the remaining patients underwent distal gastrectomy. Splenectomy was performed in 191 patients (36·5 per cent) and distal pancreatectomy in 22 (4·2 per cent). There was no significant difference in the type of gastrectomy and incidence of combined resection between the two groups. Details of patient demographics and tumour stages have been reported previously[13].

There were four hospital deaths (0·8 per cent), two in each group. Two patients suffered from rapid disease progression and died 3 and 5 months after surgery without being discharged from hospital. One patient died from pneumonia at 46 days and another died from massive bleeding from the gastroduodenal artery 24 days after operation. Complications were identified in 128 patients (24·5 per cent) and major surgical complications in 49 patients (9·4 per cent) (*Table 1*).

The results of univariate analyses of risk factors for overall postoperative complications are summarized in *Table 2*. Only pancreatic resection ($P = 0·001$) and

Table 2 Univariate and multivariate analysis of risk factors for overall complications

		No. with	Univariate analysis		Multivariate analysis	
	n	complications	Relative risk	*P*	Relative risk	*P*
Extent of lymphadenectomy						
D2	263	55	1		1	
D3	260	73	1·48 (0·99, 2·21)	0·057	0·93 (0·58, 1·51)	0·776
Splenectomy						
No	332	64	1		1	
Yes	191	64	2·11 (1·41, 3·17)	< 0·001	2·05 (0·52, 8·01)	0·304
Pancreatectomy						
No	501	115	1		1	
Yes	22	13	4·85 (2·02, 11·63)	< 0·001	5·62 (1·94, 16·27)	0·001
Extent of gastrectomy						
Distal	320	62	1		1	
Total or proximal	203	66	2·01 (1·34, 3·00)	< 0·001	0·84 (0·22, 3·27)	0·804
Invasion to adjacent organs						
T2, T3	501	123	1		1	
T4	22	5	0·90 (0·33, 2·50)	0·846	0·37 (0·11, 1·24)	0·107
Sex						
M	358	94	1		1	
F	165	34	0·73 (0·47, 1·14)	0·163	0·73 (0·45, 1·19)	0·207
Age (years)						
< 56	160	33	1		1	
56–65	207	48	1·16 (0·70, 1·92)	0·557	1·26 (0·73, 2·17)	0·403
> 65	156	47	1·66 (0·99, 2·77)	0·053	1·63 (0·92, 2·89)	0·092
Body mass index						
< 25	446	101	1		1	
≥ 25	77	27	1·85 (1·10, 3·10)	0·019	1·75 (0·99, 3·08)	0·054
Operating time (min)						
< 240	167	23	1		1	
240–297	179	43	1·98 (1·13, 3·46)	0·016	1·77 (0·96, 3·25)	0·068
> 297	177	62	3·38 (1·97, 5·78)	< 0·001	2·65 (1·34, 5·23)	0·005
Blood loss (ml)						
< 395	174	27	1		1	
395–710	174	42	1·73 (1·01, 2·97)	0·045	1·05 (0·58, 1·90)	0·886
> 710	175	59	2·77 (1·65, 4·64)	< 0·001	1·11 (0·58, 2·12)	0·754
Blood transfusion						
Yes	408	87	1		1	
No	115	41	2·04 (1·31, 3·20)	0·002	1·53 (0·92, 2·56)	0·102
Case volume*						
< 20	147	41	1		1	
≥ 20	376	87	0·78 (0·51, 1·20)	0·256	0·83 (0·51, 1·34)	0·437
Period						
1995–1998	295	75	1		1	
1999–2001	228	53	0·9 (0·59, 1·33)	0·566	0·87 (0·56, 1·35)	0·539

Values in parentheses are 95 per cent confidence intervals. *No. of patients registered.

Table 3 Univariate and multivariate analysis of risk factors for major surgical complications

	n	No. with major complications	Univariate analysis		Multivariate analysis	
			Relative risk	*P*	Relative risk	*P*
Extent of lymphadenectomy						
D2	263	23	1		1	
D3	260	26	1·16 (0·64, 2·09)	0·623	0·67 (0·32, 1·39)	0·279
Splenectomy						
No	332	20	1		1	
Yes	191	29	2·79 (1·53, 5·09)	<0·001	1·08 (0·15, 7·56)	0·941
Pancreatectomy						
No	501	43	1		1	
Yes	22	6	3·99 (1·49, 10·74)	0·003	6·90 (1·86, 25·58)	0·004
Extent of gastrectomy						
Distal	320	19	1		1	
Total or proximal	203	30	2·74 (1·50, 5·03)	<0·001	2·15 (0·31, 15·20)	0·442
Invasion to adjacent organs						
T2, T3	501	47	1		1	
T4	22	2	0·97 (0·22, 4·26)	0·964	0·37 (0·067, 2·01)	0·246
Sex						
M	358	38	1		1	
F	165	11	0·60 (0·30, 1·21)	0·150	0·57 (0·25, 1·27)	0·169
Age (years)						
<56	160	7	1		1	
56–65	207	20	2·34 (0·96, 5·67)	0·061	3·06 (1·15, 8·20)	0·026
>65	156	22	3·59 (1·49, 8·66)	0·005	4·04 (1·48, 11·02)	0·006
Body mass index						
<25	446	34	1		1	
≥25	77	15	2·93 (1·51, 5·69)	0·001	3·32 (1·54, 7·12)	0·002
Operating time (min)						
<240	167	8	1		1	
240–297	179	14	1·69 (0·69, 4·13)	0·252	1·60 (0·60, 4·27)	0·350
>297	177	27	3·58 (1·58, 8·12)	0·002	2·96 (1·03, 8·55)	0·045
Blood loss (ml)						
<395	174	10	1		1	
395–710	174	11	1·11 (0·46, 2·68)	0·822	0·47 (0·17, 1·30)	0·145
>710	175	28	3·12 (1·47, 6·65)	0·003	0·86 (0·32, 2·31)	0·767
Blood transfusion						
Yes	408	29	1		1	
No	115	20	2·75 (1·49, 5·08)	<0·001	1·99 (0·97, 4·08)	0·061
Case volume*						
<20	147	16	1		1	
≥20	376	33	0·79 (0·42, 1·48)	0·457	0·76 (0·36, 1·57)	0·454
Period						
1995–1998	295	30	1		1	
1999–2001	228	19	0·80 (0·44, 1·47)	0·475	0·83 (0·43, 1·61)	0·575

Values in parentheses are 95 per cent confidence intervals. *No. of patients registered.

prolonged operating time (patients in the upper tertile for whom the operating time was more than 297 min; $P = 0·005$) were identified as significant independent risk factors for overall complications (*Table 2*). A BMI of 25 or more was close to significance ($P = 0·054$).

The results of univariate analyses of risk factors for major surgical complications are summarized in *Table 3*. Multivariate analysis identified BMI ($P = 0·002$), pancreatic resection ($P = 0·004$), age (56–65 years, $P = 0·026$; over 65 years, $P = 0·006$) and operating time over 297 min ($P = 0·045$) as significant independent risk factors for major surgical complications (*Table 3*).

Discussion

Gastrectomy plus extended systemic lymphadenectomy (D2 resection) is the standard procedure for gastric carcinoma in Japan. This approach has resulted in superior stage-by-stage survival than that observed in most Western countries and has led to cure for a

proportion of patients with nodal disease beyond the perigastric region, although this has not been confirmed in Western randomized trials[15,16]. Although long-term follow-up revealed significantly better disease-free survival for the D2 group in the subset with node-positive cancer[17], this difference did not extend to all patients in the trial, in part owing to the unacceptably high mortality rate associated with D2 resection[8]. JCOG 9501, a Japanese multi-institutional prospective randomized trial comparing D2 with more extended resection, has superior quality control of surgical procedures and reliability of data[13] than retrospective Japanese studies and Western prospective trials.

The most significant risk factor for both surgical and overall complications in the present study was pancreatic resection, although it should be noted that this was performed in only 4·2 per cent of patients, compared with 30·3 and 15·2 per cent in the UK Medical Research Council (MRC) and Dutch trials respectively[15,16]. The rate of pancreatectomy was lower in the present series because a pancreas-preserving technique[18,19] was generally used, whereas distal pancreatectomy and splenectomy were integral parts of D2 dissection in the Dutch trial unless cancer was located in the distal stomach. The low morbidity rate in the present study may well be related to pancreas preservation[18,19]. The success of this approach has also been reported in a multicentre phase II trial of D2 dissection in Northern Italy[20].

Splenectomy, on the other hand, was not an independent determinant of risk, possibly because it was never performed with distal gastrectomy in the present series. In the Dutch randomized trial a high mortality rate after distal gastrectomy was attributed in part to necrosis of the remnant stomach as a result of splenectomy and division of the short gastric arteries[21]. The survival benefit of splenectomy performed solely to facilitate dissection of lymph nodes close to the splenic hilum has been questioned, however, and a randomized trial to explore this issue is ongoing[22].

Age was not an independent risk factor for overall complications in this study, in contrast to the Dutch trial in which age over 65 years was a significant risk factor for hospital death and overall complications[21]. This discrepancy may be attributed to the fact that only patients aged 75 years or less were eligible for inclusion in the JCOG 9501[13], whereas other trials have included older patients[15,16]. Japanese patients were, on average, 8 years younger than Dutch patients[23]; consequently the proportion of patients over 65 years of age was 29·8 per cent in the present series as opposed to 51·3 per cent in the Dutch trial[16]. This age distribution may account for the very low incidence of perioperative cardiovascular events in the present series, another factor that may have influenced the low morbidity and mortality rates.

Extended lymph node dissection may be hampered by excess bodyweight[24–26] and in the present study BMI was a significant risk factor for major surgical complications. Caucasians in general have a higher BMI than Japanese and the incidence of morbid obesity is significant among patients in the USA and Europe. Only 14·7 per cent of the present patients had a BMI of 25 kg/m^2 or greater, whereas one-third of the US population is obese (BMI over 27 kg/m^2)[27]. These data suggest that the patients' physique favours Japanese patients when major gastric cancer surgery is performed.

The extent of lymph node dissection (D2 *versus* D3), surgical volume and the period in which the operation was performed had no impact, suggesting that there were no learning curve issues. Although D2 resection has long been a standard procedure in Japan, all surgeons in the trial were experts from specialized centres who had sufficient experience with D3 resection through numerous other studies. Of the variables reflecting difficulties encountered during surgery, prolonged operating time was identified as a significant independent risk factor for both overall and major surgical complications. However, amount of blood loss and blood transfusion were significant only in univariate analysis; this may be attributable to multicolinearity, as these two factors are closely related.

Gastrectomy with extended lymphadenectomy is feasible and safe in Japan, provided that older patients with co-morbidity are excluded and pancreatectomy is reserved for lesions with direct invasion to the pancreas. Obese patients should be treated with caution, however, as they have a significant risk of developing major surgical complications. Hopefully, with careful patient selection, appropriate surgical expertise and pancreas and spleen preservation[8] where possible, equally good results, rarely achieved previously[20,28], will be realized in the West.

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References

1 Ekstrom AM, Hansson LE, Signorello LB, Lindgren A, Bergstrom R, Nyren O. Decreasing incidence of both major histologic subtypes of gastric carcinoma – a population-based study in Sweden. *Br J Cancer* 2000; **83**: 391–396.

2 Hundahl SA, Menck HR, Mansour EG, Winchester DP. The National Cancer Data Base report on gastric carcinoma. *Cancer* 1997; **80**: 2333–2341.

3 Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer* 1999; **83**: 18–29.

4 Vanhoefer U, Rougier P, Wilke H, Ducreux MP, Lacave AJ, Van Cutsem E *et al.* Final results of a randomized phase III trial of sequential high-dose methotrexate, fluorouracil, and doxorubicin *versus* etoposide, leucovorin, and fluorouracil *versus* infusional fluorouracil and cisplatin in advanced gastric cancer: a trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group. *J Clin Oncol* 2000; **18**: 2648–2657.

5 Ohtsu A, Shimada Y, Shirao K, Boku N, Hyodo I, Saito H *et al.* Randomized phase III trial of fluorouracil alone *versus* fluorouracil plus cisplatin *versus* uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: the Japan Clinical Oncology Group Study (JCOG9205). *J Clin Oncol* 2003; **21**: 54–59.

6 McNeer G, Lawrence W Jr, Ortega LG, Sunderland DA. Early results of extended total gastrectomy for cancer. *Cancer* 1956; **9**: 1153–1159.

7 Jinnai D. Evaluation of extended radical operation for gastric cancer, with regard to lymph node metastasis and follow-up results. *Jpn J Cancer Res* 1968; **3**: 225–231.

8 Kodera Y, Schwarz RE, Nakao A. Extended lymph node dissection in gastric carcinoma: where do we stand after the Dutch and British randomized trials? *J Am Coll Surg* 2002; **195**: 855–864.

9 Baba M, Hokita S, Natsugoe S, Miyazono T, Shimada M, Nakano S *et al.* Paraaortic lymphadenectomy in patients with advanced carcinoma of the upper-third of the stomach. *Hepatogastroenterology* 2000; **47**: 893–896.

10 Kunisaki C, Shimada H, Yamaoka H, Takahashi M, Ookubo K, Akiyama H *et al.* Indications for paraaortic lymph node dissection in gastric cancer patients with paraaortic lymph node involvement. *Hepatogastroenterology* 2000; **47**: 586–589.

11 Isozaki H, Okajima K, Fujii K, Nomura E, Izumi M, Mabuchi H *et al.* Effectiveness of paraaortic lymph node dissection for advanced gastric cancer. *Hepatogastroenterology* 1999; **46**: 549–554.

12 Maeta M, Yamashiro H, Saito H, Katano K, Kondo A, Tsujitani S *et al.* A prospective pilot study of extended (D3) and superextended para-aortic lymphadenectomy (D4) in patients with T3 or T4 gastric cancer managed by total gastrectomy. *Surgery* 1999; **125**: 325–331.

13 Sano T, Sasako M, Yamamoto S, Nashimoto A, Kurita A, Hiratsuka M *et al.* Gastric cancer surgery: morbidity and mortality results from a prospective randomized controlled trial (JCOG9501) comparing D2 and extended para-aortic lymphadenectomy. Japan Clinical Oncology Group Study 9501. *J Clin Oncol* 2004; **22**: 2767–2773.

14 Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma – 2nd English Edition. *Gastric Cancer* 1998; **1**: 10–24.

15 Cuschieri A, Weeden S, Fielding J, Bancewicz J, Craven J, Joypaul V *et al.* Patient survival after D1 and D2 resections for gastric cancer: long-term results of the MRC randomized surgical trial. Surgical Co-operative Group. *Br J Cancer* 1999; **79**: 1522–1530.

16 Bonenkamp JJ, Hermans J, Sasako M, van de Velde CJH. Extended lymph-node dissection for gastric cancer. Dutch Gastric Cancer Group. *N Engl J Med* 1999; **340**: 908–914.

17 Hartgrink HH, van de Velde CJH, Putter H, Bonenkamp JJ, Klein-Kranenbarg E, Songun K *et al.* Extended lymph node dissection for gastric cancer: who may benefit? Final results of the randomized Dutch Gastric Cancer Group trial. *J Clin Oncol* 2004; **22**: 2069–2077.

18 Maruyama K, Sasako M, Kinoshita T, Sano T, Katai H, Okajima K. Pancreas-preserving total gastrectomy for proximal gastric cancer. *World J Surg* 1995; **19**: 532–536.

19 Furukawa H. Hiratsuka M, Ishikawa O, Ikeda M, Imamura H, Masutani S *et al.* Total gastrectomy with dissection of lymph nodes along the splenic artery: a pancreas-preserving method. *Ann Surg Oncol* 2000; **7**: 669–673.

20 Degiuli M, Sasako M, Ponti A, Soldati T, Danese F, Calvo F. Morbidity and mortality after D2 gastrectomy for gastric cancer: results of the Italian Gastric Cancer Study Group prospective multicenter surgical study. *J Clin Oncol* 1998; **16**: 1490–1493.
21 Sasako M for the Dutch Gastric Cancer Study Group. Risk factors for surgical treatment in the Dutch Gastric Cancer Trial. *Br J Surg* 1997; **84**: 1567–1571.
22 Sano T, Yamamoto S, Sasako M for the Gastric Cancer Surgical Study Group of Japan Clinical Oncology Group. Randomized controlled trial to evaluate splenectomy in total gastrectomy for proximal gastric carcinoma: Japan Clinical Oncology Group study JCOG 0110-MF. *Jpn J Clin Oncol* 2002; **32**: 363–364.
23 Bonenkamp JJ, van de Velde CJ, Kampschoer GH, Hermans J, Hermanek P, Bemelmans M *et al.* Comparison of factors influencing the prognosis of Japanese, German, and Dutch gastric cancer patients. *World J Surg* 1993; **71**: 410–415.
24 Kodera Y, Ito S, Yamamura Y, Mochizuki Y, Fujiwara M, Hibi K *et al.* Obesity and outcome of distal gastrectomy with D2 lymphadenectomy for carcinoma. *Hepatogastroenterology* 2004; **51**: 1225–1228.
25 Dhar DK, Kubota H, Tachibana M, Koto T, Tabara H, Masunaga R *et al.* Body mass index determines the success of lymph node dissection and predicts the outcome of gastric carcinoma patients. *Oncology* 2000; **59**: 18–23.
26 Inagawa S, Adachi S, Oda T, Kawamoto T, Koike N, Fukao K. Effect of fat volume on postoperative complications and survival rate after D2 dissection for gastric cancer. *Gastric Cancer* 2000; **3**: 141–144.
27 Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1963 to 1991. *JAMA* 1994; **272**: 205–211.
28 Sue-Ling HM, Johnston D, Martin IG, Dixon MF, Lansdown MR, McMahon MJ *et al.* Gastric cancer: a curable disease in Britain. *BMJ* 1993; **307**: 591–596.

Individualized Adjuvant Chemotherapy Guided by Chemosensitivity Test Sequential to Extended Surgery for Advanced Gastric Cancer

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Abstract. *Background and Objectives: Various adjuvant chemotherapy regimens have been proposed for patients with advanced gastric cancer; however, the majority of these trials failed to show a clear survival benefit over surgery alone. In this study, the feasibility and efficacy of a strategy of extended surgery combined with individualized adjuvant chemotherapy for advanced gastric cancer with serosal invasion and nodal involvement was examined. Patients and Methods: Sixty-four patients with advanced gastric cancer underwent gastrectomy with extended lymph node dissection. After surgery, a chemosensitivity test by MTT assay, using highly purified tumor cells, was performed, and the patients received individualized adjuvant chemotherapy on the basis of the results of this chemosensitivity test. Results: Overall survival in the chemosensitivity-guided chemotherapy (CSC) group was significantly better than the standard chemotherapy (SC) and the no-chemotherapy (NC) group ($p<0.05$). In patients with stage IV disease, the 5-year survival rate was 38.1% in the CSC group and 0% in the SC + NC group, respectively, with a significant difference being observed in the two survival curves ($p<0.01$). In patients with paraaortic node involvement, survival in the CSC group was significantly better than that in the SC + NC group ($p<0.01$). On the other hand, in patients without paraaortic node involvement, no survival difference was observed between the two groups. Conclusion: The strategy of extended surgery combined with individualized adjuvant chemotherapy offers a favorable survival outcome for advanced gastric cancer patients with serosal invasion and nodal involvement.*

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Key Words: Gastric cancer, advanced, chemosensitivity, adjuvant chemotherapy, MTT assay, extended surgery, superextended lymphadenectomy.

Gastric cancer is one of the leading causes of cancer-related death, especially in Asia, Africa and parts of Europe (1, 2). Extended lymph node dissection has been performed for gastric cancer in Japan, and the survival benefit of extended surgery has been demonstrated (3, 4). Neverthless, the prognosis of patients with advanced gastric cancer has not been sufficiently improved by extensive surgery (5). Therefore, various adjuvant chemotherapy regimens have been proposed to improve the postoperative survival. However, there are only a few reports which show a clear survival benefit of adjuvant chemotherapy over surgery alone (6).

It is important to select anticancer drugs which are effective against cancer cells in order to avoid the unnecessary use of these drugs which may cause adverse effects, especially after curative operation. In this respect, *in vitro* chemosensitivity testing is important (7). A rapid colorimetric assay was described by Mosmann (8) for determining the ability of viable cells to convert a soluble tetrazolium salt, 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), into an insoluble formazan precipitate. The MTT assay is a rapid and quantitative colorimetric system for determining the chemosensitivity of human tumor cells; however, the use of this assay for solid tumor tissues has been limited because of contamination by nonmalignant cells (9). In a previous study, we determined chemosensitivity in gastric cancer and colorectal cancer, using highly purified tumor cells, and showed a correlation between this sensitivity and clinical response (9-11). Since then, we have developed a treatment plan to improve the poor prognosis of patients with advanced gastric cancer. Gastrectomy, with extended lymph node dissection, was performed for patients with advanced gastric cancer showing serosal invasion and nodal involvement. After surgery, the MTT assay, using highly purified tumor cells, was performed,

and patients received individualized adjuvant chemotherapy on the basis of the results of the chemosensitivity test.

In the present study, the feasibility and the efficacy of the strategy of extended surgery combined with individualized adjuvant chemotherapy for advanced gastric cancer with serosal invasion and nodal involvement is examined in a prospective non-randomized manner.

Patients and Methods

Patients. Sixty-four patients with advanced gastric cancer, admitted to Wakayama Medical University Hospital, Japan, between 1991 and 1996, underwent gastrectomy with extended lymph node dissection. This extended surgery was indicated for patients with advanced gastric carcinoma showing serosal invasion or N2 lymph node metastases. Total gastrectomy was performed in 49 patients, distal gastrectomy was performed in 14 patients and pancreaticoduodenectomy was performed in 1 patient. The lymph nodes, located perigastrically, around the gastric artery, the hepatic artery, the splenic artery and the celiac artery and paraaortic nodes were extensively dissected in all the patients.

The clinical stages of the 64 patients according to the TNM classification (4th edition) of malignant tumors by UICC were: 7 with stage Ib, 6 with stage II, 10 with stage IIIa, 12 with stage IIIb and 29 with stage IV. None of these patients had received any previous antitumor drugs. Surgical specimens were obtained from primary gastric lesions and the MTT assay was successfully performed in 38 patients.

Informed consent was obtained from the patient and/or the family twice, in advance of operation and of chemotherapy, in accordance with the guidelines of the Ethical Committee on Human Research, Wakayama Medical University, Japan.

Anticancer drugs. The antitumor drugs tested were cisplatin (CDDP), mitomycin C (MMC), doxorubicin (DOX) and 5-fluorouracil (5-FU). Each drug was diluted in complete medium at 10-fold therapeutic peak plasma concentration (Cmax x 10), achieved by intravenous administration of clinical doses (12), as described in our previous studies (10). The values were 10 μg/ml MMC, 100 μg/ml 5-FU, 4 μg/ml DOX and 20 μg/ml CDDP. The complete medium used consisted of RPMI-1640 (Nissui Co., Tokyo Japan) supplemented with 10% heat-inactivated fetal calf serum (GIBCO, New York, USA), 2 mM L-glutamine and antibiotics (100 units/ml of penicillin and 100 μg/ml of streptomycin).

Purification of fresh human gastric cancer cells. Freshly excised tumor tissues were processed using enzymatic digestion, as previously described (10). Briefly, tumor tissues were dissected into small pieces, which were immersed in complete medium containing collagenase (2 mg/ml, type V-S; Sigma), hyaluronidase (10 units/ml, type IV-S; Sigma), and DNase-I (0.4 mg/ml; Sigma). After a 40-min incubation at 37°C, the cells were harvested and were centrifuged on Ficoll-Hypaque (specific gravity 1.077; Pharmacia, Uppsala, Sweden) gradients at 400 xg for 30 min. The interface was collected, and suspended at a density of $1x10^6$/ml in complete medium. Then, the cell suspension was layered on discontinuous gradients consisting of 10 ml of 100% and 15 ml of 75% Ficoll-Hypaque. After centrifugation at 400 xg for 30 min, a tumor cell-rich fraction was collected from the 75% interface. The tumor cell-enriched suspension was then layered on discontinuous gradients containing 4 ml each of 25%, 15% and 10% Percoll (Pharmacia, Uppsala, Sweden) in complete medium. Centrifugation was performed at 15 xg for 7 min and tumor cells depleted of lymphoid cells were collected from the bottom and from the 25% interface. The cells thus prepared were primarily tumor cells, with less than 10% contamination by nonmalignant cells (9).

MTT assay. Chemosensitivity was assessed using the tetrazolium salt MTT (Sigma No. M2128) to measure the viability of tumor cells, as previously described (9, 10). Briefly, tumor cell suspensions ($1x10^6$ cells /ml) were added to each anticancer drug at a final concentration of Cmax x 10 in 96-well flat-bottomed microtiter plates (Corning No. 25860), and incubated at 37°C in a humidified 5% CO_2 atmosphere for 96 h. The chemosensitivity assay was assessed in triplicate. Microtiter wells containing tumor cells without anticancer drugs were used as controls for cell viability, while wells containing only complete medium were used as controls for nonspecific dye reduction. After incubation, the plates were centrifuged, the supernatants were removed and MTT solution with 10 μM of sodium succinate was added to all the wells. The plates were incubated for an additional 4 h, and dimethyl sulfoxide (DMSO) was then added to all the wells; the mixtures were pipetted thoroughly to dissolve the dark blue crystals. The plates were then read on a microplate reader (Corona Electric, MTP-32) using a test wavelength of 570 nm and a reference wavelength of 630 nm. The control wells without tumor cells had an optical density (OD) of less than 0.005, and the samples in which the OD was over 0.1 were accepted for the assay. The inhibition rate was calculated as follows:

Inhibition rate = (1 – OD drug-treated/OD control) x 100

The background of tumor cells (including dead cells) without addition of MTT had an OD of less than 0.012 after 96-h incubation, and the influence of dead tumor cells could therefore be ignored in the present study. The viability of tumor cells was maintained at 75-90%, during the 96-h incubation (10). The cut-offs were inhibition rates equal to or more than 74% (10).

Chemosensitivity test-oriented chemotherapy. The MTT assay was performed in 40 out of 64 patients, succeeding in 38. The patients received treatment according to the chemosensitivity guideline as follows: when sensitive drugs could be selected by the MTT assay, a single drug or combination of two or three drugs were chosen on the basis of these results; when no effective drugs were identified, patients primarily did not receive the adjuvant chemotherapy, or were treated with cisplatin and 5-fluorouracil (FP) at their request; when the chemosensitivity test could not be performed, the patients were treated with FP.

Six out of 38 patients did not receive chemosensitivity-guided chemotherapy after extended surgery of their own volition, although suitable drugs had been identified by the MTT assay.

Forty-two patients received adjuvant chemotherapy after surgery. Thirty-two patients were treated on the basis of the results of the MTT assay (chemosensitivity-guided chemotherapy group; CSC), while 17 patients received standard chemotherapy without any chemosensitivity information (standard chemotherapy group; SC). Patients were individually treated with the protocols shown in Figure 1. On the other hand, 15 patients did not receive any chemotherapy after surgery (no-chemotherapy group; NC).

Chemosensitivity-guided chemotherapy group (CSC)

Choose a single drug or combination therapy using two or three drugs as below on the basis of the results of chemosensitivity test (2 – 3 courses).

CDDP:	10 - 15 mg / m^2	day 1 –day 5	
MMC:	8 - 10 mg / m^2	day 1	
5-FU:	500 - 750 mg / m^2	day 1 –day 5	(continuous infusion)
ADR:	20 mg / m^2	day 1	

Chemotherapy without chemosensitivity group (standard chemotherapy group; SC).

Combination therapy using two drugs as below (2 – 3 courses)

CDDP:	10 - 15 mg / m^2	day 1 –day 5	
5-FU:	500 - 750 mg / m^2	day 1 –day 5	(continuous infusion)

Figure 1. *Treatment protocol of adjuvant chemotherapy. Patients were individually treated with different protocols. In cases where the sensitive drugs could be selected by MTT assay, either a single drug or combination of two or three drugs was chosen on the basis of the results of the chemosensitivity test. In cases where there were no sensitive drugs, patients did not receive the adjuvant chemotherapy, or were treated with cisplatin and 5-fluorouracil (FP) as self-requested. In cases where the chemosensitivity test could not be performed, patients were treated with FP.*

Statistical analysis. Quantitative results were expressed as mean±standard deviation of the mean. Statistical analysis was performed by ANOVA and Fisher's test. Background factors were compared using the Student's *t*-test, the Mann-Whitney *U*-test and the χ^2 test. The survival rates were estimated using the Kaplan-Meier method, and the differences were analyzed by using the log-rank test, to compare the resulting curves of the treatment groups. Multivariate analysis was examined according to Cox's proportional hazard model. A *p*-value of <0.05 was considered to be statistically significant. StatView 5.0 software (Abacus Concepts, Inc., Berkeley, CA, USA) was used for all statistical analyses.

Results

Patient characteristics. The clinicopathological characteristics of the patients were shown in Table I. There were no significant differences in background factors which were considered to be related to prognosis between the CSC group and the SC + NC group.

Chemosensitivity of patients with advanced gastric carcinoma. The MTT assay was performed in 40 out of 64 patients who received extended surgery, succeeding in 38, but failing in 2 patients because of low OD. The success rate of this assay was 95%. The chemosensitivity of the patients with advanced gastric carcinoma is shown in Table II. At a drug concentration of Cmax x 10, the inhibition rates of tumor cells for each of the four drugs was around 65%. There was no significant difference in chemosensitivity between differentiated and undifferentiated types.

Table I. *Clinicopathological characteristics.*

Clinicopathological characteristics	CSC group (n=32)	SC + NC group (n=32)	*p*
Age (yrs; average±SD)	57.8±13.0	57.2±11.3	NS
Gender(male/ female)	26/ 6	22/10	NS
Stage			
I	2	5	NS
II	3	3	
III	12	10	
IV	15	14	
Depth of tumor invasion			
t1	2	2	NS
t2	10	11	
t3	15	12	
Lymph node metastases			
n0	6	7	NS
n1	0	0	
n2	26	25	
Tumor type			
type 0	2	0	NS
type 1	1	1	
type 2	5	7	
type 3	18	20	
type 4	6	4	
Histological type			
differentiated	17	14	NS
undifferentiated	15	18	
Operation			
distal gastrectomy	7	7	NS
total gastrectomy	25	24	
pancreaticoduodenectomy	0	1	
Curability of surgery			
Curative	27	24	NS
Noncuratiive	5	8	

There were no significant differences in background factors which were considered to be related to the prognosis of gastric cancer patients between the CSC group and the SC + NC group.

Effect of chemosensitivity-guided adjuvant chemotherapy on survival of gastric cancer patients. The 5-year survival rate was 56.3% in the CSC group and 28.1% in the SC + NC group, respectively, presenting a significant difference in the two survival curves ($p<0.05$) (Figure 2A). The difference between the two groups was remarkable in patients with advanced stage. In patients with stage III disease, the 5-year survival rate was 66.7% in the CSC group and 30.0% in the SC + NC group, respectively, although there was no statistically

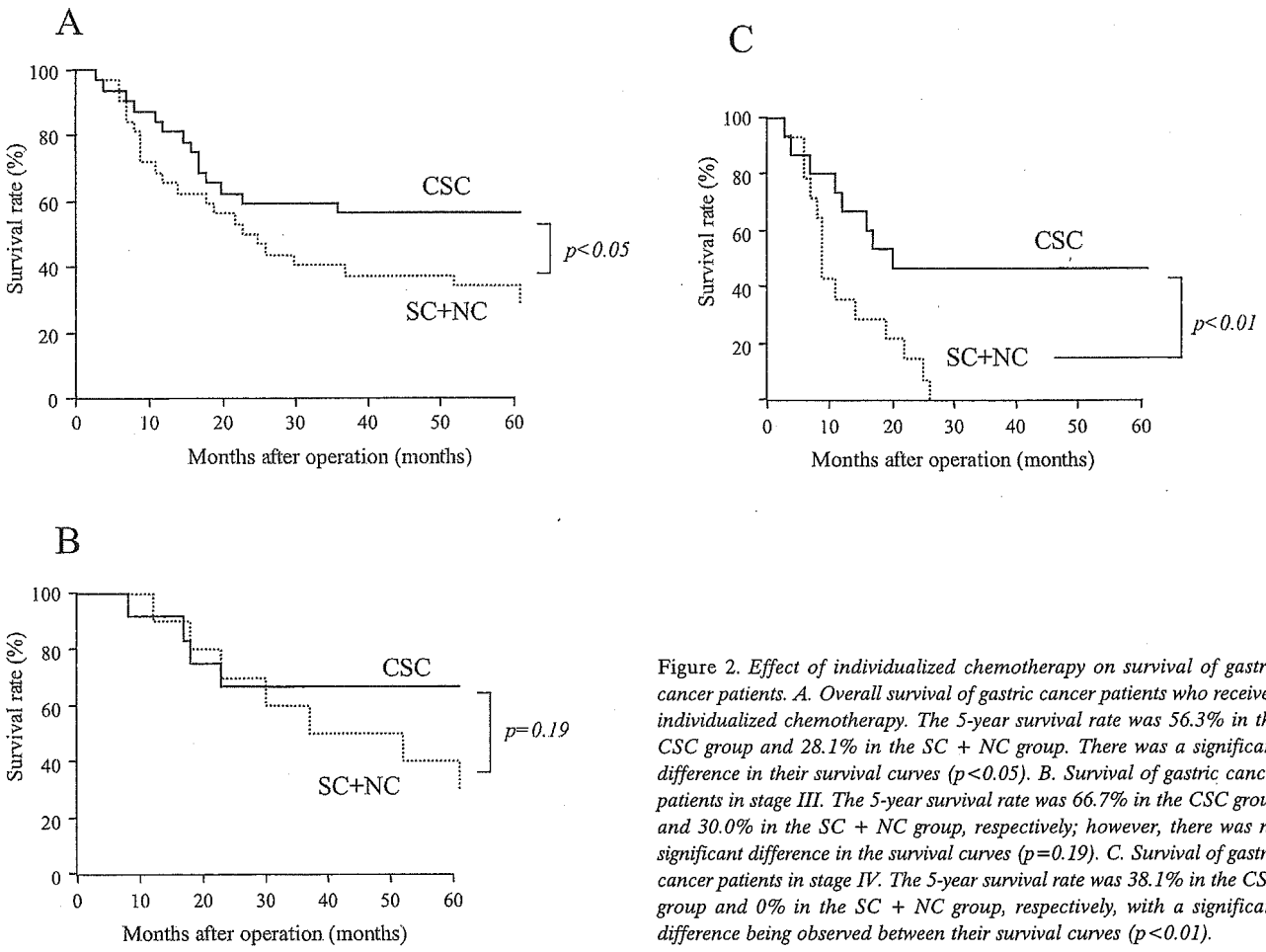

Figure 2. *Effect of individualized chemotherapy on survival of gastric cancer patients. A. Overall survival of gastric cancer patients who received individualized chemotherapy. The 5-year survival rate was 56.3% in the CSC group and 28.1% in the SC + NC group. There was a significant difference in their survival curves ($p<0.05$). B. Survival of gastric cancer patients in stage III. The 5-year survival rate was 66.7% in the CSC group and 30.0% in the SC + NC group, respectively; however, there was no significant difference in the survival curves ($p=0.19$). C. Survival of gastric cancer patients in stage IV. The 5-year survival rate was 38.1% in the CSC group and 0% in the SC + NC group, respectively, with a significant difference being observed between their survival curves ($p<0.01$).*

Table II. *Chemosensitivity of patients who received extended surgery.*

(n=38)

	Inhibition rates (%)			
	CDDP	MMC	ADR	5-FU
All cases	66±23	66±21	64±23	69±25
Differentiated type	68±19	66±21	65±21	68±25
Undifferentiated type	64±28	67±22	62±25	70±25

Data are expressed as mean + standard deviation (SD).
Differentiated type: well- or moderately- differentiated tubular adenocarcinoma, papillary adenocarcinoma.
Undifferentiated type: poorly-differentiated adenocarcinoma, signet-ring cell carcinoma.

significant difference in survival ($p=0.19$) (Figure 2B). In patients with stage IV disease, the 5-year survival rate was 38.1% in the CSC group and 0% in the SC + NC group, respectively, with a significant difference being observed between the survival curves ($p<0.01$) (Figure 2C). To analyze the characteristics of patients who benefited from the adjuvant chemotherapy, patients with stage IV were divided into two groups according to the existence of paraaortic lymph nodes metastases. In patients with paraaortic lymph node metastases, the 5-year survival rate was 42.9% in the CSC group and 0% in the SC + NC group, respectively, and there was significant difference in the survival curves ($p<0.01$) (Figure 3A). On the other hand, in patients without paraaortic lymph node metastases, no survival difference was observed between the two groups (Figure 3B).

For patients with stage IV disease, a significant difference in the survival curves was observed between the CSC group and either the SC group or the NC group ($p<0.05$), although the survival difference between the SC group and the NC group was not significant (Figure 4).

Multivariate analysis of risk factors for prolonged overall survival. Multivariate analysis of risk factors for prolonged overall survival was examined according to Cox's proportional hazard model. The risk ratio of each factor is

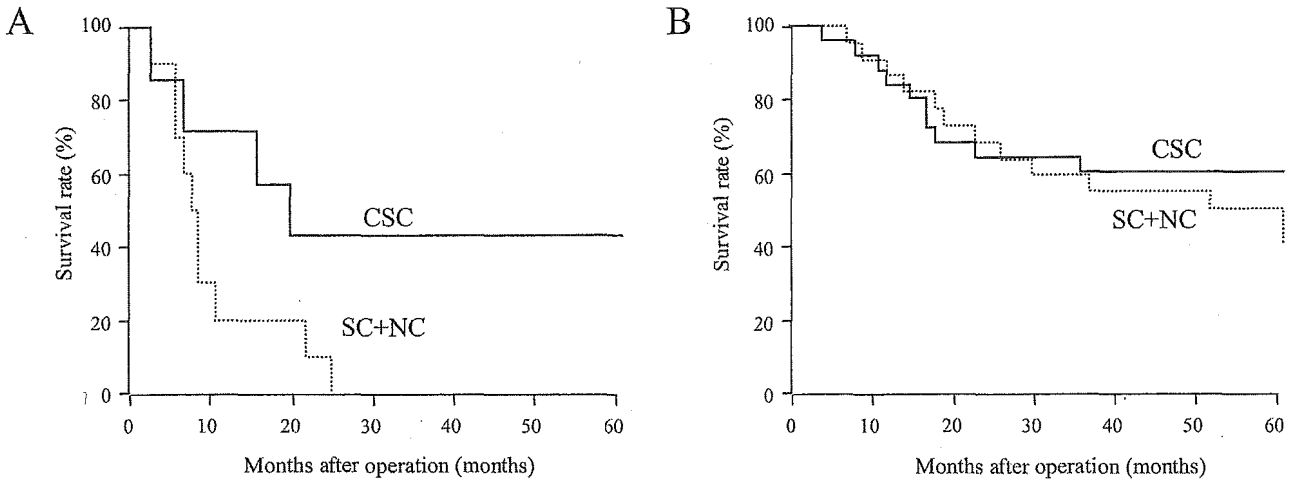

Figure 3. *Effect of individualized chemotherapy on survival of patients with and without paraaortic lymph node involvement. A. Survival of gastric cancer patients with paraaortic lymph node involvement. The 5-year survival rate was 42.9% in the CSC group and 0% in the SC + NC group, respectively, with a significant difference in the survival curves ($p<0.01$). B. Survival of gastric cancer patients without paraaortic lymph node involvement. No significant survival difference was observed between the CSC group and the SC + NC group.*

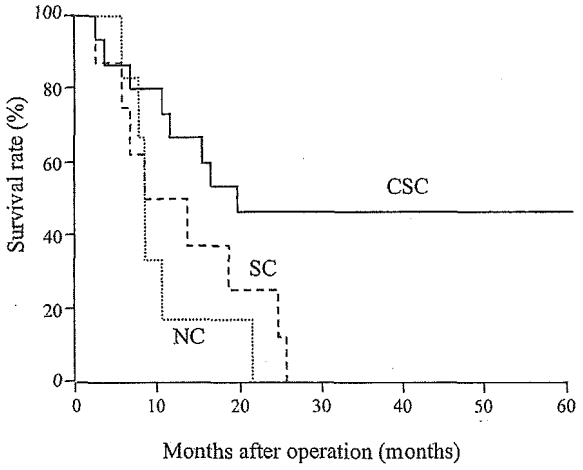

Figure 4. *Effect of individualized chemotherapy on the survival of gastric cancer patients in stage IV. When the SC + NC group was divided into the SC group and the NC group, a significant difference was observed between the survival curves of the CSC group and the SC group or the NC group ($p<0.05$). On the other hand, there was no significant difference in the survival between the SC group and the NC group.*

shown in Table III. Chemosensitivity-guided chemotherapy was an independent risk factor for overall survival.

Discussion

Surgical resection is the most common approach for the treatment of patients with advanced gastric carcinoma. Although, the benefit of D2 lymphadenectomy is yet to be clarified (13, 14), Japanese surgeons have established D2 lymph node dissection (15), and some specialized centers in other countries have shown the benefits of D2 lymph node dissection (16-22). Furthermore, some Japanese specialists have performed a pilot study of extended D4 lymph node dissection (removal of paraaortic nodes in addition to D2 dissecton) for patients with advanced gastric carcinoma (23, 24). Nevertheless, the prognosis of the patients who have paraaortic node involvement is still poor (23, 24). Therefore, we attempted to improve the postoperative survival of such patients by the combination of extensive surgery with adequate adjuvant chemotherapy.

To date, various adjuvant chemotherapy regimens have been proposed; however, the majority of trials have failed to show a clear survival benefit over surgery alone (25, 26), although several meta-analyses have shown the survival benefit of adjuvant chemotherapy after curative surgery compared with surgery alone (27-29). The most recent study conducted by the Japanese Clinical Oncology Group (JCOG) also could not show the efficacy of adjuvant chemotherapy (30).

Bearing the problem of drug resistance in mind, the MTT assay has proven to be a rapid and quantitative colorimetric system for the determination of the chemosensitivity of tumor cells, correlating with clinical response (9-11, 31, 32). This strategy was employed in choosing the regimen of adjuvant chemotherapy for advanced gastric cancer patients who underwent gastrectomy.

In this study, survival of the patients in the CSC group was significantly better than that in the SC + NC group. The difference between the two groups was more remarkable in patients with advanced stage, especially in patients with stage IV disease (5-year survival rate: 38.1%). This result is

Table III. *Multivariate analysis of risk factors for prolonged overall survival.*

Risk factors	Hazard ratio	*p*	95%CI
Chemosensitivity-guided chemotherapy	3.636	0.0045	1.491 ~ 8.863
Peritoneal metastases	2.684	0.0416	1.038 ~ 6.940
Histological type	2.344	0.0670	0.942 ~ 5.830
Depth of tumor invasion	1.289	0.4197	0.696 ~ 2.387
Lymph node metastases	1.981	0.0151	1.141 ~ 3.437
Curability of surgery	2.894	0.1386	0.709 ~ 11.816

Multivariate analysis of risk factors for prolonged overall survival was examined according to Cox's proportional hazard model. Chemosensitivity-guided chemotherapy was an independent risk factor for overall survival.

consistent with previous reports (7, 33, 34). In stage IV, it was patients with paraaortic node involvement who benefited from the adjuvant chemotherapy. These results suggest that adjuvant chemotherapy after extended surgery might prolong the survival of patients who possibly have micrometastatic lesions which were not resected during operation. Noteworthily, when the SC + NC group was divided into its two counterparts, a significant difference in the survival curves of stage IV patients was observed not only between the CSC group and the NC group, but also between the CSC group and the SC group. Adjuvant chemotherapy in the SC group failed to show a survival advantage over surgery alone, although cisplatin and 5-fluorouracil (FP) were used in most of this group. Therefore, it is suggested that the chemosensitivity test based on the MTT assay using highly purified tumor cells would be useful for planning individualized adjuvant chemotherapy, resulting in a favorable survival outcome.

Several studies are in agreement with our viewpoint. Kubota *et al.* have reported that prediction of chemosensitivity using a histoculture drug-response assay would potentially contribute to patient survival in gastric cancer (33). The collagen gel droplet embedded culture-drug sensitivity test (CD-DST) has been recently developed, and it has been reported that CD-DST can predict the response to chemotherapy with a high accuracy in breast cancer patients (35). Each method of chemosensitivity testing, including our method, has merits and demerits. Quite recently, so-called tailor-made chemotherapy has been developed using biomarkers such as multiple drug-resistant protein (MRP)-1 (36) and dihydropyrimidine dehydrogenase (DPD) (37), but superiority to bioassays are yet to be established (35).

New anticancer drugs have been recently developed, and some of them, such as TS-1, CPT-11, paclitaxel and docetaxel, are already available for gastric cancer. TS-1, in particular, is a novel oral anticancer drug and has been reported to be very effective in a phase II clinical trial (38). These new drugs, as single or combined regimens, will surely play major roles in chemotherapy against gastric cancer. However, with efficacy rates less than 50%, potential adverse effects and high costs, correct selection by chemosensitivity testing is obviously desirable.

In conclusion, chemosensitivity testing of individual gastric cancer with highly purified tumor cells using the MTT assay was useful in choosing effective anticancer drugs for adjuvant chemotherapy. The strategy of extended surgery combined with individualized adjuvant chemotherapy offers a favorable survival outcome for advanced gastric cancer with serosal invasion and nodal involvement.

References

1 Patino JF: The current management of gastric cancer. Adv Surg *27*: 1-19, 1994.

2 Thompson GB, van Heerden JA and Sarr MG: Adenocarcinoma of the stomach: are we making progress? Lancet *342*: 713-718, 1993.

3 Kodama Y, Sugimachi K, Soejima K, Matsusaka T and Inokuchi K: Evaluation of extensive lymph node dissection for carcinoma of the stomach. World J Surg *5*: 241-248, 1981.

4 Maruyama K, Gunven P, Okabayashi K, Sasako M and Kinoshita T: Lymph node metastases of gastric cancer: general pattern in 1931 patients. Ann Surg *210*: 596-602, 1989.

5 Nakajima T: Gastric cancer treatment guideline in Japan. Gastric cancer *5*: 1-5, 2002.

6 Shimada K and Ajani JA: Adjuvant chemotherapy for gastric carcinoma patients in the past 15 years. Cancer Res *86*: 1657-1668, 1999.

7 Furukawa T, Kubota T and Hoffman RM: Clinical application of the histoculture drug response assay. Clin Cancer Res *1*: 301-311, 1995.

8 Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J Immunol Methods *65*: 55-63, 1983.

9 Yamaue H, Tanimura H, Tsunoda T, Tani M, Iwahashi M, Noguchi K, Tamai M, Hotta T and Arii K: Chemosensitivity testing with highly purified tumor cells with the MTT colorimetric assay. Eur J Cancer *27*: 1258-1263, 1991.

10 Yamaue H, Tanimura H, Noguchi K, Hotta T, Tani M, Tsunoda T, Iwahashi M, Tamai M and Iwakura S: Chemosensitivity testing of fresh human gastric cancer with highly purified tumor cells using the MTT assay. Br J Cancer *66*: 794-799, 1992.

11 Yamaue H, Tanimura H, Nakamori M, Noguchi K, Iwahashi M, Tani M, Hotta T, Murakami K and Ishimoto K: Clinical evaluation of chemosensitivity testing for patients with colorectal cancer using MTT assay. Dis Colon Rectum *39*: 416-422, 1996.

12 Scheithauer W, Clark GM, Salmon SE, Dorda W, Shoemaker RH and Von Hoff DD: Model for estimation of clinically achievable plasma concentrations for investigational anticancer drugs in man. Cancer Treat Rep *70*:1379-1382, 1986.

13 Bonenkamp JJ, Songun I, Hermans J, Sasako M, Welvaart K, Plukker JT, van Elk P, Obertop H, Gouma DJ, Taat CW, van Lanschot J, Meyer S, de Graaf PW, von Meyenfeldt MF, Tilanus H and van de Velde CJH : Randomized comparison of morbidity after D1 and D2 dissection for gastric cancer in 996 Dutch patients. Lancet *345*: 745-748, 1995.

14 Cuschieri A, Fayers P, Fielding J, Craven J, Bancewicz J, Joypaul V and Cook P: Postoperative morbidity and mortality after D1 and resections for gastric cancer: preliminary results of MRC randomised controlled surgical trial. Lancet *347*: 995-999, 1996.

15 Kodera Y, Schwarz RE and Nakao A: Extended lymph node dissection in gastric carcinoma: Where do we stand after the Dutch and British randomized trials? J Am Coll Surg *195*: 855-864, 2002.

16 Volpe CM, Koo J, Miloro SM, Driscoll DL, Nava HR and Douglass HO Jr: The effect of extended lymphadenectomy on survival in patients with gastric adenocarcinoma. J Am Coll Surg *181*: 56-64, 1995.

17 Jatzko GR, Lisborg PH, Denk H, Klimpfinger M and Stettner HM: A 10-year experience with Japanese-type radical lymph node dissection for gastric cancer outside of Japan. Cancer *76*: 1302-1312, 1995.

18 Siewet JR, Bottcher K, Stein HJ and Roder JD: Relevant prognostic factors in gastric cancer. Ann Surg *228*: 449-461, 1998.

19 Degiuli M, Sasako M, Ponti A, Soldati T, Danese F and Calvo F: Morbidity and mortality after D2 gastrectomy for gastric cancer: results of the Italian Gastric Cancer Study Group prospective multicenter surgical study. J Clin Oncol *16*: 1490-1493, 1998.

20 Onate-Ocana LF, Aiello-Crocifoglio V, Mondragon-Sanchez R and Molina-Ruiz JM: Survival benefit of D2 lymphadenectomy in patients with gastric adenocarcinoma. Ann Surg Oncol *7*: 210-217, 2000.

21 Roviello F, Marrelli D, Morgagni P, de Manzoni G, Di Leo A, Vindigni C, Saragoni L, Tomezzoli A and Kurihara H: Italian Research Group for Gastric Cancer: Survival benefit of extended D2 lymphadenectomy in gastric cancer with involvement of second level lymph nodes: a longitudinal multicenter study. Ann Surg Oncol *9*: 894-900, 2002.

22 Sierra A, Regueira FM, Hernandez-Lizoain JL, Pardo F, Martinez-Gonzalez MA and Cienfuegos JA: Role of the extended lymphadenectomy in gastric cancer surgery: experience in a single institution. Ann Surg Oncol *10*: 219-226, 2003.

23 Baba M, Hokita S, Natsugoe S, Miyazono T, Shimada M, Nakano S, Takao S and Aikou T: Paraortic lymphadenectomy in patients with advanced carcinoma of the upper-third of the stomach. Hepato-Gastroenterol *33*: 893-896, 2000.

24 Maeta M, Yamashiro H, Saito H, Katano K, Kondo A, Tsujitani S, Ikeguchi M and Kaibara N: A prospective pilot study of extended (D3) and superextended (D4) in patients with T3 or T4 gastric cancer managed by total gastectomy. Surgery *125*: 325-331, 1999.

25 Nakajima T, Nashimoto A, Kitamura M, Kito T, Iwanaga T, Okabayashi K and Goto M: Adjuvant mitomycin and fluorouracil followed by oral uracil plus tegafur in serosa-negative gastric cancer: a randomized trial. Lancet *354*: 273-277, 1999.

26 Bajetta E, Buzzoni R, Mariani L, Beretta E, Bozzetti F, Bordogna G, Aitini E, Fava S, Schieppati G, Pinotti G, Visini M, Ianniello G and Di BM: Adjuvant chemotherapy in gastric cancer: 5-year results of a randomised study by the Italian Trials in Medical Oncology (ITMO) Group. Ann Oncol *13*: 299-307, 2002.

27 Hermans J and Bonenkamp JJ: In reply. J Clin Oncol *12*: 879-880, 1994.

28 Nakajima T, Ota K, Ishihara S, Oyama S, Nishi M and Hamashima N: Meta-analysis of 10 postoperative adjuvant chemotherapies for gastric cancer in CIH. Jpn J Cancer Chemother *21*: 1800-1805, 1994.

29 Mari E, Floriani I, Tinazzi A, Buda A, Belfiglio M, Valentini M, Cascinu S, Barni S, Labianca R and Torri V: A meta-analysis of published randomized trials. Ann Oncol *11*: 837-843, 2000.

30 Nashimoto A, Nakajima T, Furukawa H, Kitamura M, Kinoshita T, Yamamura Y, Sasako M, Kunii Y, Motohashi H, Yamamoto S; Gastric Cancer Surgical Study Group, Japan Clinical Oncology Group: Randomized trial of adjuvant chemotherapy with mitomycin, fluorouracil, and cytosine arabinoside followed by oral fluorouracil in serosa-negative gastric cancer: Japan Clinical Oncology Group 9206-1. J Clin Oncol *21*: 2282-2287, 2003.

31 Park JG, Kramer BS, Steinberg SM, Carmichael J and Collins JM: Chemosensitivity testing of human colorectal carcinoma cell lines using a tetrazolium-based colorimetric assay. Cancer Res *47*: 5875-5879, 1987.

32 Saikawa Y, Kubota T, Furukawa T, Suto A, Watanabe M, Kumai K, Ishibiki K and Kitajima M: Single-cell suspension assay with an MTT end point is useful for evaluating the optimal adjuvant chemotherapy for advanced gastric cancer. Jpn J Cancer Res *85*: 762-765, 1994.

33 Kubota T, Sasano N, Abe O, Nakao I, Kawamura E, Saito T, Endo M, Kimura K, Demura H, Sasano H, Nagura H, Ogawa N, Hoffman RM and the Chemosensitivity Study Group for the Histoculture Drug-Response Assay: Potential of the histoculture drug-response assay to contribute to cancer patient survival. Clin Cancer Res *1*: 1537-1543, 1995.

34 Kubota T, Egawa T, Otani Y, Furukawa T, Saikawa Y, Yoshida M, Watanabe M, Kumai K and Kitajima M: Cancer chemotherapy chemosensitivity testing is useful in evaluating the appropriate adjuvant cancer chemotherapy for stage III/IV gastric cancers without peritoneal dissemination. Anticancer Res *23*: 583-588, 2003.

35 Takamura Y, Kobayashi H, Taguchi T, Motomura K, Inaji H and Noguchi S: Prediction of chemotherapeutic response by collagen gel droplet embedded culture-drug sensitivity test in human breast cancers. Int J Cancer *98*: 450-455, 2002.

36 Loe DW, Almquist KC, Deeley RG and Cole SP: Multidrug resistance protein (MRP)-mediated transport of leukotriene C4 and chemotherapeutic agents in membrane vesicles. J Biol Chem *271*: 9675-9682, 1996.

37 Etienne MC, Cheradame S, Fischel JL, Formento P, Dassonville O, Renee N, Schneider M, Thyss A, Demard F and Milano G: Response to fluorouracil therapy in cancer patients: the role of tumoral dihydropyrimidine dehydrogenase acitivity. J Clin Oncol *13*: 1663-1670, 1995.

38 Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitani Y and Taguchi T: Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur- 0.4 M gimestat - 1 M otastat potassium) in gastric cancer patients. Eur J Cancer *34*: 1715-1720, 1998.

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Surgical Management of Small Gastrointestinal Stromal Tumors of the Stomach

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Abstract
Small gastrointestinal stromal tumors (GISTs) (<3 cm) occasionally are found in the stomach during endoscopy. There is no consensus about the surgical management of these small tumors, although this clinical issue is crucial because some of the tumors show unexpected malignant behavior. In this study, we evaluated the clinical management of patients with gastric GISTs who underwent surgical resection. Altogether, 31 patients with gastric GISTs were examined retrospectively. Surgical resection was fundamentally indicated for the patients with gastric GISTs suspected to be malignant by endoscopy or endoscopic ultrasonography (EUS). The malignant grade of the GISTs was evaluated by the mitotic rate, tumor size, and MIB-1 index. EUS was useful for differentiating benign from malignant GISTs; but by limiting the study to patients with small tumors (<3 cm), the diagnostic value of EUS was not satisfactory for defining the surgical indication. Tumors that were <50 mm were successfully treated by laparoscopic surgery. Of the 31 patients, 4 had a relapse of the disease, and 1 of those 4 patients had a small tumor (30 mm). All of the recurrences were classified in the high risk category. Surgery is indicated for gastric GISTs that are ≥20 mm or are suspected to be malignant based on EUS findings. Laparoscopic resection is feasible and is recommended as the treatment of choice for patients with tumors < 50 mm. Risk assessment can be most useful for predicting recurrence.

Gastrointestinal stromal tumors (GISTs) are the most common nonepithelial, mesenchymal neoplasms of the gastrointestinal tract.[1,2] Grossly, they appear to arise from the muscular layer, and their presumed origin from smooth muscle cells has led to the use of such terms as "leiomyoma," "leiomyosarcoma," and "leiomyoblastoma".[3] However, it has been recognized that there are a group of sarcomas arising from the gastrointestinal tract that do not have the typical features of leiomyosarcoma. Recent immunohistochemical studies have shown that up to 94% of GISTs express CD117, a KIT protein,[1,3,4] and 60% to 70% of GISTs stain for CD34.[1,5,6] Therefore, the GIST is now considered a completely separate entity from leiomyoma and leiomyosarcoma, which were previously thought to be the most common soft tissue neoplasms in the gastrointestinal tract.[6]

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GISTs occur mainly in the stomach (40–70%),[6] and small GISTs of <3 cm are occasionally found in the stomach during endoscopy because of improvements in the upper gastrointestinal endoscopic examination. However, there is no consensus about the surgical management of such small tumors. This clinical issue is crucial because some of these small tumors exhibit unexpected malignant behavior.[5] Moreover, small tumors can be resected by a laparoscopic surgical procedure in most cases,[7–9] and tumor size has been previously reported to be one of the most important prognostic factors for GISTs.[3,5,6]

In the present study, we analyzed the clinicopathologic features and evaluated clinical management in 31 patients with gastric GISTs. We also examined the feasibility of the preoperative diagnosis of GISTs using endoscopic ultrasonography (EUS). We have tried to formulate guidelines for the surgical management of GISTs, especially of tumors <3 cm.

PATIENTS AND METHODS

Patients

A series of 33 patients with submucosal tumors (SMTs) of the stomach were admitted to Wakayama Medical University Hospital between 1998 and 2003 and underwent surgical treatment. Altogether, 31 of those patients had histologically proven GISTs and were retrospectively examined in this study. Surgical resection was fundamentally indicated for the patients with gastric SMTs suspected to be malignant.

Methods

The clinical diagnosis before treating the patients was obtained via gastrointestinal endoscopy, EUS, and computed tomography (CT). EUS was mainly used for diagnosing the SMTs in 30 patients; one patient did not undergo EUS. Our criteria for endoscopic or EUS findings being interpreted as possibly malignant were as follows: ulcer formation, tumors >30 mm, asymmetric margin, heterogeneous US pattern, existence of an echo-free area, and rapid growth.

The histologic diagnosis of all tumors was obtained by microscopy with conventional hematoxylin and eosin (H&E) staining. Immunohistochemical staining for CD117, CD34, s-100 protein, vimentine, and αSMA was also carried out; and CD117- andCD34-positive tumors were diagnosed as GISTs. The malignant grades of the tumors were evaluated using the mitotic rate and tumor diameter. We used the determination that tumors <5 cm in diameter with 0 to 4 mitoses/50 high-power fields (HPFs) were considered benign, and tumors >5 cm in diameter with 0 to 4 mitoses/50 HPF were considered low-grade malignant. Tumors with >5 mitoses/50 HPF were considered malignant.[6,10]

We also evaluated each tumor using the risk categories proposed by Fletcher and colleagues.[5] Tumors <2 cm in diameter with 0 to 4 mitoses/50 HPF were considered very low risk, and tumors 2 to 5 cm in diameter with 0 to 4 mitoses/50 HPFs were low risk. Tumors <5 cm in diameter with 6 to 10 mitoses/50 HPF or tumors 5 to 10 cm in diameter with 0 to 4 mitoses/50 HPF were considered intermediate risk. Tumors >5 cm in diameter with a mitotic count higher than 5/50 HPF, those >10 cm, and those with >10 mitoses/50 HPF were classified as high risk tumors.[5]

Statistical Analysis

Quantitative results were expressed as the mean ± standard deviation of the mean (SEM). The statistical significance of the difference between the two groups was determined by Student's *t*-test. StatView 5.0 software (Abacus Concepts, Berkeley, CA, USA) was used for all statistical analyses.

RESULTS

Clinicopathologic Characteristics

The clinicopathologic characteristics of the patients are shown in Table 1. Seven patients who had symptoms (*e.g.*, epigastric pain) came to Wakayama Medical University Hospital for further examination; the other 24 patients with no symptoms underwent an ordinary checkup, at which time an abnormality of the stomach was detected. No particular differences were observed in tumor growth patterns. EUS findings showed a malignant pattern in 71% and a benign pattern in 26%. The mean tumor size was 40.2 ± 26.2 mm (range 10–120 mm). Pathologic examinations showed that 64% of the tumors were malignant, 13% were low-grade malignant, and 23% were benign based on the classifications of Amin *et al.*[10]

Risk categories were also used for the prediction of clinical behavior. Altogether, 8 cases were in the high risk group, 15 were in the intermediate risk group, 4 were in the low risk group, and 4 were in the very low risk group. Most of the cases (74%) were in the high and intermediate groups.

Surgical treatment was performed in all cases, with laparoscopic partial gastrectomy being performed in al-

Table 1.
Clinicopathologic characteristics of 31 patients

Age (years), mean ± SD	63.1 ± 13.2
Gender	
Male	13 (42%)
Female	18 (58%)
Clinical symptoms	
Anemia/bleeding	3 (10%)
Abdominal pain	4 (13%)
None	24 (77%)
Tumor location	
Upper third	23 (74%)
Middle third	8 (26%)
Lower third	0
Growth pattern	
Intragastric	8 (26%)
Intramural	11 (35%)
Extragastric	12 (39%)
EUS pattern	
Malignant	22 (71%)
Benign	8 (26%)
Not done	1 (3%)
Tumor size (mm), average ± SD	40.2 ± 26.2
Pathologic malignancy grade	
Malignant	20 (64%)
Low-grade malignant	4 (13%)
Benign	7 (23%)
Risk category	
High	8 (26%)
Intermediate	15 (48%)
Low	4 (13%)
Very low	4 (13%)
Operation	
Laparoscopic partial resection	15 (48%)
Intragastric tumor resection	7 (23%)
Open partial resection	6 (19%)
Open gastrectomy	3 (10%)

EUS: endoscopic ultrasonography.

most half of the cases (48%). Intragastric tumor resection was performed in 23% of the cases and open partial gastrectomy in 19%. Open gastrectomy was performed in only three cases (10%): one proximal gastrectomy and two total gastrectomies).

The clinical characteristics of patients with benign tumors were compared to those of patients with malignant/low grade malignant tumors. There were no significant differences (Table 2).

Correlation between EUS Findings and Pathologic Malignancy Grade

To assess whether EUS was valuable for differentiating benign from malignant gastric GISTs, a correlation between EUS findings and the pathologic diagnosis was examined. Of the 24 cases of malignant or low-grade malignant GISTs, 21 were diagnosed as malignant tumors, and 4 of the 6 benign GISTs were diagnosed as benign based on EUS findings. There was a significant correlation between EUS findings and the pathologic diagnosis ($P < 0.05$). The sensitivity and specificity were 83.3% and 66.7%, respectively, and the accuracy was 80.0% (Table 3). Clearly, limiting the results to patients with tumors <3 cm caused the diagnostic value of EUS to decline. No significant correlation between EUS and the pathologic diagnosis was recognized. More importantly, the sensitivity fell to 70.0%, and the specificity was 80.0%; therefore, the accuracy was only 73.3% (Table 4).

Correlation of Tumor Size with Pathologic Malignancy Grade and Risk Category

The size of the tumor in malignant or low-grade malignant cases ranged from 17 to 120 mm (mean 46.8 mm), which was larger than that in benign cases (mean 19.6 mm, range 11–80 mm) ($P < 0.05$) (Fig. 1A). Regarding the risk categories, the risk similarly tended to increase in proportion to tumor size. Tumor size ranged from 30 to 120 mm (mean 58.3 mm) in the high risk group, from 17 to 80 mm (mean 41.9 mm) in the intermediate risk group, from 24 to 40 mm (mean 31.3 mm) in the low risk group, and from 10 to 15 mm (mean 12 mm) in the very low risk category (Fig. 1B). However, it should be noted that some of the malignant tumors and those in the intermediate risk category were <30 mm.

Surgical Treatment

Complete resection with a macroscopic safety margin of >2 cm was accomplished in all cases. Most of the tumors that were <50 mm were treated by laparoscopic surgery. In cases treated by laparoscopic surgery, tumors with an intragastric growth pattern were treated by laparoscopic intragastric surgery,[11] and tumors showing intramural or extragastric growth patterns were treated by laparoscopic partial resection of the stomach. On the other hand, most of the tumors >50 mm were treated by open partial resection or gastrectomy. Two of the tumors <50 mm were treated by open surgery because they were accompanied by other disease, such as gastric carcinoma (Fig. 2). None of the tumors ruptured during the operation. Histologic surgical margins of resected tumors measured >15 mm in all cases. Among the 31 patients, one who also had gastric cancer underwent a regional lymph node dissection, and four patients with large tu-

Table 2.
Comparison of clinical factors between patients with benign and low-grade malignant/malignant tumors

Clinical factor	Benign (n = 7)	Low-grade/malignant (n = 24)	*P*
Age (years), mean ± SD	62.4 ± 14.5	63.3 ± 13.1	0.8819
Gender			
Male	5	8	
Female	2	16	0.0723
Clinical symptoms			
Symptomatic	2	6	
Asymptomatic	5	18	0.8493
Tumor location			
Upper third	5	18	
Middle third	2	6	0.8493
Growth pattern			
Intragastric	2	6	
Intramural	4	7	
Extragastric	1	11	0.2704
Microscopic findings			
Ulcer formation	0	7	
No ulcers	7	17	0.1044

Table 3.
Diagnostics value of EUS for the gastric GIST

	Pathologic diagnosis (n = 30)	
EUS diagnosis	Malignant/low-grade malignant	Benign
Malignant	20	2
Benign	4	4

To assess whether EUS was valuable in the diagnosis of the malignancy grade of gastric gastrointestinal stromal tumors (GISTs), a correlation between EUS findings and the pathologic diagnosis was examined. There was a significant correlation between EUS findings and pathologic diagnosis ($P < 0.05$). The sensitivity and specificity were 83.3% and 66.7%, respectively, and the accuracy was 80.0%.

Table 4.
Diagnostics value of EUS for small gastric GISTs (<3.0 cm)

	Pathologic diagnosis (n = 15)	
EUS diagnosis	Malignant/low-grade malignant	Benign
Malignant	7	1
Benign	3	4

To assess whether EUS was valuable in the diagnosis of the malignancy grade of small gastric GISTs (< 3.0 cm), a correlation between EUS findings and pathologic diagnosis was examined. No significant correlation between EUS and pathologic diagnosis was recognized. The sensitivity and specificity were 70.0% and 80.0, respectively, and the accuracy was 73.3%.

mors underwent sampling of lymph nodes adjacent to the tumors. Although most of these five patients had large tumors that were pathologically malignant and were defined as high risk, the histological examination revealed no lymph node involvement in any of them (data not shown).

Correlation of Recurrence with Malignant Grade or Risk Classification

Among 20 malignant tumors, 8 (40%) were defined as high risk, 11(55%) as intermediate risk, and just 1 (5%) as low risk in the risk categories. All of the low-grade

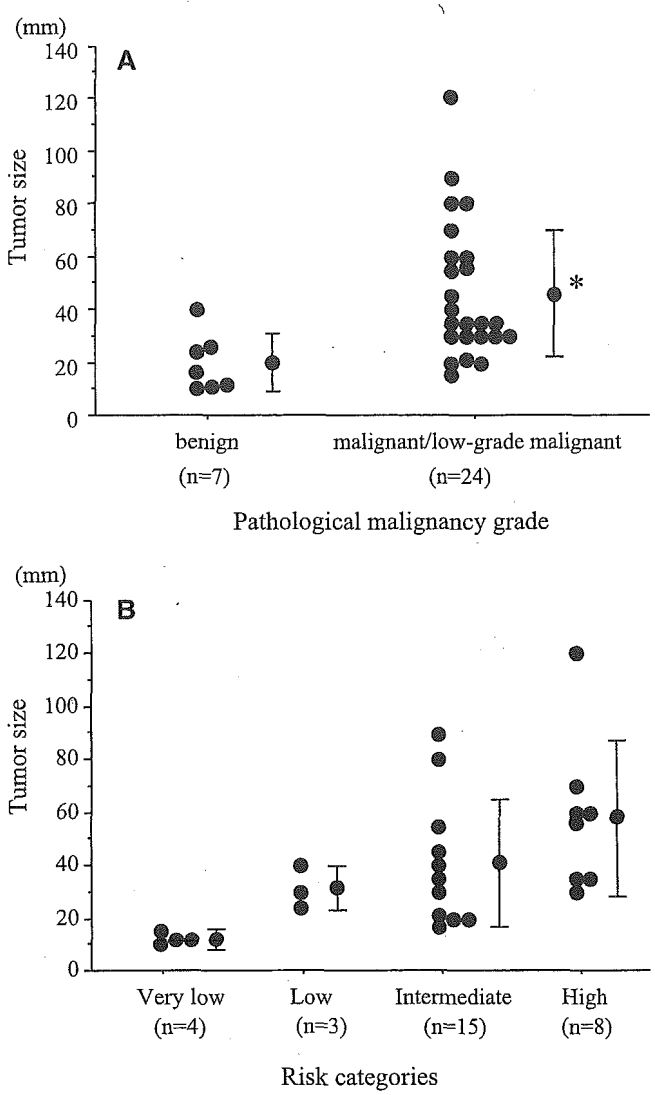

Figure 1. A. Correlation of tumor size with pathologic malignancy grade. Tumors <5 cm in diameter and with 0 to 4 mitoses/50 high-power fields (HPF) were considered benign, and tumors >5 cm in diameter and with 0 to 4 mitoses/50 HPF were low-grade malignant. Tumors with more than 5 mitoses/50 HPF were considered malignant. **B.** Correlation of tumor size with risk categories. Tumors <2 cm in diameter and with 0 to 4 mitoses/50 HPF were considered very low risk, and tumors 2 to 5 cm in diameter and with 0 to 4 mitoses/50 HPF were low risk. Tumors <5 cm in diameter and with 6 to 10 mitoses/50 HPF or tumors 5 to 10 cm in diameter and with 0 to 4 mitoses/50 HPF were intermediate risk. Tumors >5 cm in diameter and a mitotic count higher than 5 mitoses/50 HPF, tumors >10 cm, or tumors with >10 mitoses/50 HPF were classified as high risk.

malignant tumors were classified into the intermediate risk category, and all of the benign tumors were classified into the low or very low risk category (Fig. 3).

The median follow-up time was 32 months (9–68 months). No patients received adjuvant or neoadjuvant therapy with imatinib mesylate. Among the 31 patients, 4 (13%) experience a relapse, and 1 died of a recurrent

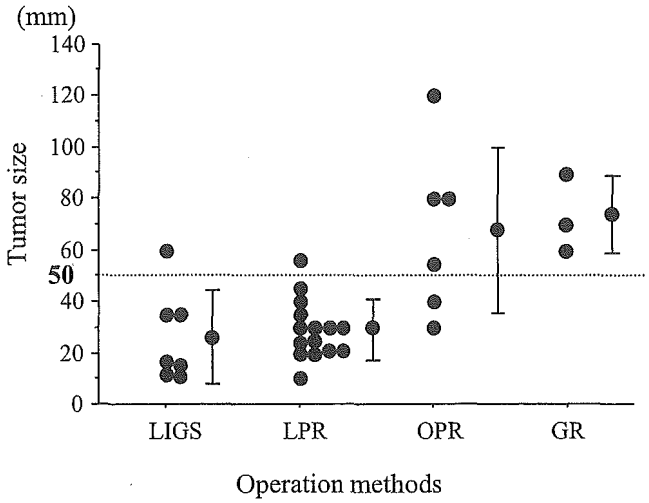

Figure 2. Surgical treatment and tumor size. Laparoscopic partial gastrectomy was performed in almost half of the cases (48%). Intragastric tumor resections were performed in 23% of cases and open partial gastrectomy in 19%. Open gastrectomy was performed in only 10% of cases. Correlation of tumor size and operative methods was evaluated. LIGS: intragastric tumor resection; LPR: laparoscopic partial resection; OPR: open partial gastrectomy; GR: open gastrectomy.

tumor. The site of recurrence was the liver in all cases. Neither lymph node recurrence nor local recurrence was observed. These recurrent cases were defined as malignant by a pathology examination and were classified in the high risk category (Table 5); moreover, the MIB-1 index of the tumors in recurrent cases was 9.2 ± 6.8 (2.4–18.1), which was significantly higher than that in the cases without recurrence 2.1 ± 1.1 (0.7–4.5) ($P < 0.0001$) (data not shown).

DISCUSSION

Endoscopic ultrasonography is considered one of the most useful tools for preoperative management of GISTs along with CT and magnetic resonance imaging (MRI).[6,12] EUS is more useful than those examinations for assessing small tumors. First, it is superior to other imaging modalities for measuring tumor size accurately. Moreover, it is a valuable method not only in the differential diagnosis of a submucosal tumor[13,14] but also for differentiating benign from malignant tumors. Yamada and colleagues reported of the usefulness of EUS for defining malignant myogenic tumors and identifying tumor diameter, shape, ulceration depth, heterogeneity, and anechoic spaces as predictors of malignancy.[15] It has been reported that the EUS findings associated with malignancy in the stromal cell tumors were tumor size (diameter >4 cm), irregular extraluminal border, echo-

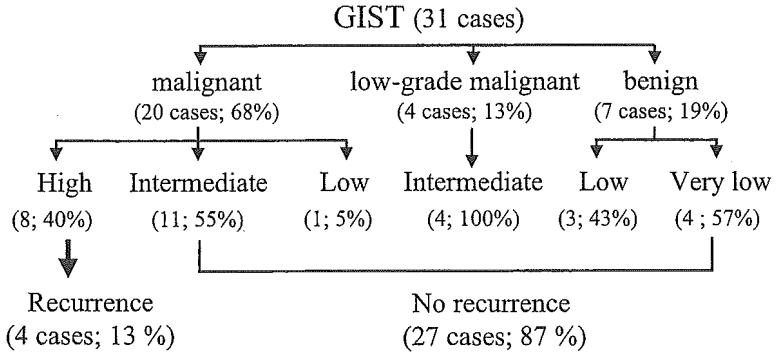

Figure 3. Correlation of recurrence with malignancy grade and with risk classification.

Table 5.
Characteristics of the recurrent cases

Patient (years/gender	Tumor size (%)	Recurrent site (mm)	Time of recurrence	Pathologic (months)	Risk category malignancy grade	MIB-1 index
78/M	70	Liver	15	Malignant	High	5.7
49/M	30	Liver	18	Malignant	High	10.7
62/M	60	Liver	24	Malignant	High	18.1
72/F	56	Liver	24	Malignant	High	2.4

The median follow-up time was 32 months (9 to 68 months). Altogether, 4 (1%) of 31 patients and were classified in a high risk category.

genic foci, and cystic spaces.[12] We have reported the differential diagnosis of myogenic tumors of the gastrointestinal tract,[16] and our data were similar to those in the two reports mentioned above. Most of the myogenic tumors previously diagnosed as smooth muscle tumors are thought to have been GISTs,[17] and therefore the predictive criteria of EUS findings could be applied.

In the present study, the preoperative differential diagnosis of EUS for gastric GISTs according to our criteria showed that the sensitivity and specificity were 83.3% and 66.7%, respectively, and the accuracy 80.0%. When limiting the results to patients with tumors <3 cm, the sensitivity was as low as 70.0% and the accuracy was only 73.3%, suggesting that the preoperative diagnostic value of EUS was not satisfactory for defining the surgical indication.

Recently, EUS-guided fine-needle aspiration (FNA) biopsy has been reported to be efficient in the diagnosis of GIST,[18,19] although it is not satisfactory in terms of differentiating benign from malignant GISTs at this time. Therefore, we did not use EUS-guided FNA as a routine examination in this study. However, we do think that the technique will be useful in the near future for differentiating benign from malignant tumors by improving the molecular diagnosis.

During the surgical management of gastric GISTs, the decision to operate is an important issue, especially for small, asymptomatic tumors that are found incidentally. There have been no reports clearly mentioning surgical indications for gastric GISTs. In this study, we established operative indications prospectively in the cases suspected to be malignant based on endoscopic or EUS findings. This is consistent with a previous report of gastric myogenic tumor.[20] However, we should keep in mind that the false-negative rate for a preoperative EUS diagnosis was fairly high (16.7% for all tumors) and 30.0% for small tumors. In fact, 10 tumors were <30 mm, and 4 of them were classified in malignant/borderline risk or intermediate risk categories; two of those four tumors (the sizes of which were 20 mm) were diagnosed as benign by EUS. Therefore, we cannot say definitively that tumors diagnosed as benign by EUS are not candidates for surgical intervention. It has been reported that even in patients with small tumors (20–50 mm) with a low mitotic rate who underwent complete resection there have been recurrences.[21,22]

The overall 5-year survival rate for patients with primary gastric GISTs who underwent complete resection ranges from 20% to 63%, with a recurrence rate of around 50% (19–76%).[23,24] In addition, the median survival times for patients whose tumors recur after complete resection are short, typically only 9 to 16 months.[23,25] Therefore, we propose that tumors that are ≥20 mm should undergo surgery, regardless of the EUS findings.

Complete surgical resection with an adequate margin remains the definitive treatment for gastric GISTs. Few reports have mentioned adequate surgical margins of gastric GISTs. It has been reported that a 2 cm margin seems sufficient for gastric GISTs.[25] Although the optimum width of the safety margin has not been defined, several investigators support the idea that it is enough for complete resection to resect a tumor with a tumor-free margin.[20,26] It has also been reported that an extended operation has no advantage over local excision for gastric leiomyosarcoma.[27]

Based on these points of view, laparoscopic wedge resection would be the most appropriate operative method for gastric GISTs.[20,24,26] Laparoscopic intragastric surgery[11] is also a suitable method for tumors exhibiting an intragastric growth pattern. These methods are thought to be minimally invasive because of a satisfactory postoperative course and a good quality of life after surgery.[7–9,11,26] However, it is important to avoid accidental rupture of tumors during laparoscopic procedures because tumor rupture before or during surgery is a statistically significant prognostic factor indicating a poor outcome.[28] As large tumors tend to rupture, inadequate laparoscopic handling could cause unexpected rupture of a tumor. Hence we should not insist on using laparoscopic resection, especially for large tumors.

In the present study, all patients with tumors <50 mm that were not accompanied by other diseases, such as gastric carcinoma, were treated successfully by laparoscopic surgery. On the other hand, most of the tumors >50 mm were treated by open surgery. There were no tumor ruptures during operation, and neither lymph node recurrence nor local recurrence was observed. We propose that laparoscopic resection be considered the treatment of choice for gastric GISTs that are <50 mm.

The malignancy grade is usually evaluated by the mitotic rate and tumor diameter. In the present study, the method proposed by Amin and colleagues[10] was used. Therefore, large tumors were mostly categorized as borderline malignant or malignant. Recently, Fletcher and colleagues have devised a classification of patients with GIST based on the risk of metastasis. All GISTs should be considered as having some low malignant potential, and they should be described in terms of risk assessment, rather than using distinct benign and malignant categories.[5,6] We classified each tumor according to these risk categories and compared the conventional malignancy grade to the risk category. The results showed a good correlation between those two classifications: 40% of the malignant tumors were defined as high risk, and all of the benign tumors were classified into low or very low risk categories. It is noteworthy that all of the recurrent cases were defined as malignant by the pathology examination and were also classified in the high risk category. Four of the eight high risk cases (50%) experienced a relapse; and, according to the risk category, we could predict that the small tumor (3 cm diameter), as shown in Table 4, metastasized to the liver within 2 years. Therefore, risk assessment can be considered to be most useful for predicting disease recurrence in the present circumstances.

During the past several years, many investigators have attempted to define more objective indicators. Although several studies have shown that the MIB-1 index is an independent factor of a poor outcome,[29–31] it does not provide better prognostication than the conventional mitosis count.[32] In the present study, although the MIB-1 index of the tumors in recurrent cases was significantly higher than that in the cases without recurrence, one patient with a tumor (with the MIB-1 index as low as 2.4) developed liver metastases within 2 years. Therefore, the MIB-1 index is not a better indicator than the mitosis count or the tumor size at this stage. Recently, it was reported that c-*kit* mutation is an independent prognostic factor.[33] However, it has not yet been proven clinically useful on an individual-case basis.

CONCLUSIONS

Gastric GISTs ≥20 mm or that are suspected to be malignant based on EUS findings are candidates for surgery. Laparoscopic resection is feasible and is recommended as the treatment of choice for gastric GISTs. However, for tumors >50 mm, the suggested treatment is open surgery to avoid an accidental rupture. Risk assessment is thought to be most useful for predicting recurrence of disease in the present circumstances.

REFERENCES

1. Hirota S, Isozaki K, Moriyama Y, *et al.* Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998;279:577–580.
2. Sircar K, Hewlett BR, Huizinga JD, *et al.* Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors. Am J Surg Pathol 1999;23:377–389.
3. Pidhorecky I, Cheney RT, Kraybill WG, *et al.* Gastrointestinal stromal tumors: current diagnosis, biologic behavior, and management. Ann Surg Oncol 2000;7:705–712.
4. Seidal T, Edvardsson H. Expression of c-kit (CD117) and Ki67 provides information about the possible cell of origin

and clinical course of gastrointestinal stromal tumours. Histopathology 1999;34:416–424.
5. Fletcher CDM, Berman JJ, Corless C, *et al.* Diagnosis of gastrointestinal stromal tumors: a consensus approach. Hum Pathol 2002;33:459–465.
6. Joensuu H, Fletcher C, Dimitrijevic S, *et al.* Management of malignant gastrointestinal stromal tumors. Lancet Oncol 2002;3:655–664.
7. Kimata M, Kubota T, Otani Y, *et al.* Gastrointestinal stromal tumors treated by laparoscopic surgery: report of three cases. Surg Today 2000;30:177–180.
8. Basso N, Rosato P, De Leo A, *et al.* Laparoscopic treatment of gastric stromal tumors. Surg Endosc 2000;14:524–526.
9. Walsh RM, Ponsky J, Brody F, *et al.* Combined endoscopic/laparoscopic intragastric resection of gastric stromal tumors. J Gastrointest Surg 2003;7:386–392.
10. Amin MB, Ma CK, Linden MD, *et al.* Prognostic value of proliferating cell nuclear antigen index in gastric stromal tumors. Am J Clin Pathol 1993;100:428–432.
11. Taniguchi E, Kamiike W, Yamanishi H, *et al.* Laparoscopic intragastric surgery for gastric leiomyoma. Surg Endosc 1997;11:287–289.
12. Chak A, Canto MI, Rosch T, *et al.* Endosonographic differentiation of benign and malignant stromal cell tumors. Gastroenterol Endosc 1997;45:468–473.
13. Yasuda K, Nakajima M, Yoshida S, *et al.* The diagnosis of submucosal tumors of the stomach by endoscopic ultrasonography. Gastroenterol Endosc 1989;35:10–15.
14. Rosch T, Lorenz R, Dancygier H, *et al.* Endosonographic diagnosis of submucosal upper gastrointestinal tract tumors. Scand J Gastroenterol 1992;27:1–8.
15. Yamada Y, Kida M, Sakaguchi T. A study on myogenic tumors of the upper gastrointestinal tract by endoscopic ultrasonography: with special reference to the differential diagnosis of benign and malignant lesions. Dig Endosc 1992;4:396–408.
16. Terashita S, Tanimura H, Nagai Y, *et al.* Clinical evaluation of endoscopic ultrasonography in the differential diagnosis for submucosal tumor of gastrointestinal tract. Gastroenterol Endosc 1992;34:342–351.
17. Miettinen M, Sarlomo-Rikala M, Lasota J. Gastrointestinal stromal tumors: recent advances in understanding of their biology. Hum Pathol 1999;30:1213–1220.
18. Vander Noot MR 3rd, Eloubeidi MA, *et al.* Diagnosis of gastrointestinal tract lesions by endoscopic ultrasound-guided fine-needle aspiration biopsy. Cancer 2004;102:157–163.
19. Gu M, Ghafari S, Nguyen PT, Lin F. Cytologic diagnosis of gastrointestinal stromal tumors of the stomach by endoscopic ultrasound-guided fine-needle aspiration biopsy: cytomorphologic and immunohistochemical study of 12 cases. Diagn Cytopathol 2001;25:343–350.
20. Katai H, Sasako M, Sano T, *et al.* Surgical treatment for gastric leiomyosarcoma. Ann Chir Gynaecol 1998;87:293–296.
21. Miettinen M, Furlong M, Sarlomo-Rikala M, *et al.* Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the rectum and anus. Am J Surg Pathol 2001;25:1121–1131.
22. Hasegawa T, Matsuno Y, Shimoda T, *et al.* Gastrointestinal stromal tumor: Consistent CD117 immunostaining for diagnosis, and prognostic classification based on tumor size and MIB-1 grade. Hum Pathol 2002;33:669–676.
23. DeMatteo RP, Lewis JJ, Leung D, *et al.* Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. Ann Surg 2000;231:51–58.
24. Lehnet T. Gastrointestinal sarcoma (GIST): a review of surgical management. Ann Chir Gynaecol 1998;87:297–305.
25. Blanke CD, Eisenberg BL, Heinrich MC. Gastrointestinal stromal tumors. Curr Treat Options Oncol 2001;2:485–491.
26. Yoshida M, Otani Y, Ohgami M, *et al.* Surgical management of gastric leiomyosarcoma: evaluation of the propriety of laparoscopic wedge resection. World J Surg 1997;21:440–443.
27. Kimura H, Yonemura Y, Kadoya N, *et al.* Prognostic factors in primary gastrointestinal leiomyosarcoma: a retrospective study. World J Surg 1991;15:771–776.
28. Ng EH, Pollock RE, Munsell MF, *et al.* Prognostic factors influencing survival in gastrointestinal leiomyosarcomas: implications for surgical management and staging. Ann Surg 1992;215:68–77.
29. Toquet C, Le Neel JC, Guillou L, *et al.* Elevated (>or = 10%) MIB-1 proliferative index correlates with poor outcome in gastric stromal tumor patients: a study of 35 cases. Dig Dis Sci 2002;47:2247–2253.
30. Fujimoto Y, Nakanishi Y, Yoshimura K, *et al.* Clinicopathologic study of primary malignant gastrointestinal stromal tumor of the stomach, with special reference to prognostic factors: analysis of results in 140 surgically resected patients. Gastric Cancer 2003;6:39–48.
31. Özgüç H, Yilmazlar T, Yerci Ö, *et al.* Analysis of prognostic and immunohistochemical factors in gastrointestinal stromal tumors with malignant potential. J Gastrointest Surg 2005;9:418–429.
32. Wong NA, Young R, Malcomson RD, *et al.* Prognostic indicators for gastrointestinal stromal tumours: a clinicopathological and immunohistochemical study of 108 resected cases of the stomach. Histopathology 2003;43:118–126.
33. Kim TW, Lee H, Kang YK, *et al.* Prognostic significance of c-kit mutation in localized gastrointestinal stromal tumors. Clin Cancer Res 2004;10:3076–3081.