Recurrence-free survival of patients with duodenal gastrointestinal stromal tumors (GISTs) and nonduodenal GISTs in (A) low-risk and intermediate-risk group and (B) high-risk group.

an independent prognostic factor for GISTs. This result may indicate that the duodenal GISTs are biologically different from other GISTs.

Over the past several years, site-specific differences in appearance, morphology, and clinical outcome have been identified in GISTs. It has been reported that the proportion of CD34-positive tumors and the frequency of c-kit mutations are different depending on location.[14,15] An earlier study reported that CD34 positivity was more frequent in malignant tumors than in borderline or benign tumors.[16] Another study reported that CD34 positivity in patients with recurrence is higher than those without recurrence, although the difference was not statistically significant.[17] In this study, the proportion of CD34-positive patients with duodenal GISTs was even lower than that in patients with stomach GISTs. Thus, we cannot explain the poor survival of patients with duodenal GISTs by CD34 positivity alone. In contrast, earlier studies showed that mutations of exon 9 were more common in patients with small intestinal GISTs than in those with stomach GISTs.[18,19] GISTs with exon 9 mutations are often clinically and pathologically malignant, and this subgroup of patients is often resistant to imatinib. In our population, a duodenal GIST patient with an exon 9 mutation showed early metastases to the liver after surgery. The positivity rate of c-kit exon 9 mutations may contribute to the poor survival of patients with duodenal GISTs. In this study, however, we did not analyze c-kit mutation sites for about half of all GIST cases, so we could not evaluate the association between survival and the location of c-kit mutation.

In comparison of clinicopathologic characteristics among 3 location types of GISTs, clinical symptom was the most significant finding. Many duodenal GIST patients had symptomatic complaints that were mainly associated with bleeding from tumor, whereas the proportion of stomach GIST patients who had any clinical symptoms in diagnosis was low (28%). Most of asymptomatic patients with stomach GISTs were diagnosed in medical screening or follow-up of other diseases. In Japan, medical screening with upper gastrointestinal endoscopy or x-ray has been widespread because of high prevalence of gastric cancer, and it may contribute to early detection of asymptomatic stomach GISTs. These features may induce the survival difference between the duodenal and nonduodenal GISTs. However, the tumor location was an independent prognostic factor after adjusting for the presence of clinical symptoms in the multivariate Cox analyses.

Surgery remains the mainstay of treatment for patients with primary GISTs without distant metastasis. A recent retrospective study to compare the survivals of duodenal GIST patients after pancreaticoduodenectomy with those after limited resection reported that the disease-free survivals were similar between 2 surgical procedures.[6] In this study, both the 2 cases who received pancreaticoduodenectomy are alive without recurrence, whereas 2 of 4 patients who received limited duodenal resection had recurrence after surgery. Complete gross resection with an intact pseudocapsule may be the most important thing to treat duodenal GISTs, and so we should not hesitate to perform combined resection such as pancreaticoduodenectomy to achieve gross resection, even though the surgical procedure is highly invasive.

Limitations of this study include its retrospective design and small sample size. As survival analyses with small number of patients sometimes mislead the results, we should therefore be careful in evaluating its results. However, to our knowledge, this is the first study to focus on the survival of patients with duodenal GISTs, and the difference of RFS between duodenal and nonduodenal GISTs was remarkable. In the future, prospective studies using larger numbers of patients will be needed.

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# Influence of Bursectomy on Operative Morbidity and Mortality After Radical Gastrectomy for Gastric Cancer: Results of a Randomized Controlled Trial

**Hiroshi Imamura · Yukinori Kurokawa · Junji Kawada · Toshimasa Tsujinaka · Shuji Takiguchi · Yoshiyuki Fujiwara · Masaki Mori · Yuichiro Doki**

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**Abstract**

*Background* Bursectomy, a procedure dissecting the peritoneal lining covering the pancreas and the anterior plane of the transverse mesocolon, has been commonly performed with radical gastrectomy for gastric cancer patients. Although possibly improving the prognosis of gastric cancers, adverse events related to bursectomy should be evaluated in prospective studies.

*Methods* This prospective randomized controlled trial was conducted by experienced surgeons in 11 Japanese institutions. Patients with T2 or T3 gastric adenocarcinoma were intraoperatively randomized to radical gastrectomy plus D2 lymphadenectomy either with or without bursectomy. Postoperative morbidity and mortality were compared between the two groups.

*Results* A total of 210 patients were assigned to the bursectomy group (104 patients) and the nonbursectomy group (106 patients) between July 2002 and January 2007. Background characteristics were well balanced. Intraoperative blood loss was greater in the bursectomy group than in the nonbursectomy group (median 475 vs. 350 ml, $p = 0.047$), whereas other surgical factors did not vary significantly. The overall morbidity rate was 14.3% (30 patients), the same for the two groups. Likewise, the incidence of major postoperative complications, including pancreatic fistula, anastomotic leakage, abdominal abscess, bowel obstruction, hemorrhage, and pneumonia, were not significantly different between the two groups. The medians of the amylase level of the drainage fluid on postoperative day 1 were similar for the two groups (median 282 vs. 314 IU/L, $p = 0.543$). The hospital mortality rate was 0.95%: one patient per group.

*Conclusions* Experienced surgeons could safely perform a D2 gastrectomy with an additional bursectomy without increased major surgical complications.

## Introduction

More than half of the new cases of gastric cancer occur in eastern Asia [1]. The surgical intervention for gastric cancers has rapidly developed in Japan. An extended radical lymphadenectomy, which is almost identical to the present D2 dissection, along with bursectomy was established as the standard treatment for advanced gastric cancers during the early 1960s [2, 3]. Bursectomy is a traditional surgical procedure to dissect the peritoneal lining covering the pancreas and the anterior plane of the transverse mesocolon with an omentectomy [4, 5]. This procedure is recommended in the Japanese Gastric Cancer Treatment Guidelines as part of the radical surgery for gastric cancer to remove micrometastases disseminated into the bursa omentalis [6]. As gastric cancer in the posterior wall sometimes shows peritoneal dissemination only in the bursa omentalis, its resection may improve survival [7].

On the other hand, a bursectomy causes some surgical stress when performed in addition to a D2 lymph node

H. Imamura
Department of Surgery, Sakai Municipal Hospital, Sakai, Japan

Y. Kurokawa (✉) · J. Kawada · S. Takiguchi · Y. Fujiwara · M. Mori · Y. Doki
Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: ykurokawa@gesurg.med.osaka-u.ac.jp

T. Tsujinaka
Department of Surgery, Osaka National Hospital, Osaka, Japan

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dissection. Therefore, the possible increase in the incidence of postoperative complications, including pancreatic fistula formation, intestinal obstruction, and hemorrhage, may be concerning. As the safety of a D2 lymph node dissection is still controversial in Western countries [8, 9], we should also carefully evaluate the safety of bursectomy. To elucidate the safety and usefulness of the bursectomy, we conducted a multiinstitutional randomized controlled trial. We hereby present our operative morbidity and mortality data, the secondary endpoints of this trial. The final analysis of survival data is scheduled to take place in 2012.

## Patients and methods

### Patients

Patient eligibility criteria for this study were as follows: (1) histologically proven primary adenocarcinoma of the stomach; (2) a preoperative and intraoperative classification of T2N0, T3N0, T2N1, or T3N1 according to 13th edition of the Japanese Classification of Gastric Carcinoma [10]; (3) a lack of noncurative surgical factors except for positive lavage cytology; (4) no Borrmann type 4 (linitis plastica) cases; (5) no prior chemotherapy or radiation therapy; (6) ages 20 to 80 years with a performance status of 0 to 2 according to the Eastern Cooperative Oncology Group (ECOG) scale; (7) no history of gastrectomy or other malignancy during the last 5 years. All patients gave written informed consent before undergoing randomization.

When the surgeon confirmed the above eligibility criteria immediately after the initial laparotomy, patients were then intraoperatively randomized to the bursectomy group (a D2 gastrectomy with bursectomy) or the nonbursectomy group (without bursectomy). Randomizations were made by the minimization method according to sex, clinical T stage (cT2 vs. cT3), and gastrectomy (total vs. distal subtotal gastrectomy).

### Surgery

In both the bursectomy and nonbursectomy groups, the surgeon performed a total or distal subtotal gastrectomy and D2 lymph node dissection as a standard treatment for advanced gastric cancers [10]. With total gastrectomy for T2 or deeper tumors in the proximal third of the stomach, the spleen was removed in principle for splenic hilar lymphadenectomy. Pancreatectomy was confined to those patients whose pancreas was involved by tumor.

An omentectomy was performed for both groups in this study. In the bursectomy group, the peritoneal lining of the bursa omentalis was removed en bloc as much as possible from the anterior plane of the transverse mesocolon and the pancreas. In the caudal area of the bursa omentalis, the anterior lesion was removed with the minor omentum at the edge of the left lobe of the liver. The posterior and right-sided lesions were removed with lymph node dissection along the common hepatic artery (no. 8a), the splenic artery (no. 11p/d), the left gastric artery (no. 7), and in the hepatoduodenal ligament (no. 12a). As complete removal of the left side of the bursa omentalis did not allow a distal subtotal gastrectomy, pancreatic serosa was removed up to the proximal half of the splenic artery (no. 11p). For the transverse colon mesentery, the peritoneum was removed up to the left gastroepiploic artery (no. 4sb). In the nonbursectomy group, the right anterior surface of the transverse colon mesentery was partially removed around the root of the right gastroepiploic artery (no. 6). Only a small amount of peritoneum could be removed for lymph node dissection. Thus, the bursa omentalis peritoneal lining was preserved as much as possible in the nonbursectomy group. The type of reconstruction and the indication of prophylactic cholecystectomy were not specified in the protocol.

Patients were enrolled from 11 hospitals belonging to the Osaka University Clinical Research Group for Gastroenterological Surgery. More than 50 gastrectomies were performed each year in these 11 hospitals. All operations were performed or supervised by senior surgeons who were members of the Japanese Gastric Cancer Association. During the planning of the study, all participating surgeons reached an agreement concerning the technical details of bursectomy.

### Postoperative evaluation

Operative methods and pathology results were recorded according to the 13th edition of the Japanese Classification of Gastric Carcinoma [10]. The number of dissected lymph nodes was measured by pathology. Drainage fluid was collected via an operatively placed drain on postoperative day (POD) 1 for measuring the amylase level. The six Representative data for the six major morbidities—pancreatic fistula, anastomotic leakage, abdominal abscess, bowel obstruction, hemorrhage, pneumonia—were prospectively collected. A pancreatic fistula was defined by a drainage output on or after POD 5 with an amylase content more than three times the upper normal serum value. Pneumonia, anastomotic leakage, abdominal abscess, and bowel obstruction were diagnosed radiologically or clinically. Postoperative hemorrhage requiring a transfusion was recorded as morbidity. Any other complications requiring pharmacologic or surgical treatment were recorded on a free format. Operative morbidity until 3 months after surgery was also analyzed in this study. Operating time, blood loss, duration of hospital stay after surgery, and reoperation details were also recorded. Hospital mortality

was defined as postoperative death of any cause within 30 days or death during the same hospitalization.

Patients were followed every 3 months until 5 years after the operation. Adjuvant therapy was not permitted before a recurrence of cancer.

### Statistical Analysis

The primary endpoint was overall survival (OS). Secondary endpoints were recurrence-free survival, operative morbidity, and POD 1 drainage amylase levels. We planned initially to recruit 200 patients, with an alpha error of 0.1 and statistical power of 80%. This allowed detection of a 10% margin of noninferiority for the nonbursectomy group under the estimation of a 60% 5-year OS in the bursectomy group. The projected accrual period and follow-up period were 3 years and 5 years, respectively. After registration of 204 patients, we amended the sample size and analysis to correct the estimation of the 5-year OS in the bursectomy group as 75% and to reduce alpha error. The amended sample size was 464, with an alpha error of 0.05 and statistical power of 80%, with an 8-year accrual period (total) and 5-year follow-up.

In January 2007, the positive result of a large-scale randomized controlled trial to evaluate adjuvant S-1 chemotherapy for stage II/III gastric cancer patients was reported [11, 12]. Since then, adjuvant S-1 chemotherapy has been a new standard treatment for stage II/III gastric cancer patients in Japan. However, because any adjuvant treatment including S-1 was not allowed after surgery in our study, we decided to close the accrual of our study in January 2007.

The operative morbidity and mortality rates were based on the proportion of the number of cases divided by all registered patients based on the intention-to-treat principle. The differences in proportion between the two groups were evaluated using Fisher's exact test or chi-squared test. The differences of continuous variables, including age, body mass index, tumor size, operating time, blood loss, and the number of dissected lymph nodes for the two groups were tested with a Mann-Whitney U-test. All $p$ values were two-sided, and statistical analysis was done using SPSS Statistics software, version 17.0 (SPSS, Chicago, IL, USA).

## Results

### Patients and surgery

Between July 2002 and January 2007, a total of 210 patients were randomly divided into 104 in the bursectomy group and 106 in the nonbursectomy group (Fig. 1). One patient in the bursectomy group did not undergo bursectomy, and one in the nonbursectomy group underwent

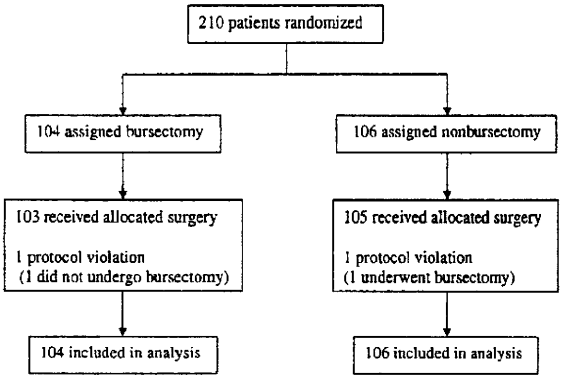


**Fig. 1** CONSORT flowchart for patients

bursectomy. Most of the baseline characteristics were well balanced (Table 1). The bursectomy group had slightly older patients than the nonbursectomy group (median 65 vs. 63 years, $p = 0.099$). The number of patients with pathologically positive nodes was slightly higher in the bursectomy group than in the nonbursectomy group (52.9% vs. 43.4%, $p = 0.214$).

The operative details are shown in Table 2. A total gastrectomy was performed on 22 (21.2%) patients in the bursectomy group and on 27 (25.5%) patients in the nonbursectomy group. About one-half of patients in each of the two groups underwent a Roux-en-Y reconstruction procedure. A combined resection of other organs was performed for 103 patients in total. The resected organs were the gallbladder in 98 patients, spleen in 26 patients, part of the pancreas in 1 patient, the colon in 1 patient, the left adrenal gland in 1 patient, and the diaphragm in 1 patient. It was of note that although the difference was not statistically significant the number of patients with a combined resection was greater in the nonbursectomy group than in the bursectomy group (42.3 vs. 55.7%, $p = 0.055$). When we evaluated the operating time after dividing the patients into two subgroups, either with or without a combined resection of other organs, the bursectomy required a longer operating time (median 27 min in patients with a combined resection, 26 min in patients without a combined resection). The amount of blood loss significantly increased in the bursectomy group compared to the nonbursectomy group (median 475 vs. 350 ml, $p = 0.047$). There was no significant difference between the two groups regarding the number of dissected lymph nodes.

### Operative morbidity and mortality

The overall operative morbidity rate was 14.3% (30 patients), which was the same in the two groups (Table 3). Prespecified complications, including pancreatic fistula, anastomotic leakage, abdominal abscess, bowel obstruction,

**Table 1** Patient and tumor characteristics

| Characteristic | Bursectomy ($n$ = 104) | Nonbursectomy ($n$ = 106) | $p$* |
|---|---|---|---|
| Age (years) | | | 0.099 |
| Median | 65 | 63 | |
| Range | 31–79 | 34–78 | |
| Sex | | | 0.761 |
| Male | 73 | 77 | |
| Female | 31 | 29 | |
| Body mass index | | | 0.653 |
| Median | 22.3 | 22.5 | |
| Range | 15.7–28.9 | 15.6–29.4 | |
| Tumor size (cm) | | | 0.311 |
| Median | 4.3 | 4.5 | |
| Range | 0.9–11.0 | 1.5–12.0 | |
| Histological type | | | 0.784 |
| Differentiated | 47 | 50 | |
| Undifferentiated[a] | 57 | 56 | |
| Clinical T stage[b] | | | 0.572 |
| cT2 | 61 | 67 | |
| cT3 | 43 | 39 | |
| Clinical N stage[b] | | | 1.000 |
| cN0 | 59 | 61 | |
| cN1 | 45 | 45 | |
| Pathologic T stage[b] | | | 0.902 |
| pT1 | 17 | 19 | |
| pT2 | 62 | 64 | |
| pT3-4 | 25 | 23 | |
| Pathologic N stage[b] | | | 0.119 |
| pN0 | 49 | 60 | |
| pN1 | 37 | 24 | |
| pN2-3 | 18 | 22 | |
| Residual tumor | | | 1.000 |
| R0 | 101 | 102 | |
| R1 | 3 | 4 | |

* The $p$ values were calculated by Fisher's exact test for sex, histological type, clinical T stage, clinical N stage, and residual tumor; by the chi-squared test for pathologic T stage and pathologic N stage; and by the Mann–Whitney $U$-test for age, body mass index, and tumor size

[a] Undifferentiated type included one endocrine cell carcinoma case in the nonbursectomy group

[b] T stage and N stage were according to the 13th edition of the Japanese Classification of Gastric Carcinoma

**Table 2** Profile of surgical treatment

| Treatment | Bursectomy ($n$ = 104) | Nonbursectomy ($n$ = 106) | $p$* |
|---|---|---|---|
| Gastrectomy | | | 0.515 |
| Total | 22 | 27 | |
| Distal subtotal | 82 | 79 | |
| Reconstruction method | | | 0.705 |
| Roux-en-Y | 48 | 55 | |
| Billroth I | 54 | 49 | |
| Other[a] | 2 | 2 | |
| Combined resection of other organs | | | 0.055 |
| Present | 44 | 59 | |
| Gallbladder | 41 | 57 | |
| Spleen | 12 | 14 | |
| Other[b] | 1 | 2 | |
| Absent | 60 | 47 | |
| Operating time (min) | | | 0.368 |
| Median | 222 | 221 | |
| Range | 134–488 | 111–360 | |
| Blood loss (ml) | | | 0.047 |
| Median | 475 | 350 | |
| Range | 80–3970 | 55–2901 | |
| No. of dissected lymph nodes | | | 0.417 |
| Median | 38 | 37 | |
| Range | 11–98 | 7–97 | |

* $p$ values were calculated by Fisher's exact test for gastrectomy and combined resection of other organs (present or absent); by chi-squared test for the reconstruction method; and by the Mann–Whitney $U$-test for operating time, blood loss, and the number of dissected lymph nodes

[a] Others included one Billroth II method and one intestinal interposition method in the bursectomy group and two Billroth II methods in the nonbursectomy group

[b] Others included one adrenal gland in the bursectomy group and one pancreas and one diaphragm in the nonbursectomy group

**Table 3** Postoperative morbidity

| Morbidity | Bursectomy ($n$ = 104) | Nonbursectomy ($n$ = 106) | $p$* |
|---|---|---|---|
| Any complication | 15 | 15 | 1.000 |
| Pancreatic fistula | 3 | 7 | 0.332 |
| Anastomotic leakage | 4 | 3 | 0.720 |
| Abdominal abscess | 3 | 8 | 0.214 |
| Bowel obstruction | 2 | 1 | 0.620 |
| Hemorrhage | 1 | 0 | 0.495 |
| Pneumonia | 1 | 1 | 1.000 |

* The $p$ values were calculated by Fisher's exact test

hemorrhage, and pneumonia, did not significantly differ between the two groups. Among the 10 patients with a pancreatic fistula, 6 underwent splenectomy, but no patients underwent pancreaticosplenectomy. Ten patients suffered from other complications, including two cases of chylous lymphorrhea, two of delayed gastric emptying without obstruction, and one case of afferent loop syndrome, acute cholecystitis, acute enteritis, arteriosclerosis obliterans of the leg, drug-induced hepatitis, and anastomotic stricture. The incidence of these miscellaneous complications tended

to be more frequent in the bursectomy group than in the nonbursectomy group (7.7 vs. 1.9%, $p = 0.057$). The median amylase levels in the drainage fluid on POD 1 were 282 IU/L in the bursectomy group and 314 IU/L in the nonbursectomy group ($p = 0.543$). Reoperation was required in four patients (1.9%): two for intestinal obstruction, one for afferent loop syndrome in the bursectomy group, and one for anastomotic leakage in the nonbursectomy group. The median hospital stay after surgery was 16 days in the bursectomy group and 15 days in the nonbursectomy group ($p = 0.744$).

There were two hospital deaths (0.95%). One patient in the bursectomy group and one patient in the nonbursectomy group died of sepsis after anastomotic leakage and pancreatic fistula formation, respectively. All other patients recovered from surgery and were discharged from the hospital.

## Discussion

Two factors are necessary for bursectomy to be accepted as a standard treatment for advanced gastric cancers: safety and oncologic benefit. Only a randomized clinical trial can scientifically evaluate this proposition, and we are the first worldwide to conduct such a trial. This article is an early report of this trial with respect to operative safety. We found that overall morbidity and mortality were equivalent with and without bursectomy. Although the amount of surgical blood loss was significantly increased with bursectomy, overall we concluded that this procedure is safe and acceptable.

The safety of surgical treatments strongly depends on the surgeon's experience. Specific training is required to perform any surgical procedure, particularly when it is done for cancer treatment. There have been clinical trials studying the extent of lymph node dissection during gastric surgery. Two European randomized trials comparing D1 with D2 lymphadenectomy concluded that D2 was not acceptable as a standard treatment because D2 was associated with higher morbidity and mortality than D1 [8, 9]. On the other hand, two randomized trials comparing D1 with D2 and D2 with D3 lymphadenectomy in eastern Asia demonstrated that both D2 and D3 gastrectomy could be performed with low operative risk [13, 14]. This finding can be explained by the high volume of gastric cancer patients treated at that hospital and the high prevalence of gastric cancer in eastern Asia. In this study, all the patients were enrolled from an institution in which more than 50 gastrectomies were performed each year. In our trial the surgical procedures being performed by experienced surgeons accounted for the low mortality rates (0.95%) and low morbidity rates (14.3%).

Among various adverse events after surgery, we were concerned about the increased incidence of pancreatic fistulas after bursectomy because bursectomy requires resection of the capsule covering the pancreas [15]. However, we did not observe a significant increase in the incidence of pancreatic fistulas or inappropriate amylase levels in the postoperative drainage fluid, a surrogate marker of a pancreas fistula. This suggests that a pancreatic fistula is not caused by removal of a pancreatic capsule but may be caused by lymph node dissection adjacent to the pancreas parenchyma.

The next concern included the possibility of adhesion formation. Intestinal obstruction is the representative symptom of adhesion. In this study, two bursectomy patients and one nonbursectomy patient suffered from postoperative bowel obstruction, but there was no significant difference between the two groups. As 3 months' observation after surgery was not enough to evaluate the incidence of intestinal obstruction, a longer observation is necessary to draw a conclusion. Adhesion to the mesocolon and pancreas may cause specific local symptoms, such as delayed gastric emptying or afferent loop syndrome. It is of note that both delayed gastric emptying (two patients) and afferent loop syndrome (one patient) were observed only in the bursectomy group. Although this also did not reach statistical significance, careful observation is required in a larger cohort study.

In general, omentectomy and bursectomy are simultaneously performed for the same purpose, but their clinical pictures are somehow different. As the great omentum has numerous milky spots, which absorb ascites and actively incorporate cancer cells, peritoneal metastasis is frequently observed [16]. On the other hand, bursa omentalis, which is a semi-closed cavity, allows exfoliated cancer cells to remain. As for the surgical aspects, omentectomy is not difficult and does not increase the operating time or the blood loss. In contrast, the bursectomy technique is complicated and increases the operating time and bleeding. Considering the balance between the risk and benefit of each surgical procedure, we performed an omentectomy for all patients and randomly assigned each case to either with or without bursectomy. If we cannot find a benefit of bursectomy in this trial, we should elucidate the significance of omentectomy in the next step.

## Conclusions

This study showed that experienced surgeons could safely perform a D2 gastrectomy with bursectomy. Although bursectomy resulted in more blood loss, the major operative complications and hospital deaths were not increased. Regarding the survival benefit of this procedure, we must

wait for the results of the final analysis when the data have matured sufficiently.

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**Conflict of interest** The authors declare no conflicts of interest.

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# Effect of S-1 Adjuvant Chemotherapy on Survival following Recurrence and Efficacy of First-Line Treatment in Recurrent Gastric Cancer

Hiroko Hasegawa[a] Kazumasa Fujitani[b] Yukinori Kurokawa[b] Motohiro Hirao[b]
Shoichi Nakazuru[a] Eiji Mita[a] Toshimasa Tsujinaka[b]

Departments of [a]Gastroenterology and [b]Surgery, National Osaka Medical Center, Osaka, Japan

**Key Words**
Adjuvant chemotherapy · S-1 · Recurrent gastric cancer · Overall survival · Efficacy of chemotherapy

**Abstract**
***Background:*** As S-1 monotherapy has recently become the standard adjuvant regimen for stage II-III gastric cancer patients after curative gastrectomy in Japan, the question whether adjuvant S-1 affects the subsequent clinical course of relapsed patients has attracted great concern. ***Patients and Methods:*** We retrospectively evaluated the effect of adjuvant S-1 on survival following recurrence and efficacy of first-line treatment in patients with recurrent gastric cancer after curative gastrectomy. A total of 89 patients were evaluated. Thirty patients received adjuvant S-1 (cohort A), 10 patients were given adjuvant chemotherapy with other oral 5-FU agents (cohort B) and 49 patients received no adjuvant chemotherapy (cohort C). ***Results:*** Median survival time following recurrence was 287 days in cohort A, 451 days in B and 547 days in C, with a significant difference between A and C ($p = 0.0034$). Response rates of the first-line chemotherapy after recurrence were 6.7, 30.0 and 42.9% in cohorts A, B and C, respectively, with a significant difference between A and C ($p = 0.0007$). On multivariate analysis, S-1 adjuvant chemotherapy was independently associated with poor prognosis after recurrence (hazard ratio 2.64). ***Conclusion:*** S-1 adjuvant chemotherapy significantly reduced survival and response to first-line chemotherapy following recurrence in patients with recurrent gastric cancer.

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## Introduction

Although several meta-analyses have suggested a survival benefit provided by adjuvant chemotherapy for gastric cancer [1–6], there have been only a few treatments with their efficacy established in large clinical trials. Postoperative radiotherapy with 5-FU plus leucovorin has become a standard adjuvant therapy in the US [7], while peri-operative triplet regimen with epirubicin, cisplatin and 5-FU is standard in the UK [8]. Recently in Japan, the ACTS-GC trial has verified the efficacy of S-1 adjuvant chemotherapy after curative gastrectomy for stage II-III disease [9]. However, around 30% of patients still develop recurrence afterwards despite adjuvant S-1. As the number of patients relapsing after S-1 adjuvant chemotherapy increases, it becomes of great concern whether adjuvant S-1 affects the subsequent clinical behavior of the recurrent disease.

This retrospective study was conducted to evaluate the effect of S-1 adjuvant chemotherapy, in comparison with other 5-FU agents or no adjuvant chemotherapy, on sur-

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Fax +41 61 306 12 34
E-Mail karger@karger.ch
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Kazumasa Fujitani, MD
Department of Surgery, National Osaka Medical Center
2-1-14 Hoenzaka
Chuo-ku, Osaka 540-0006 (Japan)
Tel. +81 6 6942 1331, Fax +81 6 6943 6467, E-Mail fujitani@onh.go.jp

vival following recurrence and efficacy of first-line chemotherapy given at the time of relapse in patients with recurrent gastric cancer after curative gastrectomy.

## Patients and Methods

*Patients*

A total of 95 patients with recurrent gastric cancer after curative gastrectomy were found at our institution between April 1999 and October 2008. Among them, 89 patients enrolled in this retrospective study fulfilled the following criteria: (1) histologically proven recurrent gastric adenocarcinoma; (2) stage II, III or IV primary disease without any distant metastasis in accordance with the guidelines of the Japanese Gastric Cancer Association [10]; (3) either adjuvant chemotherapy with S-1 or other oral 5-FU agents (UFT or 5'-FUDR) lasting more than 4 weeks or no adjuvant treatment; (4) performance status of 2 or less on the Eastern Cooperative Oncology Group scale; (5) adequate bone marrow function (white blood cell count 4,000–12,000/mm$^3$, platelet count ≥100,000/mm$^3$ and hemoglobin ≥8.0 g/dl), hepatic function (total bilirubin ≤1.5 mg/dl, serum transaminases ≤100 μ/l) and renal function (serum creatinine ≤ the upper institutional limit); (6) no other severe medical conditions; (7) no other concurrent active malignancy.

*Overall Survival, Efficacy of First-Line Chemotherapy and Statistics*

Overall survival (OS) after recurrence was defined as the time from the date of recurrence to the date of death from any cause or the last follow-up. OS was calculated using the Kaplan-Meier method and compared with the log-rank test. Univariate and multivariate analyses were performed by the Cox proportional hazards model to identify variables independently associated with poor prognosis after recurrence.

During the first-line chemotherapy after recurrence, each patient with a measurable lesion was assessed for an objective response to treatment according to the Response Evaluation Criteria in Solid Tumors [11] with computed tomography scans performed every 2 to 3 months until disease progression. Disease control rate (DCR) represented the percentage of patients with complete response, partial response or stable disease (SD). Patients only with nonmeasurable lesions were evaluated as stable disease if neither complete disappearance (complete response) nor obvious progression of the recurrent disease were observed on computed tomography scans.

Differences in proportion were evaluated with the $\chi^2$ test and the differential significance of age was estimated by the Kruskal-Wallis test. Statistical results were considered to be significant with a p value of less than 0.05.

## Results

*Patient Characteristics*

Eighty-nine patients were categorized into the 3 cohorts shown in table 1. Thirty patients in cohort A, 18 males and 12 females with a median age of 62.5 years (range: 32–83), received S-1 adjuvant chemotherapy. S-1 was given orally using a standard dose and schedule (80 mg/m$^2$/day, for 28 consecutive days followed by a 14-day rest, repeated for 1 year) [9]. Nine patients completed the planned 1-year administration of adjuvant S-1, while 11 patients discontinued the treatment within the first 6 months and 10 patients in the second 6 months after the initiation of S-1 adjuvant chemotherapy. The reasons for treatment withdrawal were treatment toxicity in 1, and recurrent disease in 20 patients. The median duration of adjuvant S-1 administration was 211 days. In cohort B, 10 patients, 6 males and 4 females with a median age of 63.0 years (range: 35–78), were given adjuvant chemotherapy with oral 5-FU agents other than S-1. UFT (a combination of uracil and tegafur at a molar ratio of 4:1) was administered at a dose of 400 mg/body/day in 6 patients and 5'-DFUR (5'-deoxy-5-fluorouridine) at a dose of 800 mg/body/day in 4 patients. Two patients completed the planned 1-year administration of adjuvant UFT/5'-DFUR, while 3 patients discontinued the treatment within the first 6 months and 5 patients in the second 6 months after the initiation of adjuvant chemotherapy. The rea-

**Table 1.** Patient characteristics

| | Cohort A S-1 adjuvant | Cohort B oral 5-FU | Cohort C no adjuvant | p value |
|---|---|---|---|---|
| Patients | 30 | 10 | 49 | |
| Gender | | | | 0.5254 |
| Male | 18 | 6 | 35 | |
| Female | 12 | 4 | 14 | |
| Age, years | | | | 0.8537 |
| Median | 62.5 | 63 | 59 | |
| Range | 32–83 | 35–78 | 42–84 | |
| Histology (Lauren's) | | | | 0.841 |
| Intestinal | 9 | 4 | 17 | |
| Diffuse | 21 | 6 | 32 | |
| Stage | | | | 0.0053 |
| II | 2 | 4 | 11 | |
| III | 13 | 5 | 32 | |
| IV | 15 | 1 | 6 | |
| Measurable lesions | | | | 0.6584 |
| Present | 19 | 6 | 26 | |
| Absent | 11 | 4 | 23 | |
| Metastatic sites | | | | 0.2531 |
| 1 | 25 | 10 | 45 | |
| ≥2 | 5 | 0 | 4 | |
| DFI | | | | 0.105 |
| <1 year | 19 | 5 | 19 | |
| ≥1 year | 11 | 5 | 30 | |

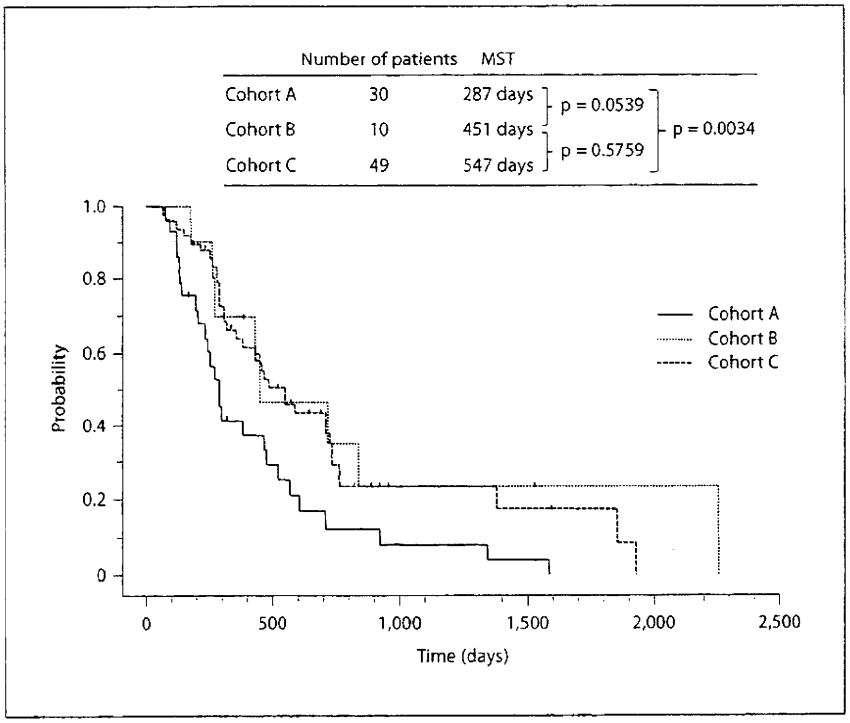


**Fig. 1.** OS after recurrence.

sons for treatment withdrawal were patient refusal in 1 and recurrent disease in 7 patients. The median duration of adjuvant 5-FU agent administration was 180 days. Forty-nine patients in cohort C, 35 males and 14 females with a median age of 59.0 years (range: 42–84), received no adjuvant chemotherapy. Histologically, around one third of patients had intestinal-type adenocarcinoma and two thirds had diffuse-type adenocarcinoma in each cohort. As for the initial stage of the primary tumor after curative gastrectomy, stage IV disease was significantly more frequent in cohort A than in the other cohorts ($p = 0.0053$). A measurable recurrent lesion was seen in 50–60% of each cohort and multiple metastatic sites were present in 10% of all patients. The disease-free interval (DFI), which was defined as the time from the date of surgery to the date of recurrence, was less than 1 year in approximately 40–60% of patients in either cohort.

*Overall Survival*

OS after recurrence was compared among the three cohorts. After a median follow-up time of 380 days from the date of recurrence (319 days in 71 dead patients and 560 days in 18 alive patents), the median survival time (MST) was 287 days in cohort A, 451 days in B and 547 days in C. OS was significantly shorter in cohort A than in cohort C ($p = 0.0034$), while there was no significant difference between cohorts B and A or C, as shown in figure 1. In cohort A, the duration of S-1 adjuvant chemotherapy was <6 months in 11 patients, 6 to <12 months in 10 and 12 months in 9. No significant difference in OS (MST, 246 vs. 287 vs. 464 days; $p = 0.4963$) was observed according to the duration of S-1 adjuvant chemotherapy, as shown in figure 2.

*Efficacy of First-Line Chemotherapy*

Regimens of the first-line chemotherapy delivered after recurrence are shown in table 2. Nine patients (30.0%) in cohort A received S-1-based therapy (S-1 monotherapy [12, 13] in 3, S-1 plus cisplatin [14] in 3, S-1 plus irinotecan [15] in 3, S-1 plus paclitaxel [16] in 0), although 9 patients were treated with paclitaxel monotherapy administered in a weekly fashion [17] and 12 patients with irinotecan-based therapy (irinotecan monotherapy [18] in 5, irinotecan plus cisplatin [19] in 7). In cohort B, 5 patients (50.0%) received S-1-based therapy, with 4 patients being treated with paclitaxel monotherapy and 1 patient with irinotecan plus cisplatin. In cohort C, 42 patients (85.7%) received S-1-based therapy, 4 patients were given paclitaxel monotherapy and 3 patients were given irinotecan plus cisplatin. It seemed inevitable for various regimens to

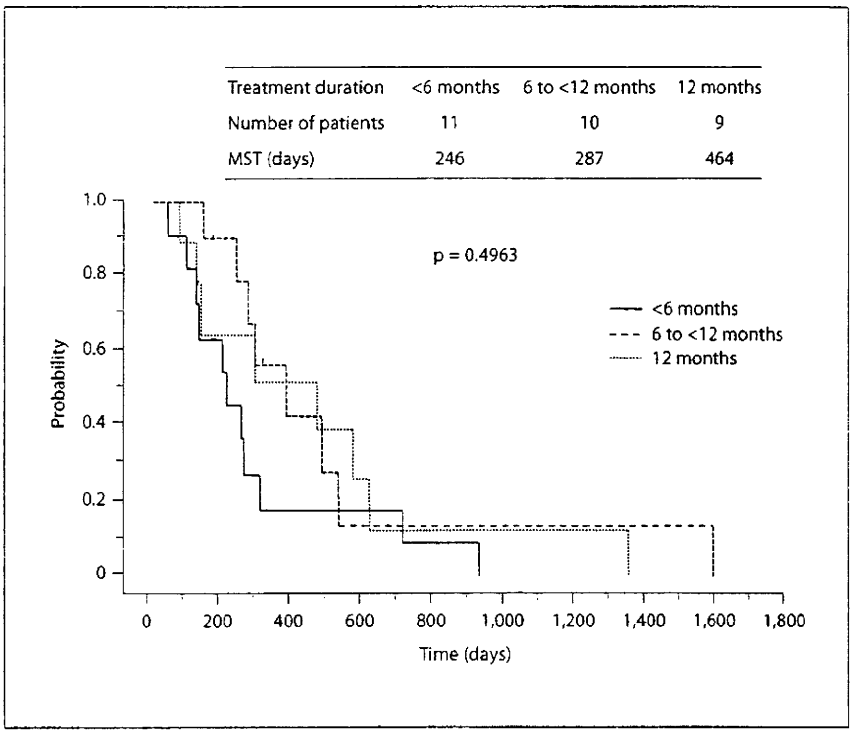


**Fig. 2.** OS according to the duration of S-1 adjuvant chemotherapy.

**Table 2.** Regimens of first-line chemotherapy after recurrence

| | Cohort A (n = 30 ) | Cohort B (n = 10) | Cohort C (n = 49) |
|---|---|---|---|
| S-1-based therapy | 9 | 5 | 42 |
| S-1 monotherapy | 3 | 3 | 26 |
| S-1 + cisplatin | 3 | 0 | 4 |
| S-1 + irinotecan | 3 | 1 | 7 |
| S-1 + paclitaxel | 0 | 1 | 5 |
| Weekly paclitaxel | 9 | 4 | 4 |
| Irinotecan-based therapy | 12 | 1 | 3 |
| Irinotecan monotherapy | 5 | 0 | 0 |
| Irinotecan + cisplatin | 7 | 1 | 3 |

have been given as the first-line treatment because it was obscure whether non-S-1-based therapy was more appropriate for patients relapsed after adjuvant S-1 or which should be chosen as a non-S-1 agent between paclitaxel and irinotecan for patients with recurrent gastric cancer. However, there was a tendency to prefer choosing S-1-based therapy as the first-line treatment after recurrence in cohort C, while non-5-FU regimens were more likely to be chosen in cohorts A and B. The best response to the first-line chemotherapy after recurrence was compared among these 3 cohorts, as shown in table 3. Response rates (RR) were 6.7% [95% confidence interval (CI) 0.8–22.1], 30.0% (95% CI 6.7–65.3), and 42.9% (95% CI 28.8–57.8) in cohorts A, B and C, respectively, with a significant difference between A and C (p = 0.0007). DCR were 50.0% (95% CI 31.3–68.7), 80.0% (95% CI 44.4–97.5) and 89.8% (95% CI 77.8–96.6) in cohorts A, B and C, respectively, with a significant difference between A and C (p = 0.0001).

*Prognostic Factors for OS*

The results of univariate and multivariate analyses of various factors, such as gender, age, histology, initial stage and presence of measurable lesion, number of metastatic sites, DFI and type of adjuvant chemotherapy for OS following recurrence are summarized in table 4. Among these, absence of a measurable lesion [hazard ratio 2.18 (95% CI 1.28–3.72)], presence of multiple metastatic sites [hazard ratio 2.89 (95% CI 1.28–6.52)] and S-1 adjuvant chemotherapy [hazard ratio 2.64 (95% CI 1.35–4.75)] were identified as significant independent factors for poor prognosis after recurrence.

**Table 3.** Efficacy of first-line chemotherapy

| | CR | PR | SD | PD | Total | RR, % | DCR, % |
|---|---|---|---|---|---|---|---|
| Cohort A | 0 | 2 | 13 | 15 | 30 | 6.7 (0.8–22.1) | 50 (31.3–68.7) |
| Cohort B | 2 | 1 | 5 | 2 | 10 | 30 (6.7–65.3) | 80 (44.4–97.5) |
| Cohort C | 4 | 17 | 23 | 5 | 49 | 42.9 (28.8–57.8) | 89.8 (77.8–96.6) |

Figures in parentheses are 95% CI.

**Table 4.** Prognostic factors for OS

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | patients | MST, days | p | hazard ratio | 95% CI | p |
| Gender | | | | | | |
| Male | 59 | 466 | 0.4212 | | | 0.791 |
| Female | 30 | 303 | | 1.082 | 0.606–1.930 | |
| Age, median 61 | | | | | | |
| ≥61 | 46 | 455 | 0.5071 | | | 0.91 |
| <61 | 43 | 446 | | 0.969 | 0.559–1.680 | |
| Histology | | | | | | |
| Intestinal | 30 | 455 | 0.5388 | | | 0.948 |
| Diffuse | 59 | 446 | | 0.981 | 0.556–1.733 | |
| Stage | | | | | | |
| II | 17 | 304 | 0.4825 | 1.115 | 0.444–2.800 | 0.744 |
| III | 50 | 455 | | 1.274 | 0.667–2.432 | |
| IV | 22 | 479 | | | | |
| Measurable lesion | | | | | | |
| Present | 51 | 547 | 0.0243 | | | 0.004 |
| Absent | 38 | 285 | | 2.181 | 1.279–3.720 | |
| Metastatic sites | | | | | | |
| 1 | 80 | 455 | 0.0154 | | | 0.011 |
| ≥2 | 9 | 268 | | 2.89 | 1.281–6.524 | |
| DFI | | | | | | |
| <1 year | 43 | 351 | 0.365 | 1.349 | 0.786–2.315 | 0.277 |
| ≥1 year | 46 | 521 | | | | |
| Adjuvant chemotherapy | | | | | | |
| None | 49 | 547 | 0.0061 | | | 0.008 |
| S-1 | 30 | 287 | | 2.635 | 1.346–4.747 | |
| Oral 5-FU | 10 | 451 | | 0.98 | 0.422–2.274 | |

## Discussion

Although adjuvant chemotherapy with S-1 has recently become the standard treatment for stage II-III gastric cancer patients after curative gastrectomy in Japan based on the result of the ACTS-GC trial [9], nearly 30% of patients still relapse, despite the adjuvant S-1 treatment. Since the total number of patients with recurrence after adjuvant S-1 is increasing, it is of great concern to discern whether adjuvant S-1 affects the subsequent clinical course of the patients after recurrence. We, therefore, retrospectively evaluated the effect of adjuvant S-1 on survival following recurrence and the efficacy of first-line chemotherapy given at the time of relapse in patients with recurrent gastric cancer.

As shown in figure 1, patients initially treated with adjuvant S-1 had shorter survival following the recurrence than those receiving no adjuvant treatment (MST 287 vs.

547 days, p = 0.0034). Similarly, adjuvant chemotherapy was reported to have a negative impact on outcome after recurrence in other types of cancer such as colon and breast [20, 21]. As for the results of subset analysis of cohort A shown in figure 2, there may be some controversies. MST of the patients who relapsed after completion of 12 months of S-1 adjuvant chemotherapy was 464 days, equivalent to that of 451 days in cohort B. Although the duration of S-1 adjuvant chemotherapy showed no effect on OS after recurrence, this lack of statistical difference between the subgroups might be due to the small sample size. However, at least, the patients who discontinued S-1 adjuvant chemotherapy within 12 months because of recurrence were very unlikely to be salvaged by the additional chemotherapy given at the time of relapse. Although there was an imbalance of initial stage of the primary tumor between cohorts A and C, as shown in table 1, MSTs at stage II-III and IV in cohort A were 237 and 479 days, respectively, while they were 588 and 290 days in cohort C, respectively, with no significant difference between stage II-III and IV. Furthermore, on multivariate analysis in table 4, S-1 adjuvant chemotherapy but not initial stage was confirmed as an independent prognostic factor for OS after recurrence. Absence of a measurable lesion and presence of multiple metastatic sites also significantly correlated with inferior survival on multivariate analysis. MSTs of patients whose metastatic lesions involved the peritoneum (n = 36), bone/skin (n = 6), lymph nodes (n = 34) and liver (n = 19) were 285, 209, 609 and 426 days, respectively. Prior receipt of S-1 adjuvant chemotherapy as well as absence of a measurable lesion and presence of multiple metastatic sites contributed to the poor prognosis following tumor recurrence. These prognostic factors identified in this study might become useful factors of stratification for future clinical trial design in patients with recurrent gastric cancer.

With respect to the efficacy of first-line chemotherapy given at the time of relapse, patients who had received S-1 adjuvant chemotherapy showed a significantly lower RR than those receiving no adjuvant treatment: 6.7 versus 42.9% (p = 0.0007) as shown in table 3. Likewise, in patients with recurrent breast cancer, adjuvant chemotherapy was demonstrated to be a significant factor in predicting a poor response to first-line chemotherapy after recurrence [21]. As for the choice of first-line regimen given at the time of relapse, about two thirds of patients in cohort A received non-S-1-based therapy after adjuvant S-1. Although 1 retrospective study reported the invalidity of S-1-based chemotherapy as first-line treatment for recurrent disease after adjuvant S-1 in terms of a significantly lower RR, DCR as well as shorter progression-free survival compared to non-S-1-based chemotherapy [22], it still remains a problem to be clarified prospectively whether patients failing S-1 adjuvant chemotherapy should subsequently be treated with non-S-1-based regimens. In fact, in cohort A, patients treated with non-S-1-based chemotherapy showed an MST of 287 days with RR of 9.5% and DCR of 52.4%, while those with S-1-based regimens demonstrated an MST of 268 days with RR of 0% and DCR of 33.3%, with no significant difference among them. These findings suggest that patients who recurred following S-1 adjuvant chemotherapy must have extremely aggressive tumors refractory to any kind of further chemotherapy.

The poor outcome following relapse in patients who had received adjuvant S-1 might be speculatively interpreted as follows. While noncurative adjuvant chemotherapy might eradicate sensitive tumor cells, adjuvant S-1 could screen and select biologically more aggressive cellular clones with intrinsic resistance to cytotoxic agents that progress more quickly once recurrence is identified, or could induce acquired cellular resistance to further chemotherapy, like anthracyclines which induce the development of multidrug resistance [23]. In either case, the tumor mass would be constituted mainly of resistant cells at the time of relapse or, as a consequence, a poor response to first-line chemotherapy and a shorter OS would be expected following recurrence. In a recent report [24], adjuvant S-1, compared to surgery alone, was shown to deteriorate recurrence-free survival as well as OS after curative gastrectomy when confined to patients with high intratumoral mRNA expression of thymidylate synthase (TS). Although high TS expression is well correlated with resistance to 5-FU [25] derived from S-1, these findings suggest that biologically more aggressive cancer cells could be induced by the S-1 administration in a tumor with high TS expression.

Irrespective of types of regimens, adjuvant chemotherapy was reported to be significantly associated with a low probability of response to first-line chemotherapy and shorter survival following recurrence in patients with recurrent breast cancer [21]. However, in the present study, adjuvant treatment with 5-FU agents other than S-1 showed modest effects on OS and RR compared to adjuvant S-1, though adjuvant S-1 adversely affected OS and RR in recurrent gastric cancer patients, as shown in figure 1 and table 3. It is not clear whether this difference in adverse effect between S-1 and other 5-FU agents depends on the ability in inducing chemoresistant cells of the respective agent.