Patients and methods

Patients

Clinical data were prospectively recorded according to *The Japanese classification of gastric carcinoma* [20]. We reviewed the case records of consecutive patients treated by the staff surgeons at the National Cancer Center Hospital between 1993 and 2000.

A total of 1291 patients with early gastric were treated, of whom 965 patients underwent PPG or distal gastrectomy; 380 patients underwent DGBI between 1993 and 1999 and 234 patients underwent PPG between 1995 and 2000. Between 1993 and 1999, the Billroth-I technique was gradually phased out and a Roux-en-Y reconstruction method was used after distal gastrectomy. DGBI is no longer used. The first PPG was carried out at our institute in 1995, and the number of patients undergoing this procedure has increased each year.

We identified 234 patients following PPG and 241 patients following DGBI who had undergone at least one endoscopy and abdominal ultrasonography within 3 years of surgery.

We excluded 20 patients who developed a second cancer, 2 who died of other causes, 4 who had developed or died from a gastric remnant cancer, and 8 whose surgery had been performed by surgeons who did not participate in this study. The gastric remnant cancers had developed in the remnant gastric body, not in the remnant pyloric cuff. Questionnaires identifying postoperative symptoms were sent to 212 patients following PPG and 229 following DGBI. Finally, 194 patients in the PPG group (mean age, 56.8 years) and 203 patients in the DGBI group (mean age, 58.7 years) with completed questionnaires were evaluated.

Operative procedures

PPG. The indication for PPG was early gastric cancer located in the middle third of the stomach. A pyloric cuff of 2.5 cm to 6.0 cm in length was retained. When the tumor was located in the lower to middle body of the stomach, the length of the remnant pyloric cuff was longer because of the smaller proximal remnant to make the total volume of the gastric remnant large enough. The hepatic and pyloric branches of the vagal nerve, and the right gastric vessels, were preserved up to the first branch to the stomach wall. The celiac branch of the vagal nerve and the infrapyloric artery and vein were also preserved in some patients, with complete dissection of the subpyloric lymph node and left gastric or celiac lymph nodes.

DGBI. All the patients underwent Kocher's maneuver to mobilize the duodenum, thereby minimizing the tension at the gastroduodenal anastomosis. All patients underwent a D1 + β or D2 lymph node dissection.

Methods of symptom evaluation

Questionnaires to compare the two procedures were completed at two time points–in 2000 for those who underwent DGBI or PPG before 1997, and in 2004 for those who underwent PPG or DGBI between 1998 and 2000.

The questionnaire used in this study consisted of 37 questions for the patients to answer pertaining to gastrointestinal complaints and symptoms. The questionnaire asked about postoperative symptoms, including those corresponding to early dumping syndrome (within 30 min after meals; based on the diagnostic criteria for dumping syndrome established by the Japanese Society of Gastroenterological Surgery) [21], those related to late dumping syndrome (2 to 3 h after meals [cold sweat, dizziness, syncope, general malaise, tremor]), and those associated with disturbed gastric emptying between meals (abdominal distention, epigastric discomfort, continuous fullness, continuous nausea, rumbling, heartburn, hiccup, belching, continuous abdominal pain). Other questions, about meal volume, bowel movement, flatus, and overall satisfaction with the operation were included in the questionnaire (Table 1).

Postoperative follow-up

Postoperative follow-up included clinical and laboratory examinations every 6 months for the first 2 years and annually thereafter. Body weight was measured and any changes were recorded. Relative body weight (present/preoperative) was calculated in each subject. Endoscopy was performed and the findings graded as previously reported [22]. Grade B or worse esophagitis according to the Los Angeles classification was regarded as positive. Transabdominal ultrasound was performed as part of the routine follow-up.

Statistical methods

Statistical analyses were performed with SPSS statistical software (Chicago, IL, USA). Student's *t*-test and the χ^2 test were used for comparisons between the two groups. Statistical significance was defined as $P < 0.05$.

Table 1. Questionnaire survey about postoperative symptoms established by the Japanese Society of Gastroenterological Surgery [21]

(1) Early dumping syndrome (symptoms within 30 min after a meal)

Systemic symptoms
1. Do you break into a cold sweat?
2. Do you have palpitations?
3. Do you have dizziness?
4. Do you feel numbness or lose consciousness?
5. Does your face look red?
6. Does your face look pale?
7. Do you feel hot over the whole body? Do you have a sensation of heat in the whole body?
8. Do you feel general malaise and weakness?
9. Do you suffer from drowsiness?
10. Do you have headaches or does your head feel heavy?
11. Do you have pain in your chest?

Abdominal symptoms
1. Does your stomach rumble?
2. Do you have a stomachache?
3. Do you have diarrhea?
4. Do you have nausea?
5. Do you suffer from vomiting?
6. Do you have abdominal distension?
7. Do you have abdominal discomfort?

(2) Late dumping syndrome (symptoms a couple of hours after a meal)
1. Do you have cold sweats?
2. Do you have dizziness?
3. Do you lose consciousness or have convulsions?
4. Do you feel general fatigue and/or languor?
5. Do you have finger tremor?

(3) Emptying disturbance (symptoms between meals)
1. Do you have early satiety?
2. Do you have a heavy feeling in your stomach?
3. Do you have nausea?
4. Do you belch excessively?
5. Do you have abdominal distension?
6. Do you have regurgitation?
7. Do you have heartburn?
8. Do you have hiccups?
9. Do you have epigastric pain?

(4) Others
1. Are you satisfied with your treatment so far?
2. Tell us about the size of your daily meals (including between-meal snacks).
3. Tell us about your present bowel habits (diarrhea and constipation).
4. Tell us about any changes in your bowel habit.
5. Do you have excessive flatus?

Results

Patients and operative characteristics

The patients in the two groups were equally matched (Table 2).

Questionnaire

The incidences of symptoms corresponding to early dumping syndrome, including dizziness, stomach rumbling, diarrhea, and vomiting were significantly lower in those who underwent PPG than in those who underwent DGBI ($P < 0.05$; Table 3). There were no significant differences in the incidence of symptoms of late dumping syndrome and symptoms associated with disturbance in gastric emptying between the PPG and DGBI groups (Tables 4, 5). The incidence of bowel disturbance was significantly higher in the DGBI group than in the PPG group, excessive flatus was significantly less common in the PPG than in the DGBI group. There was no significant difference between the two groups in average meal volume or in the proportion of those who felt satisfied or dissatisfied with the operation (Table 6).

Change in body weight

The relative body weights (present/preoperative) were 90.2 ± 9.7% and 93.9 ± 7.3% in the DGBI and PPG groups, respectively. The loss of body weight was significantly less in the PPG group than in the DGBI group ($P < 0.0001$ by Student's t-test; Table 7).

Endoscopic findings

Residual food in the remnant stomach was more frequently observed in the PPG group than in the DGBI group. There was no significant difference in other endoscopic findings between the groups (Table 8).

Gallstones

Gallstones appeared following gastrectomy in 10.8% of those who underwent PPG and in 13.3% of those who underwent DGBI. There was no significant difference between the groups (Table 9).

Discussion

Various reconstructive procedures, such as Billroth-I, Billroth-II, and jejunal pouches [23] have been used in an attempt to improve the symptoms for patients following distal gastrectomy. Billroth-I and -II reconstructions have been performed most commonly because of their simplicity. However, they often lead to duodenogastric reflux and gastritis and produce symptoms after distal gastrectomy that adversely affect the quality of life for these patients [24, 25]. The PPG procedure with vagal nerve preservation can be performed safely with a low incidence of major complications and a better

Table 2. Characteristics of the two groups of patients who underwent pylorus-preserving gastrectomy (PPG) and distal gastrectomy reconstructed by the Billroth-I method (DGBI)

	PPG $n = 194$	DGBI $n = 203$
Male:Female	121:73	127:76
Resection of stomach	1/2 — 2/3	2/3
Lymph node dissection	D2 — No. 5	D1 + β or D2
Anastomosis	Gastro — gastro	Gastro — duodenum
Pylorus ring	Preserved	Absent
Food passage through duodenum	Yes	Yes
Hepatic branch of vagus	194	6
Pyloric branch	194	0
Celiac branch	99	4

Table 3. Outcome of the questionnaire on symptoms suggestive of early dumping syndrome

Symptoms	PPG $n = 194$	DGBI $n = 203$	P value
Cold sweat	1	2	0.589
Palpitation	2	4	0.443
Dizziness	0	8	0.005
Numbness	2	2	0.964
Facial redness	3	0	0.075
Facial pallor	1	1	0.974
Heat	4	1	0.161
General malaise	4	8	0.274
Sleepiness	8	10	0.701
Headache	1	6	0.065
Chest pain	3	5	0.516
Rumbling	7	26	0.001
Abdominal pain	3	10	0.059
Diarrhea	6	26	0.000
Nausea	2	8	0.064
Vomiting	0	5	0.028
Abdominal fullness	21	25	0.643
Discomfort	16	18	0.825

Table 4. Outcome of the questionnaire on symptoms suggestive of late dumping syndrome

Symptoms	PPG $n = 194$	DGBI $n = 203$	P value
Cold sweat	1	2	0.166
Dizziness	2	1	0.328
Syncope	0	0	0.974
General malaise	2	9	0.316
Tremor	3	5	0.278

Table 5. Outcome of the questionnaire on symptoms corresponding to gastric emptying disturbance after meals

Symptoms	PPG $n = 194$	DGBI $n = 203$	P value
Abdominal distension	19	27	0.275
Epigastric discomfort	20	15	0.305
Continuous fullness	20	26	0.437
Continuous nausea	1	6	0.065
Rumbling	9	3	0.066
Heartburn	14	13	0.748
Hiccup	3	5	0.516
Belching	14	24	0.119
Continuous abdominal pain	5	3	0.436

Table 6. Outcome of the questionnaire on other symptoms

Questions	PPG $n = 194$	DGBI $n = 203$	P value
Intake volume < 50% of preoperative value	27	29	0.916
Bowel disturbance	27	51	0.005
Frequent flatus	94	137	0.000
Overall dissatisfaction with operation	3	3	0.955
Overall satisfaction with operation	159	150	0.053

Table 7. Body weight changes in the two groups of patients who underwent pylorus-preserving gastrectomy (PPG) and distal gastrectomy reconstructed by the Billroth-I method (DGBI)

Relative body weight	PPG	DGBI
Present/preoperative (%)	93.9 ± 7.3	90.2 ± 9.7

DGBI vs PPG, $P < 0.001$

Table 8. Endoscopic findings in the two groups of patients who underwent pylorus-preserving gastrectomy (PPG) and distal gastrectomy reconstructed by the Billroth-I method (DGBI)

Findings	PPG $n = 194$	DGBI $n = 203$	P value
Esophagitis	12	5	0.143
Gastritis of remnant stomach	24	17	0.191
Residual food	42	27	0.028
Bile reflux	14	17	0.667

Table 9. Gallstone formation in the two groups of patients who underwent pylorus-preserving gastrectomy (PPG) and distal gastrectomy reconstructed by the Billroth-I method (DGBI)

	Incidence	Percentage
PPG	21/194	10.8
DGBI	27/203	13.3

DGBI vs PPG, $P = 0.449$

postoperative outcome than the Billroth-type reconstructions [2, 6, 26]. However, long-term postoperative evaluation including symptom scoring has not been assessed in detail for large numbers of patients following PPG.

Most published studies on the functional outcome after PPG have been performed at 1 year postoperatively, with only two studies with late results following surgery [18, 19]. Kodama et al. [10] reported that early morbidity did not differ between PPG and DGBI, and that the most frequent complication was gastric stasis after PPG. Similar findings were reported in another study [11–13].

Shibata et al. [19] reported long-term results including those for 36 patients after PPG, which revealed that the occurrence of early dumping syndrome was lower in the PPG group than in DGBI patients and that there was no difference between the two groups in body weight change, food intake, and overall satisfaction with the operation. Tomita et al. [18] also reported long-term results, including those for 10 patients 5-years after PPG without preservation of the vagal nerve. In their study, the only weakness of the PPG procedure was a sensation of epigastric fullness due to gastric stasis of food in the remnant stomach. They also reported that results showing post-gastrectomy syndrome after the PPG without nerve preservation were the same as those after the PPG with nerve preservation. The number of patients with long-term results after PPG in both these reports was too small to provide good postoperative symptom data following the PPG operation.

In the present study, nearly 200 patients were subject to long-term evaluation following PPG. Our study showed that the incidences of early dumping syndrome and postoperative body weight loss were significantly lower in the PPG than in the DGBI group. Contrary to previous studies, the incidence of gastric stasis with PPG was the same as that with DGBI [19]. In the long-term, it may be important to preserve the vagal nerve for improved gastric emptying.

It has been reported that patients undergoing DGBI without preservation of the hepatic and pyloric branches of the vagal nerve are at increased risk of developing cholecystolithiasis, with an incidence of 10% to 40% [10]. Nabae et al. [27] suggested that preservation of pyloroduodenal myoneural continuity during PPG would help maintain a normal sphincter of Oddi and gallbladder. The data in our study corroborate these previous reports [10, 27] in that the incidence of gallstones was lower in the PPG group. Almost all patients with DGBI in the present study underwent gastrectomy with vagal denervation.

In conclusion, the long-term results indicate that PPG has some advantages over DGBI in terms of postoperative symptoms, and that the incidence of gastric stasis is not problematic following PPG. These data suggest that PPG has an improved postoperative long-term outcome and should be the recommended procedure for early gastric cancer located in the middle third of the stomach.

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References

1. Moriwaki Y, Kunisaki C, Kobayashi S, Harada H, Imai S, Kido Y, et al. Progressive improvement of prognosis for patients with gastric cancer (dynamic stage grouping) with increasing survival interval from initial staging: how much longer can a given survivor expect to live? Surgery 2003;133:135–40.
2. Kodera Y, Yamamura Y, Kanemitsu Y, Shimizu Y, Hirai T, Yasui K, et al. Lymph node metastasis in cancer of the middle-third stomach: criteria for treatment with a pylorus-preserving gastrectomy. Surg Today 2001;31:196–203.
3. Higashi H, Natsugoe S, Ishigami S, Uenosono Y, Matsumoto M, Nakajo A, et al. Distribution of lymph node metastasis including micrometastasis in gastric cancer with submucosal invasion. World J Surg 2003;27:455–9.
4. Maehara Y, Kakeji Y, Oda S, Takahashi I, Akazawa K, Sugimachi K. Time trends of surgical treatment and the prognosis for Japanese patients with gastric cancer. Br J Cancer 2000;83: 986–91.

5. Adachi Y, Shiraishi N, Shiromizu A, Bandoh T, Aramaki M, Kitano S. Laparoscopy-assisted Billroth I gastrectomy compared with conventional open gastrectomy. Arch Surg 2000;135: 806–10.
6. Osugi H, Fukuhara K, Takada N, Takemura H, Kinoshita H. Reconstructive procedure after distal gastrectomy to prevent remnant gastritis. Hepatogastroenterology 2004;51:1215–8.
7. Gotoda T, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, et al. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. Gastric Cancer 2000;3:219–25.
8. Soetikno RM, Gotoda T, Nakanishi Y, Soehendra N. Endoscopic mucosal resection. Gastrointest Endosc 2003;57:567–79.
9. Maki T, Shiratori T, Hatafuku T, Sugawara K. Pylorus-preserving gastrectomy as an improved operation for gastric ulcer. Surgery 1967;61:838–42.
10. Kodama M, Koyama K, Chida T, Arakawa A, Gennady T. Early postoperative evaluation of pylorus-preserving gastrectomy for gastric cancer. World J Surg 1995;19:456–61.
11. Nakae Y, Akehira K, Inoue K, Iiyama H, Sato M, Masuya Y, et al. Postoperative evaluation of pylorus-preserving gastrectomy for early gastric cancer. Hepatogastroenterology 2000;47: 590–5.
12. Imada T, Rino Y, Takahashi M, Suzuki M, Tanaka J, Shiozawa M, et al. Postoperative functional evaluation of pylorus-preserving gastrectomy for early gastric cancer compared with conventional distal gastrectomy. Surgery 1998;123:165–70.
13. Yamaguchi T, Ichikawa D, Kurioka H, Ikoma H, Koike H, Otsuji E, et al. Postoperative clinical evaluation following pylorus-preserving gastrectomy. Hepatogastroenterology 2004;51:883–6.
14. Nishikawa K, Kawahara H, Yumiba T, Nishida T, Inoue Y, Ito T, et al. Functional characteristics of the pylorus in patients undergoing pylorus-preserving gastrectomy for early gastric cancer. Surgery 2002;131:613–24.
15. Sawai K, Takahashi T, Fujioka T, Minato H, Taniguchi H, Yamaguchi T. Pylorus-preserving gastrectomy with radical lymph node dissection based on anatomical variations of the infrapyloric artery. Am J Surg 1995;170:285–8.
16. Isozaki H, Okajima K, Momura E, Ichinona T, Fujii K, Izumi N, et al. Postoperative evaluation of pylorus-preserving gastrectomy for early gastric cancer. Br J Surg 1996;83:266–9.
17. Hotta T, Taniguchi K, Kobayashi Y, Johata K, Sahara M, Naka T, et al. Postoperative evaluation of pylorus-preserving procedures compared with conventional distal gastrectomy for early gastric cancer. Surg Today 2001;31:774–9.
18. Tomita R, Fujisaki S, Tanjoh K. Pathophysiological studies on the relationship between postgastrectomy syndrome and gastric emptying function at 5 years after pylorus-preserving distal gastrectomy for early gastric cancer. World J Surg 2003;27: 725–33.
19. Shibata C, Shiiba K, Funayama Y, Ishii S, Fukushima K, Mizoi T, et al. Outcomes after pylorus-preserving gastrectomy for early gastric cancer: a prospective multicenter trial. World J Surg 2004; 28:857–61.
20. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma. Second English edition. Gastric Cancer 1998;1: 10–24.
21. Nagao F, Hayashi S, Yamaguchi Y, Shiratori T, Ohkubo T, Okajima K, et al. Symposium "Early dumping syndrome". Jpn J Gastroenterol Surg 1972;4:1–20.
22. Kubo M, Sasako M, Gotoda T, Ono H, Fujishiro M, Saito D, et al. Endoscopic evaluation of the remnant stomach after gastrectomy: proposal for a new classification. Gastric Cancer 2002;5: 83–9.
23. Matsumoto K, Uchida Y, Noguchi T, Hashimoto T, Hiraoka Y, Kubo N. A device in reconstruction method after distal gastrectomy: special reference to double tract method with jejunal pouch. J Jpn Surg Soc. 1997;98:565–70.
24. Kim BJ, O'Connell T. Gastroduodenostomy after gastric resection for cancer. Am Surg 1999;65:905–7.
25. Svensson JO. Duodenogastric reflux after gastric surgery. Scand J Gastroenterol 1983;18:729–34.
26. Fukuhara K, Osugi H, Takada N, Takemura M, Higashino M, Kinoshita H. Reconstructive procedure after distal gastrectomy for gastric cancer that best prevents duodenogastroesophageal reflux. World J Surg 2002;26:1452–7.
27. Nabae T, Takahashi S, Konomi H, Deng ZL, Yokohata K, Chijiiwa K, et al. Effect of prepyloric gastric transection and anastomosis on sphincter of Oddi cyclic motility in conscious dogs. J Gastroenterol 2001;36:530–7.

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Identification of the high-risk group for metastasis of gastric cancer cases by *vascular endothelial growth factor receptor-1* overexpression in peripheral blood

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Identification of an isolated tumour cell with metastatic ability is important for predicting the recurrence and prognosis of gastric cancer. A biological marker for evaluating the metastatic ability of gastric cancer cells has not yet been identified. We assessed *vascular endothelial growth factor receptor-1* mRNA expression by quantitative real-time reverse transcriptase-polymerase chain reaction. *Vascular endothelial growth factor receptor-1* mRNA in peripheral blood was more highly expressed in perioperative metastasis-positive and postoperative recurrence cases than in normal control cases, early cancer cases and nonmetastatic advanced cancer cases. The peripheral blood *vascular endothelial growth factor receptor-1* mRNA-positive group was associated with advanced clinical stage, deep invasion beyond the muscularis propria, lymphatic involvement, vascular involvement, lymph node metastasis, positive peritoneal lavage cytology, preoperative metastasis and postoperative recurrence. Flow cytometry analysis disclosed that vascular endothelial growth factor receptor-1 expressing cells in the peripheral blood were more abundant in cancer cases with metastases than in cases without metastases. Our data suggest that the amount of positive cells may provide information on the clinical features of gastric cancer, especially in regard to gastric cancer metastasis.
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Vascular endothelial growth factor (VEGF) plays an important role in cancer progression. The receptors of VEGF consist of VEGFR1 (Flt-1), VEGFR2 (KDR/Flk-1) and VEGFR3 (Flt-4). VEGFR1 is a receptor not only for VEGF, but also for placental growth factor (PGF), and is associated with tumour progression and neovascularization (Pajusola *et al*, 1992; Galland *et al*, 1993; Ferrara & Davis-Smyth, 1997; Shibuya *et al*, 1999; Hiratsuka *et al*, 2001). Furthermore, there are splicing variants from the original VEGFR1 protein. Kendall and Thomas (1993) reported that soluble VEGFR1 (sVEGFR1) is one of the splicing variants derived from the membrane penetrating type VEGFR1 protein, and therefore there is considerable difference between the soluble protein and the one in this study. The sVEGFR1 competitively inhibits the binding between VEGFR1 and its ligands, such as VEGF and PGF. Moreover, Clark *et al* (1998) reported that sVEGFR1 exists specifically in serum from pregnant women, and does not exist in serum from men and nonpregnant women. For that reason we did not assess sVEGFR1 protein in this study.

Recently, Kaplan *et al* (2005) reported that bone marrow progenitor cells expressing VEGFR1 play an important role in the development of malignant metastatic foci. Their finding suggests that VEGFR1-expressing cells in the bone marrow or peripheral blood may be related to cancer metastasis and recurrence. There have been no reports studying the clinical and pathological significance of *VEGFR1* mRNA expression in the bone marrow and peripheral blood of cancer patients up to the present time. We have therefore studied its significance in gastric cancer patients, and have demonstrated that patients with high *VEGFR1* mRNA expression in bone marrow or peripheral blood have significantly higher metastasis and recurrence rates than those with low *VEGFR1* mRNA expression.

MATERIALS AND METHODS

Patients

Ninety gastric cancer patients who underwent surgical treatment in the National Cancer Center Hospital, Japan, from 2001 to 2004 were enrolled in the study. Sixteen patients with no history of cancer, who underwent abdominal operation in our hospital from 2001 to 2004, were recruited as noncancer controls. The mean postoperative period was 9.8 months, ranging from 4 to 24 months. The clinical stages and pathologic features of primary tumours were defined according to the criteria of the Japanese classification of gastric carcinoma (Japanese Research Society for Gastric

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Cancer, 1999). The ages of the 68 male and 38 female patients ranged from 31 to 85 years. Written informed consent was obtained from all patients. The total 106 patients were divided into the following four groups: group 1 consisted of noncancer patients ($n=16$); group 2 consisted of early cancer patients, with tumours that had invaded less than the entire submucosal layer ($n=30$); group 3 consisted of advanced cancer patients, where there was evidence of deep invasion beyond the muscularis propria and no preoperative distant metastasis ($n=30$); and group 4 comprised patients with metastasis and recurrence, where there were distant metastases at the time of operation (i.e., liver and/or lung metastasis, peritoneal dissemination) ($n=18$), or, patients up to April 2005, who developed postoperative recurrence (i.e., peritoneal dissemination and distant metastasis) ($n=12$). Additionally, the 18 gastric cancer patients with metastases at the time of operation had palliative therapies (gastrointestinal reconstruction and control of bleeding), to improve patient quality of life.

Bone marrow and blood sampling

Aspiration of both peripheral blood and bone marrow was conducted under general anaesthesia immediately before surgery. The bone marrow aspirate was obtained from the sternum using a bone marrow aspiration needle (MDTECH, Gainesville, FL, USA), and peripheral blood was obtained through a venous catheter. The first 1 ml of both peripheral blood and bone marrow was discarded to avoid contamination by epidermal cells. A 1 ml sample of peripheral blood and bone marrow was each immediately mixed vigorously with 4 ml of ISOGEN-LS (NIPPON GENE, Toyama, Japan) and stored at −80°C until RNA extraction.

Total RNA extraction and first-strand cDNA synthesis

Total RNA was extracted according to the ISOGEN-LS manufacturer's protocols. All the clinical samples obtained from the National Cancer Center Hospital were sent to our institute. The reverse transcriptase reaction (RT) was performed as described previously (Mori *et al*, 1995; Masuda *et al*, 2002). First-strand cDNA was synthesized from 2.7 μg of total RNA in a 30 μl reaction mixture containing 5 μl 5× RT reaction buffer (BRL, Gaithersburg, MD, USA), 200 μM dNTP, 100 μM solution random hexadeoxynucleotide primer mixture, 50 U of RNasin (Promega, Madison, WI, USA), 2 μl 0.1 M dithiothreitol and 100 U of Molony murine leukaemia virus RT (BRL). The mixture was incubated at 37°C for 60 min, heated to 95°C for 10 min, and then chilled on ice.

Quantitative RT–PCR

The sequences of *VEGFR1* mRNA were as follows: sense primer, 5′-TCATGAATGTTTCCCTGCAA-3′; and antisense primer, 5′-GGAG GTATGGTGCTTCCTGA-3′. Ribosomal protein S27a (RPS27A) was used as an internal control. The sequences of RPS27A primers were as follows: sense primer, 5′-TCGTGGTGGTGCTAAGAAAA-3′; and antisense primer, 5′-TCTCGACGAAGGCGACTAAT-3′. Real-time monitoring of PCR reactions was performed using the LightCycler™ system (Roche Applied Science, Indianapolis, IN, USA) and SYBR green I dye (Roche Diagnostics). Monitoring was performed according to the manufacturer's instructions, as described previously (Masuda *et al*, 2003; Ogawa *et al*, 2004). In brief, a master mixture was prepared on ice, containing 500 ng of cDNA of each gene, 2 μl of LC DNA Master SYBR green I mix, 50 ng of primers and 2.4 μl of 25 mM $MgCl_2$. The final volume was then adjusted to 20 μl with water. After the reaction mixture was loaded into the glass capillary tube, PCR was carried out under the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 8 s, annealing at 68°C for 8 s and extension at 72°C for 2 s. After amplification, the products were subjected to a temperature gradient from 72 to 95°C at 0.1°C/s, under continuous fluorescence monitoring to produce a melting curve of the products.

Flow cytometry analysis

To determine VEGFR1 protein expression in peripheral blood, we obtained preoperatively 10 ml of heparanized peripheral blood from gastric cancer patients with or without distant metastasis. Blood mononuclear cells were obtained by Ficoll density centrifugation at 1500 g for 30 min. Phycoerythrin-conjugated monoclonal antibody against human VEGFR1 was purchased from R&D Systems (Minneapolis, MN, USA). Mononuclear cells (1×10^6) were stained with VEGFR1 antibody after Fc receptor blocking, and analysed by the BD FACS Vantage™ SE flow cytometry system. The data were analysed using CellQuest soft ware (BD Biosciences, San Jose, CA, USA).

Data analysis

The expression levels of *VEGFR1* and *RPS27A* mRNA were determined by comparison with the cDNA from Human Universal Reference Total RNA (Clontech, Palo Alto, CA, USA). After proportional baseline adjustment, the fit point method was employed to determine the cycle in which the log-linear signal was first distinguishable from the baseline, and then that cycle number was used as a crossing-point value. The standard curve was produced by measuring the crossing point of each standard value and plotting it against the logarithmic value of the concentrations. Concentrations were calculated by plotting their crossing points against the standard curve, and were adjusted by *RPS27A* content. Taking into consideration the clinical application of the current study, the 95% confidence interval was used as the upper limit of a normal case cutoff value (bone marrow, 0.12; peripheral blood, 0.059). The 95% value of a normal case according to the reference intervals of the Clinical and Laboratory Standards Institute (Sasse, 2000) was established, and the reference limit was regarded as the cutoff value. Levels higher or lower than the cutoff value were considered 'positive' and 'negative', respectively. The sensitivity and specificity of the data were determined to evaluate the legitimacy of the cutoff value.

Statistics

For continuous variables, the data were expressed as the mean ± s.d. The relationships between *VEGFR1* mRNA expression and the clinicopathological factors were analysed using the χ^2-test and Kruskal–Wallis test. All tests were analysed using JMP software (SAS Institute Inc., Cary, NC, USA). Statistical significance was determined from two-sided tests as $P<0.05$.

RESULTS

Expression of *VEGFR1* mRNA in peripheral blood and bone marrow of surgical gastric cancer patients

Figure 1 shows peripheral blood *VEGFR1* mRNA levels of the four groups. In peripheral blood, the mean expression level of *VEGFR1* mRNA in group 4 (0.099 ± 0.055) was significantly higher than all other groups ($P<0.0001$; group 1 (0.033 ± 0.05), group 2 (0.044 ± 0.039) and group 3 (0.045 ± 0.039)). It is of note that there was no significant difference in *VEGFR1* expression levels in the 18 cases with metastasis at the time of operation compared with the 12 cases with postoperative recurrence; however, 30 cases with recurrence/metastasis expressed a significantly higher level of *VEGFR1* than the 60 gastric cancer cases without metastasis/recurrence (Figure 2). In bone marrow samples, there was no clear relationship between the expression level of *VEGFR1* mRNA and the progression of gastric cancer cases.

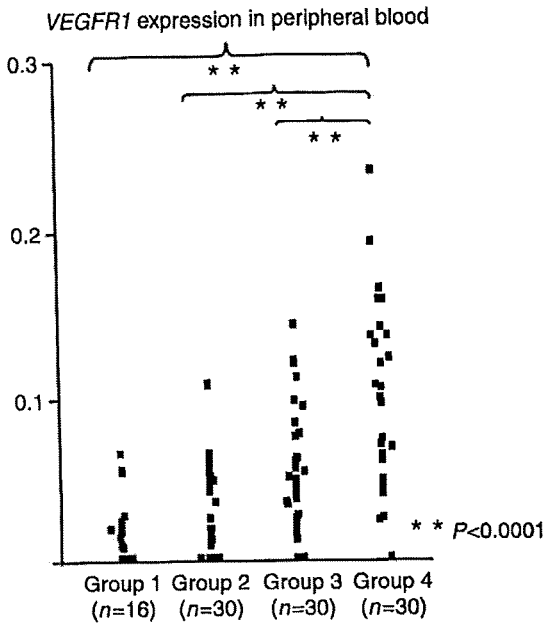

Figure 1 *VEGFR1* mRNA expression in peripheral blood from gastric cancer cases. Group 1 consisted of noncancer patients ($n=16$). Group 2 consisted of early cancer patients, with tumours that invaded less than the sub-mucosal layer ($n=30$). Group 3 consisted of advanced cancer patients, where there was evidence of deep invasion beyond the muscularis propria and no preoperative distant metastasis ($n=30$). Group 4 was the metastasis and recurrence patient group, where there were distant metastases at the time of operation (i.e., liver and/or lung metastasis, peritoneal dissemination) and who developed postoperative recurrence (e.g., peritoneal dissemination and distant metastasis) ($n=30$). The mean expression level of *VEGFR1* mRNA in group 4 was significantly higher than all other groups ($P<0.0001$, respectively).

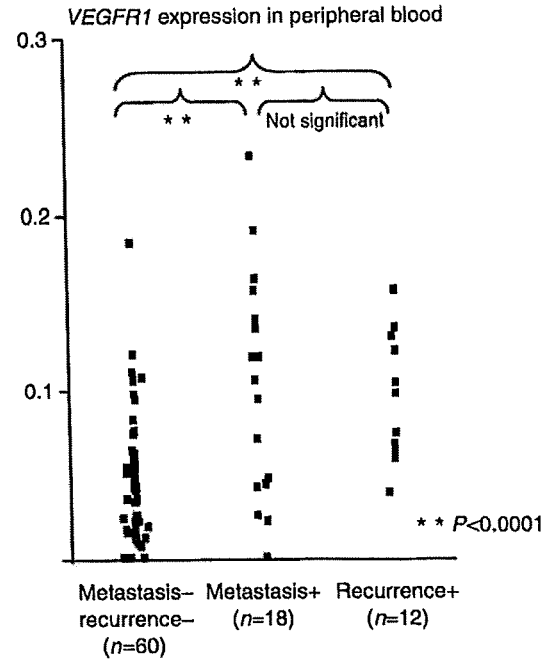

Figure 2 *VEGFR1* expression with or without metastasis and recurrence. There is no significant difference between patients with metastasis at the time of operation and patients with postoperative recurrence. The higher *VEGFR1* expression was observed in patients with metastasis pre- and postoperatively in comparison with gastric cancer patients without metastasis ($P<0.0001$, respectively).

VEGFR1 expression and clinicopathological features of gastric cancer patients with surgery

The correlations between the results for the *VEGFR1* mRNA levels and clinicopathological variables are summarized in Table 1. By the predetermined cutoff values for bone marrow, 0.12, and peripheral blood, 0.059, of the 90 patients there were 23 (25.6%) and 34 (37.8%) estimated to be positive for *VEGFR1* mRNA in bone marrow and peripheral blood, respectively. Sensitivities with these cutoff values were 66.7% in peripheral blood and 46.7% in bone marrow, and specificities were 76.7% in peripheral blood and 85.0% in bone marrow. In the peripheral blood, a significantly higher number of *VEGFR1* mRNA positive cases belonged to the following clinical subgroups: those in stages 3 and 4 ($P<0.001$), invasion deeper than the muscularis propria ($P<0.01$), lymphatic involvement ($P<0.001$), vascular involvement ($P<0.0001$), lymph node metastases ($P<0.0001$), positive peritoneal lavage cytology ($P<0.001$), perioperative overt metastases (e.g., liver or lung $P<0.05$) and postoperative recurrence ($P<0.01$). In contrast, in bone marrow, there was a significant difference observed that correlated with the pathological stage ($P<0.05$), the incidence of lymphatic involvement ($P<0.05$), lymph node metastases ($P<0.01$), positive peritoneal lavage cytology ($P<0.05$) and the presence of postoperative recurrence ($P<0.01$).

VEGFR1 expression in blood by flow cytometry

According to flow cytometry analysis, VEGFR1-positive cells in the lymphocytes and monocytes of mononuclear cells isolated from the peripheral blood of gastric cancer patients with metastasis were increased over patients without metastasis (9.8, *vs* 2.5% in representative study case) (Figure 3). In particular, VEGFR1-positive cells in the fraction of monocytes in FS/SS plots were more abundant in the metastatic patient (9.7%) than in the nonmetastatic patient (2.4%).

DISCUSSION

In this study, we studied *VEGFR1* mRNA expression in the bone marrow and peripheral blood of patients with gastric cancer. Patients with metastases and/or recurrence expressed higher levels of *VEGFR1* mRNA in the peripheral blood than nonmetastatic and nonrecurring patients. Patients with a high level of *VEGFR1* mRNA expression in peripheral blood showed deeper tumour invasion in the primary organ, positive vascular vessel or lymphatic vessel invasion, positive lymph node metastasis and positive peritoneal lavage cytology. Thus, the expression of *VEGFR1* mRNA in the peripheral blood may be associated with metastasis and recurrence of gastric cancer.

The source of *VEGFR1* mRNA in peripheral blood or bone marrow in gastric cancer patients has not yet been elucidated. We initially speculated that the original cells expressing VEGFR1 in gastric cancer patients are the haematopoietic progenitor cells (HPCs). Lyden *et al* (2001) reported that the progression of tumour vessels needs the cooperation of VEGFR1-positive HPCs and VEGFR2-positive endothelial progenitor cells. In addition, Kaplan *et al* (2005) reported that bone marrow-derived HPCs express VEGFR1 home to tumour-specific premetastatic sites, and form cellular clusters that provide a permissive niche for incoming tumour cells before the arrival of tumour cells. Another possible source may be mature vessel-derived endothelial cells, which might be the largest source of VEGFR1-expressing circulating endothelial cells (CECs) (Mancuso *et al*, 2001; Beerepoot *et al*, 2004). Contrary to our expectation, there was no significant difference in the number of VEGFR1-expressing cells of $CD133^{+/-}$ $CD31^{+}$

Table 1 Relationship between clinicopathological variables and the VEGFR1 mRNA expression in peripheral blood bone and marrow from gastric cancer cases

	VEGFR1 -bone marrow				VEGFR1 -peripheral blood			
	Positive		Negative		Positive		Negative	
Features	n	(n=23)	(n=67)	P value	n	(n=34)	(n=56)	P-value
Sex								
Male	61	16	45	0.83	61	26	35	0.17
Female	29	7	22		29	8	21	
Age (mean±s.d.)		62.7±13.4	59.6±11.1	0.14		62.5±11.2	59.2±12	0.1
Stage								
1 and 2	55	9	46	<0.05	55	13	42	<0.001
3 and 4	35	14	21		35	21	14	
Invasion depth								
Slighter than submucus	28	4	24	0.09	28	5	23	<0.01
Deeper than muscularis propria	60	19	43		62	29	33	
Lymphatic involvement								
Negative	58	10	48	<0.05	58	14	44	<0.001
Positive	32	13	19		32	20	12	
Vascular involvement								
Negative	65	14	51	0.16	65	16	49	<0.0001
Positive	25	9	16		25	18	7	
Lymph node metastasis								
Negative	62	10	52	<0.01	62	15	47	<0.0001
Positive	28	13	15		28	19	9	
Pentoneal lavage cytology								
Negative	71	14	57	<0.05	71	20	51	<0.001
Positive	19	9	10		19	14	5	
Pentoneal dissemination								
Negative	82	20	62	0.42	82	30	52	0.46
Positive	8	3	5		8	4	4	
Perioperative overt metastasis								
Negative	72	16	56	0.15	72	23	49	<0.05
Positive	18	7	11		18	11	7	
Postoperative recurrence								
Negative	78	16	62	<0.01	78	25	53	<0.01
Positive	12	7	5		12	9	3	

s.d. = standard deviation.

cells except the monocytes between a gastric cancer patient with metastasis and a patient without metastasis (data not shown). Our present study revealed that the original cells producing VEGFR1 may be monocytes. Bone marrow and peripheral blood samples contain many white blood cells, including monocytes, in addition to a few circulating CECs or HPCs. In patients with advanced cancer, IL-10 or IL-12 are expressed more frequently than in patients with early cancer or normal volunteers (Sugai *et al*, 2004). These findings indicate that the monocyte–macrophage lineage is activated in patients with advanced cancer, and *VEGFR1* may be expressed by this lineage (Sawano *et al*, 2001). In general, the metastatic pathway in gastric cancer is not haematogenous, and therefore, the responsible cells expressing VEGFR1 may be monocytes and not HPCs and CECs. A last possibility is that VEGFR1 originates from circulating cancer cells in the bone marrow or peripheral blood (Fan *et al*, 2005; Yang *et al*, 2006). However, the number of circulating cancer cells is very low, which allow us to ignore the possibility that cancer cell are the origin of VEGFR1 expression.

To our knowledge, this study is the first to describe the detection of *VEGFR1* mRNA in the circulating blood of cancer patients. It will be very important to determine in advanced cases which cells produce *VEGFR1* mRNA in the peripheral blood. In brief, using flow cytometry analysis to detect VEGFR1-expressing cells in blood, we found that the number of VEGFR1-positive cells in peripheral blood was distinctly larger in a gastric cancer case with metastasis than in a case without metastasis. In gastric cancer cases with metastases, VEGFR1-positive monocytes were more abundant than the other cells, including CECs and HPCs. Because there are much fewer CECs and HPCs than monocytes, the *VEGFR1* mRNA which we detected in this study may be of monocyte origin. That VEGFR1-positive HPCs and CECs may also contribute to gastric cancer progression has been supported by several reports. Additional study is needed to verify the function of individual VEGFR1-expressing cells.

In conclusion, the evaluation of *VEGFR1* mRNA in the peripheral blood samples of gastric cancer patients could be very important, because it may be a valuable marker for cancer metastasis or recurrence. When considering the clinical application of this marker, it is a fortuitous finding, because from a practical standpoint, it is easier to obtain peripheral

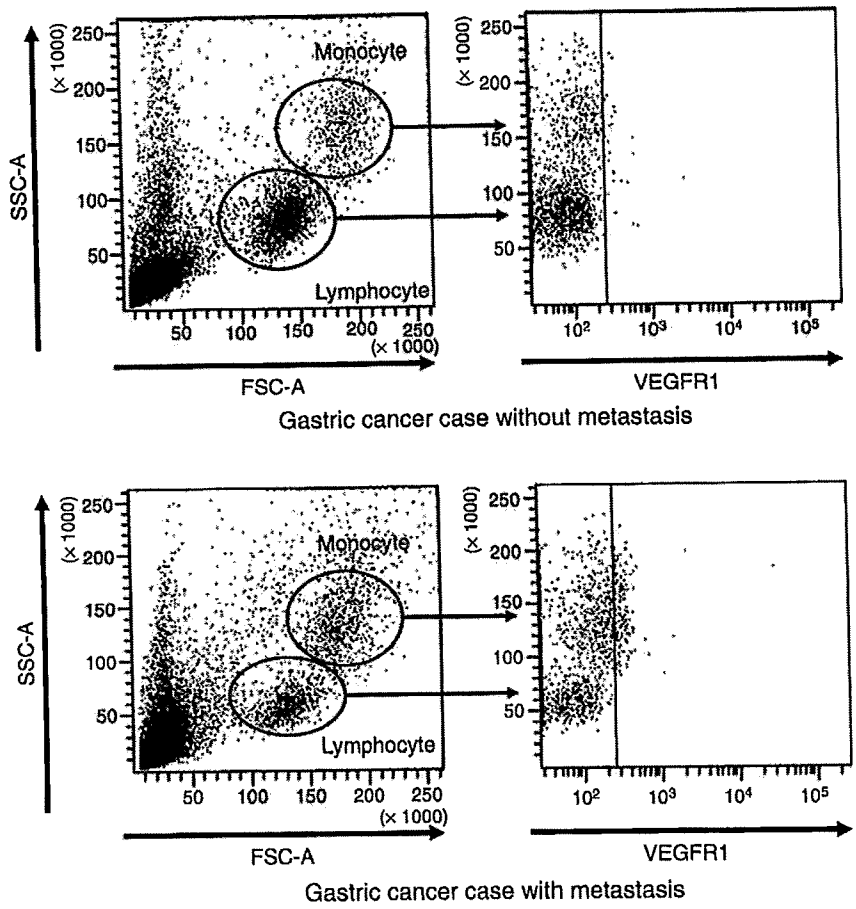

Figure 3 *VEGFR1* expression in blood of gastric cancer cases with or without metastasis. The number of *VEGFR1*-positive cells in a representative gastric cancer case with metastasis was more abundant (9.8%) than a case without metastasis (2.5%). Particularly, *VEGFR1*-positive cells in the fraction of monocytes in FS/SS plots were more abundant than those cells in the fraction of lymphoid cells.

blood samples than bone marrow samples. In addition, our final goal will be to evaluate the protein level of VEGFR1 in blood samples of cancer patients, to determine its practical use as a tumour marker.

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REFERENCES

Beerepoot LV, Mehra N, Vermaat JS, Zonnenberg BA, Gebbink MF, Voest EE (2004) Increased levels of viable circulating endothelial cells are an indicator of progressive disease in cancer patients. *Ann Oncol* **15:** 139–145

Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, Lammoglia R, Charnock-Jones DS (1998) A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod* **59:** 1540–1548

Fan F, Wey JS, McCarty MF, Belcheva A, Liu W, Bauer TW, Somcio RJ, Wu Y, Hooper A, Hicklin DJ, Ellis LM (2005) Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells. *Oncogene* **24:** 2647–2653

Ferrara N, Davis-Smyth T (1997) The biology of vascular endothelial growth factor. *Endocr Rev* **18:** 4–25

Galland F, Karamysheva A, Pebusque MJ, Borg JP, Rottapel R, Dubreuil P, Rosnet O, Birnbaum D (1993) The FLT4 gene encodes a transmembrane tyrosine kinase related to the vascular endothelial growth factor receptor. *Oncogene* **8:** 1233–1240

Hiratsuka S, Maru Y, Okada A, Seiki M, Noda T, Shibuya M (2001) Involvement of Flt-1 tyrosine kinase (vascular endothelial growth factor receptor-1) in pathological angiogenesis. *Cancer Res* **61:** 1207–1213

Japanese Research Society for Gastric Cancer (1999) *Japanese Classification of Gastric Carcinoma*. Kanehara: Tokyo

Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* **438:** 820–827

Kendall RL, Thomas KA (1993) Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci USA* **90:** 10705–10709

Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R, Rafii S (2001) Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med* **7:** 1194–1201

Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F (2001) Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. *Blood* **97:** 3658–3661

Masuda TA, Inoue H, Nishida K, Sonoda H, Yoshikawa Y, Kakeji Y, Utsunomiya T, Mori M (2003) Cyclin-dependent kinase 1 gene expression is associated with poor prognosis in gastric carcinoma. *Clin Cancer Res* **9:** 5693–5698

Masuda TA, Inoue H, Sonoda H, Mine S, Yoshikawa Y, Nakayama K, Nakayama K, Mori M (2002) Clinical and biological significance of S-phase kinase-associated protein 2 (Skp2) gene expression in gastric carcinoma: modulation of malignant phenotype by Skp2 overexpression, possibly via p27 proteolysis. *Cancer Res* 62: 3819–3825

Mori M, Mimori K, Inoue H, Barnard GF, Tsuji K, Nanbara S, Ueo H, Akiyoshi T (1995) Detection of cancer micrometastases in lymph nodes by reverse transcriptase-polymerase chain reaction. *Cancer Res* 55: 3417–3420

Ogawa K, Utsunomiya T, Mimori K, Tanaka Y, Tanaka F, Inoue H, Murayama S, Mori M (2004) Clinical significance of elongation factor-1 delta mRNA expression in oesophageal carcinoma. *Br J Cancer* 91: 282–286

Pajusola K, Aprelikova O, Korhonen J, Kaipainen A, Pertovaara L, Alitalo R, Alitalo K (1992) FLT4 receptor tyrosine kinase contains seven immunoglobulin-like loops and is expressed in multiple human tissues and cell lines. *Cancer Res* 52: 5738–5743

Sasse EA (2000) How to define and determine reference intervals in the clinical laboratory: approved guideline. Clinical and Laboratory Standards Institute: Wayne, Pennsylvania

Sawano A, Iwai S, Sakurai Y, Ito M, Shitara K, Nakahata T, Shibuya M (2001) Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. *Blood* 97: 785–791

Shibuya M, Ito N, Claesson-Welsh L (1999) Structure and function of vascular endothelial growth factor receptor-1 and -2. *Curr Top Microbiol Immunol* 237: 59–83

Sugai H, Kono K, Takahashi A, Ichihara F, Kawaida H, Fujii H, Matsumoto Y (2004) Characteristic alteration of monocytes with increased intracellular IL-10 and IL-12 in patients with advanced-stage gastric cancer. *J Surg Res* 116: 277–287

Yang AD, Camp ER, Fan F, Shen L, Gray MJ, Liu W, Somcio R, Bauer TW, Wu Y, Hicklin DJ, Ellis LM (2006) Vascular endothelial growth factor receptor-1 activation mediates epithelial to mesenchymal transition in human pancreatic carcinoma cells. *Cancer Res* 66: 46–51

Tailoring treatments for curable gastric cancer

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Although its incidence is decreasing worldwide, gastric cancer is still a major cause of death. There is remarkable geographic variation, with 60 per cent of cases arising in Eastern Asia. In Japan and Korea, public access to endoscopy is assured and almost half of newly diagnosed patients are detected at an early stage. Surgeons in these countries have been able to develop new and exciting minimally invasive therapeutic options. In the West, on the other hand, most patients still present with advanced disease and the treatment options are limited. Furthermore, Western patients are often obese and unfit for surgery, making optimal gastrectomy difficult. Wherever they are in the world, however, surgeons must lead the treatment strategy for potentially curable gastric cancer because without resection there will be no cure.

Depth of tumour invasion (T) and lymph node metastasis are the most important prognostic factors, and they correlate closely with each other[1]. Clinical staging of lymph node status is unreliable, especially for early tumours, while the preoperative diagnosis of T1, and intraoperative distinction between T1/2 and T3/4, can be made quite accurately. So, unless extensive nodal metastasis is clinically evident, the T-stage serves as a key factor in therapeutic planning.

T1 tumours, or early gastric cancers, have a low risk of nodal metastasis and a gastrectomy with limited lymphadenectomy is sufficient for cure. Pylorus- and/or vagus-preserving gastrectomy, and laparoscopic surgery, are recent options in Japan and Korea. Some T1 tumours are even resected at endoscopy, without surgery[2]. The rationale for endoscopic mucosal resection derives from a meticulous analysis of the lymph node status of a large number of patients treated by gastrectomy; when an endoscopically resected tumour satisfies certain criteria, one can be confident that the patient is very unlikely to have nodal metastasis because hundreds of tumours in the same category have had no associated nodal metastasis. Surgeons should be aware of this option for early tumours, since the avoidance of gastrectomy has significant quality of life benefits for patients.

T2 gastric cancer might be regarded as localized disease, but it is associated with more frequent (over 50 per cent) and extensive nodal metastasis than T1, so sufficient lymphadenectomy should be planned. Systematic dissection of the nodes around the coeliac artery and its branches (D2) permits resection of the positive nodes associated with most T2 tumours. Hepatic metastases are rare. T1 and T2 gastric cancers are localized lesions that can be cured by surgery alone, and surgeons should take that responsibility.

Once the tumour penetrates the serosa (T3) or invades adjacent organs (T4), it begins to spread by routes other than the lymphatic system, notably through peritoneal dissemination and in the portal–hepatic blood. Furthermore, lymph node metastasis from T3/4 tumours sometimes overwhelms the regional network, with cancer cells entering the systemic circulation to cause bone and lung metastases. These are effectively beyond the surgeon's reach. In addition to these metastases, the primary lesion becomes larger and more infiltrative and the chance of obtaining an R0 resection diminishes. As a consequence, more than half the patients with T3/T4 tumours develop local or systemic recurrence of disease, which is almost always fatal.

Some surgeons are inclined to regard T3 and T4 gastric cancers as incurable, but the role of surgery should not be underestimated, even at these stages. Some local recurrence may be prevented by careful gastrectomy. Gastric and duodenal stump recurrence at least should be preventable by careful pre- and intraoperative histological examination of the resection margins. Other local recurrence can be attributed to residual lymph node metastasis around the coeliac artery. Complete clearance of the tumour-bearing nodes by D2 lymphadenectomy should diminish this problem and prolong survival. Japanese surgeons have believed this to be so for many years and two recent randomized controlled trials have now provided evidence to support the 'D2 concept' both directly and indirectly. One is the Taipei single-institution study comparing D1 and former D3 (current D2); this was completed without operative mortality and showed a significant survival benefit for D2[3]. The other is the American Intergroup study in which chemoradiation therapy to the gastric bed after limited lymphadenectomy (D0/D1) significantly decreased the local recurrence rate and increased long-term survival[4]. This can be

interpreted as showing that radiotherapy eliminated residual lymph node metastasis, which would have been removed by D2 resection.

The Intergroup study seems to have changed the standard care for gastric cancer in the USA, but its impact has been weak in Japan and Korea, where D2 lymphadenectomy is routinely and safely performed, and where local recurrence is not a major pattern of relapse. D2 lymphadenectomy is, however, technically demanding, with a pronounced learning curve. Patient fitness for surgery is another important factor for a safe operation, and patient obesity hampers the performance of even the most experienced surgeons[5]. When a safe D2 procedure cannot be expected due to any of these factors, adjuvant chemoradiotherapy might prove an adequate substitute. Surgeons now have alternatives for local tumour control and it is they who should assume responsibility for designing the best treatment for each patient.

Many randomized trials of adjuvant chemotherapy have failed to produce solid evidence of effect in patients with resectable cancers who are at high risk of systemic recurrence. However, the MAGIC trial in Europe has recently shown that a significant survival benefit accrues from peri-operative combination chemotherapy[6]. The role of lymphadenectomy is obscure in this trial because it was not standardized and simply left up to the choice of the individual surgeon. One must interpret the results as demonstrating that peri-operative chemotherapy has enough power to offset the influence of surgical diversity. Since the publication of this trial it has become more important than ever for surgeons to consider the treatment options for their patients before they operate.

In conclusion, the result of treatment for locally advanced gastric cancer is the sum of the effect of local tumour control by surgery, with or without radiotherapy and/or systemic chemotherapy. The role of each treatment modality varies according to the stage of disease, individual patient risk, surgical volume, available chemotherapy regimens and quality of radiotherapy. Evidence of the effect of different combinations of treatments should be established for each clinical circumstance and surgeons should play a key role here.

References

1 Sasako M, Sano T, Katai H, Maruyama K. Radical surgery. In: *Gastric Cancer*. Sugimura T, Sasako M, eds. Oxford University Press: Oxford, 1997; 223–248.

2 Soetikno R, Kaltenbach T, Yeh R, Gotoda T. Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract. *J Clin Oncol* 2005; **23**: 4490–4498.

3 Wu CW, Hsiung CA, Lo SS, Hsieh MC, Chen JH, Li AF *et al.* Nodal dissection for patients with gastric cancer: a randomised controlled trial. *Lancet Oncol* 2006; **7**: 309–315.

4 Macdonald JS, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN *et al.* Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med* 2001; **345**: 725–730.

5 Kodera Y, Sasako M, Yamamoto S, Sano T, Nashimoto A, Kurita A *et al.* Identification of risk factors for the development of complications following extended and superextended lymphadenectomies for gastric cancer. *Br J Surg* 2005; **92**: 1103–1109.

6 Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M *et al.* Perioperative chemotherapy *versus* surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11–20.

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Surgical Treatment of Advanced Gastric Cancer: Japanese Perspective

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Key Words
Esophagogastric junction · Gastric cancer, advanced · Surgical treatment

Abstract
The results of clinical trials regarding surgery of curable advanced gastric cancer and esophagogastric junction (EGJ) tumors are reviewed and summarized. Four clinical trials have evaluated D2 dissection for curable gastric cancer in the West. Two large trials in the UK and the Netherlands failed to prove the efficacy of D2 dissection. However, these trials had critical weak points. As they were carried out in a number of hospitals where there was no experience with this surgery, the quality of surgery and postoperative care were very poor making the hospital mortality unacceptably high. After these trials, an Italian group started a phase II study in 8 hospitals with a relatively high volume to confirm the safety of this procedure for Caucasians. They achieved 3% mortality, which was much smaller than that of even D1 in the former trials. These results first highlighted the importance of learning and hospital volume in D2 dissection. Survival results of the Dutch trial showed some difference between D1 and D2, but the difference was not statistically significant. This was attributed to the high hospital mortality and poor quality of surgery, especially low compliance of D2 and the high rate of extension of D1, making this comparison similar to that between D1.3 and D1.7. The results of the phase III study by the Italian group are awaited. Recently a Taiwanese trial proved the benefit of D2 dissection over D1 in a phase III trial. This was a single institutional trial with a sample size of 221 patients. The 5-year survival rate of D2 and D1 was 59.5 and 53.6%, respectively ($p = 0.04$). The Dutch trials for EGJ tumors showed a large difference in overall survival between the transthoracic and transhiatal approach for Siewert type 1 and 2 tumors, but this was not statistically significant, most likely due to the small sample size. In the subgroup analysis, they demonstrated that there was no survival difference in Siewert type 2 but a large difference in Siewert type 1. A Japanese study showed that there is no benefit to the thoraco-abdominal approach over the transhiatal approach for EGJ tumors whose invasion in the esophagus is 3 cm or less. These two trials clearly demonstrated that mediastinal dissection through a right thoracotomy is recommendable for Siewert type 1, while the transhiatal approach should be considered as standard for Siewert type 2.

Background

In the guidelines of the Japan Gastric Cancer Association, standard surgery for curable advanced gastric cancer is defined as a more than 2/3 gastrectomy with D2 dissection [1]. With the results of several important

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Table 1. Morbidity and mortality after D2 dissection and hospital volume

Trial	Type	n	Number of patients per hospital per year	Mortality %	Morbidity %	Reference
Hong Kong	RCT	30	7.5	3	57	Robertson et al. [7]
MRC	RCT	200	1.5	13	46	Cuschieri et al. [8]
Dutch	RCT	331	1.0	10	43	Bonenkamp et al. [2]
Taiwanese	RCT	211	18.5	0	17	Wu et al. [16]
IGCSG	Phase II	191	8.0	3	21	Degiuli et al. [4]
IGCSG	RCT	82	4.3	0	16	Degiuli et al. [6]
Italian study	Retro	451	21.5	2	17	Roviello et al. [9]

RCT = Randomized controlled trial; MRC = Medical Research Council; IGCSG = Italian Gastric Cancer Study Group.

clinical trials, not only in surgery but also multidisciplinary treatment, this policy of the Japanese guidelines might be challenged. In this article, the Japanese perspective of curative surgery for advanced gastric cancer is explained.

Results of European Trials

There have been four European clinical trials on D2 dissection for curable gastric cancer [2–5]. Three of them were phase III trials and the remainder was the only phase II trial in the world. The phase III trials were carried out by the Medical Research Council (MRC) [3], the Dutch Gastric Cancer Group (DGCG) [2] and the Italian Gastric Cancer Study Group (IGCSG) [5]. The first two trials have already shown negative results, while the long-term results of the last one are awaited. After the first two large phase III trials showed quite high hospital mortality after D2 dissection on Caucasians, the IGCSG started with a phase II study to confirm the safety of the D2 dissection in their population [4].

Morbidity and Mortality of D2 Dissection in These Trials

The Dutch and the MRC studies showed extremely high hospital mortality after D2 dissection, 10 and 13%, respectively. Such a high mortality is no longer accepted for any cancer surgery today. These results were heavily criticized and attributed to a very low hospital volume [6]. Table 1 shows the clear negative correlation between hospital volume and hospital mortality after D2 dissection in the literature. This high mortality was also attributed to splenectomy and pancreatectomy. Especially in the MRC trial, many surgeons thought that D2 distal gastrectomy included splenectomy, and splenectomy was carried out in many distal gastrectomy cases [10]. This was based on the misunderstanding of the definition of D2 gastrectomy by the Japanese Research Society for Gastric Cancer [11]. In Japan, splenectomy was included in D2 dissection only when a total gastrectomy was carried out. Together with thorough lymph node dissection of the lesser curvature, splenectomy causes serious ischemia of the remnant stomach, necrosis of the remnant stomach or anastomotic leakage. This was also the case in the DGCG trial [12]. In the multivariate analysis of hospital mortality, splenectomy was one of the factors most responsible for mortality. The lack of experience in treating major surgical complications after D2 dissection, namely, anastomotic leakage, pancreatic fistula (juice leak) or intra-abdominal abscess, led to a much higher mortality than a Japanese specialist center where a few hundred patients were treated yearly (table 2) [6]. With less than a few cases yearly, surgeons can never learn how to treat these major complications to avoid treatment-related death. This high mortality after D2 dissection in the Dutch trial might also be attributed to the greater fragility of the Dutch compared with the Japanese. However, the results of another Dutch trial comparing a transthoracic esophagogastrectomy via right thoracotomy with a transhiatal approach for esophagogastric junction (EGJ) tumors showed a much lower mortality in the both treatment arms, 4% for the former and 2% for the latter [13]. This trial was carried out exclusively in two major cancer hospitals which have a reasonably high hospital volume. This suggests that high mortality in the D1/D2 trial was not attributed to the fragility of the Dutch patients but to the very low hospital volume.

Table 2. Mortality after postoperative major surgical complications

Complication	Dutch trial (n = 711)			NCCH trial (1982–1987; n = 1,197)			p
	deceased patients	affected patients	%	deceased patients	affected patients	%	
Leakage	19	46	41.3	12	84	14.3	0.0005
Distal	9	22	40.1	2	23	8.7	0.012
Total	10	24	41.7	10	60	16.7	0.0047
Abscess or pancreatic fistula	19	91	20.9	2	75	2.7	0.0004

NCCH = National Cancer Center Hospital.

After these two trials with dismal short-term results, the IGCSG started a phase II trial to confirm the safety. Actually a 3% mortality was found in 8 hospitals with a total of 191 patients [4]. They avoided the routine use of distal pancreatectomy in cases of total gastrectomy; instead they adopted pancreas-preserving total gastrectomy, the so-called Maruyama technique [5]. Thus they avoided splenectomy in distal gastrectomy and distal pancreatectomy in total gastrectomy. The morbidity and mortality shown by the phase II study was confirmed by the results of the interim analysis of the IGCSG phase III trial. Hospital mortality was 1.3% after D1 but 0% after D2 gastrectomy in this study [6].

Survival Results after D2 Dissection

In the MRC trial, the survival curve of D2 was never better than that of D1 until the end of the trial. In the Dutch trial, the survival curve of D2 caught up with that of D1 after 4 years and remained superior, but the difference between D1 and D2 survival never reached statistical significance. Practically, in the MRC trial, there was no quality control of surgery and the quality seemed poor due to the mortality. In the Dutch trial, there were several efforts to control the quality of performance including direct tuition of the D2 dissection in the operation theater and quality evaluation by the number of dissected nodes. According to their results, there were many cases in the D1 group where more extended dissection than D1 was actually carried out and many patients in the D2 group underwent less than D2 dissection [14]. Eventually they compared D1.3 versus D1.7, for example, minimizing the difference between the arms. Low-quality surgery together with a much higher mortality immediately after surgery could explain why D2 dissection was not found to be beneficial. In fact, the Italian group showed much better survival results in their phase II trial than those of the Dutch trial [15]. The 5-year survival rates for stages IA, IB, II, IIIA and IIIB were 93, 88, 60, 40 and 20%, respectively, while those in the Dutch trial were 81, 61, 42, 28 and 13%, respectively. Survival results of the phase III study by the IGCSG are awaited.

Results of Taiwanese Trial

Recently a Taiwanese hospital published the results of a phase III study comparing D1 versus D2/3 surgery for curable gastric cancer in a single institution [16]. Their D3 includes lymph node stations in the hepatoduodenal ligament, on the superior mesenteric vein, behind the common hepatic artery and on the posterior pancreatic surface in addition to D2 dissection, according to the 1st English Edition of the Japanese Classification of Gastric Carcinoma [17]. They showed statistically significant improvement in survival by D2/3 surgery over D1. The 5-year overall survival of D2/3 and D1 was 59.5 and 53.6%, respectively ($p = 0.04$; fig. 1). This study included only three surgeons at a single institution, therefore the quality of surgery in this study seemed to be more identical than in multicenter trials. This is the first randomized controlled study which showed significantly better overall survival of D2/3 surgery than D1 in the world. There are several remarkable differences between this study and the Dutch study. Due to the much higher hospital volume and good quality control at a single institution, the hospital mortality after D2/3 was 0% in this study, while it was as high as 10% in the Dutch trial. More patients in the Taiwanese study had antral tumors and underwent distal subtotal gastrectomy than the Dutch trial. The proportion of those who underwent distal subtotal gastrectomy in this study and the Dutch study was 76 and 66%, respectively. Due to the rather small sample size and

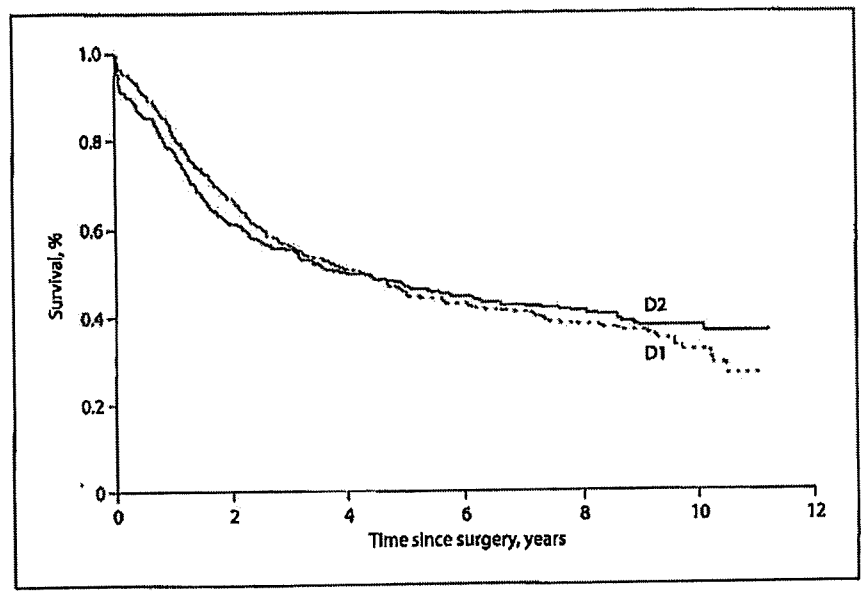

Fig. 1. Overall survival curves of the entire patient population by treatment groups in the Dutch trial.

modest survival benefit, this study cannot be considered as solid evidence for the superiority of D2 over D1 dissection.

Results of Adjuvant Chemoradiotherapy

A phase III study comparing surgery alone with postoperative adjuvant chemoradiotherapy (CRT), the INT0116/SWOG9008, showed a large survival benefit of CRT for curable gastric cancer; the median survival time of surgery alone was 27 months, compared with 36 months for CRT [18]. The hazard ratio for death was 1.35 (95% CI 1.09–1.66; p = 0.005). In this trial, the tested arm included curative surgery and radiation therapy of 45 Gy with combination chemotherapy using fluorouracil and leucovorin (5 courses of 5-day continuous infusion, including 2 courses of concomitant administration). However, detailed analysis of the type of surgery revealed that 54 and 36% of the patients underwent D0 and D1 surgery, respectively, while only 10% underwent D2 dissection. Although there was no statistically significant interaction between the subgroups divided by the degree of lymph node dissection and the effect of treatment, a benefit from treatment was observed only in the D0 or D1 group in the subset analysis [19]. In the retrospective detailed analysis, the researchers of this study found that surgical undertreatment clearly undermined the survival of patients [20]. Thus this study for the first time proved the efficacy of local control by radiation for gastric cancer and proved that limited surgery alone cannot be sufficient treatment for this cancer.

The patient population enrolled in the test arm of this study was by chance quite similar to the population enrolled in a Japanese clinical trial comparing surgery alone with surgery followed by adjuvant CTX (JCOG9206-2) [21]. Table 3 shows the tumor and patient characteristics of the 2 groups. Most of the prognostic factors, i.e., histological type, tumor location, age, tumor size, and, most important, tumor depth, were reasonably comparable between the groups. Although these 2 groups were the patients of two different trials with two different treatment methods, they are identical and therefore the treatment results are more or less comparable. The 5-year overall survival was 42 and 61% in the INT0116 and JCOG9206-2, respectively. This suggests strongly that D2 surgery alone might produce better survival than limited surgery followed by CRT and that the effect of adjuvant CTX might not be expected after D2 as suggested by the subgroup analysis.

Surgical Treatment for Esophagogastric Junction Tumors

Hulscher et al. [13] reported the results of a phase III trial for Siewert type 1 and 2 tumors, comparing two surgical approaches, a transthoracic esophagogastrectomy

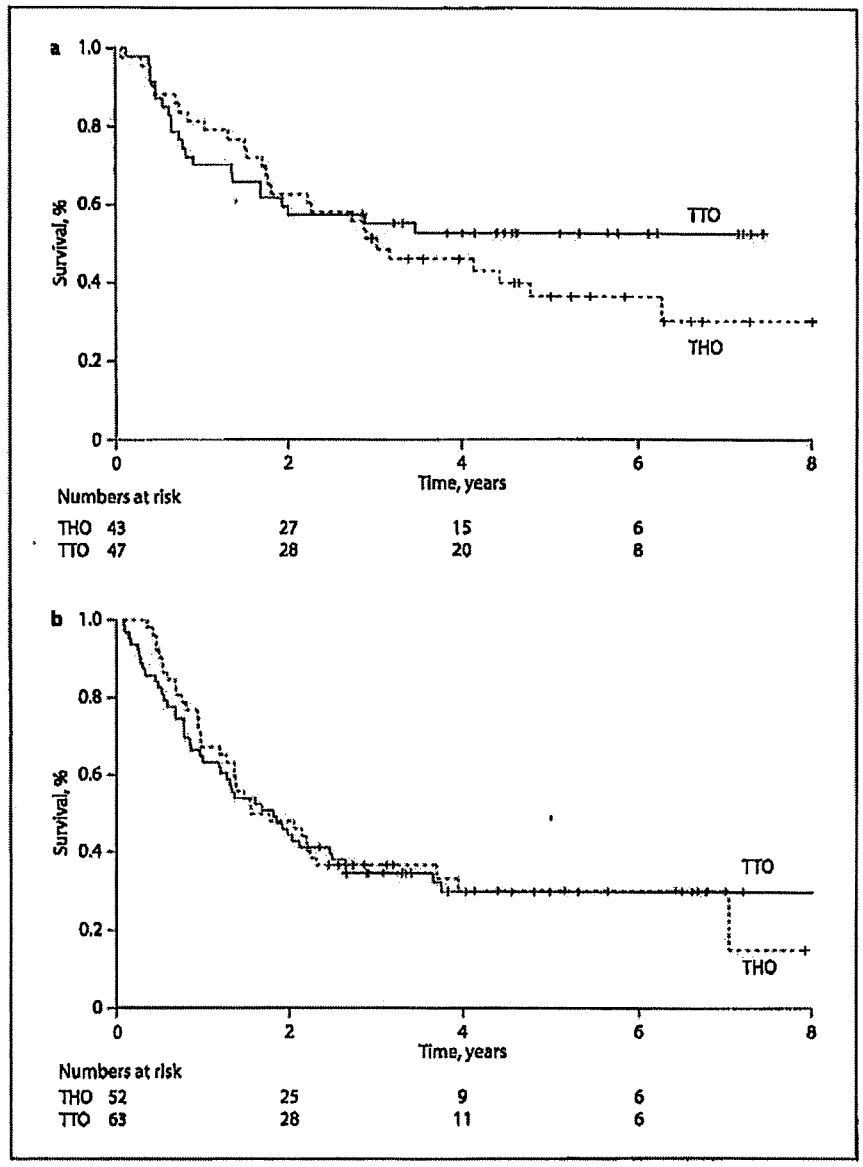

Fig. 2. Overall survival curves in patients with Siewert type 1 (**a**) and Siewert type 2 (**b**) tumors, by treatment groups. THO = Transthoracic esophagectomy; TTO = transhiatal esophagectomy.

via right thoracotomy with transhiatal one. The overall survival in the entire study population did not show statistically significant differences between the 2 groups. However, the actual difference in the survival curves was impressive and the overall 5-year survival rate was 29% for the transhiatal approach and 39% for the transthoracic one ($p = 0.38$; fig. 1). In the subgroup analysis according to the Siewert classification, the difference in overall 5-year survival was as large as 17% (95% CI –3 to 37%) for Siewert type 1 ($n = 90$), while it was only 1% for Siewert type 2 ($n = 115$; fig. 2) [22]. Due to the small sample size, this study was not able to show any statistically significant difference, but the results strongly suggest that thorough mediastinal dissection via right thoracotomy is needed for Siewert type 1 but not for type 2. With higher morbidity after transthoracic dissection, the transhiatal approach might be better treatment for Siewert type 2.

Sasako et al. [23] reported the results of a phase III trial for Siewert type 2 and 3 tumors, comparing a left thoraco-abdominal approach versus a transhiatal one. All these tumors were diagnosed to have esophageal in-

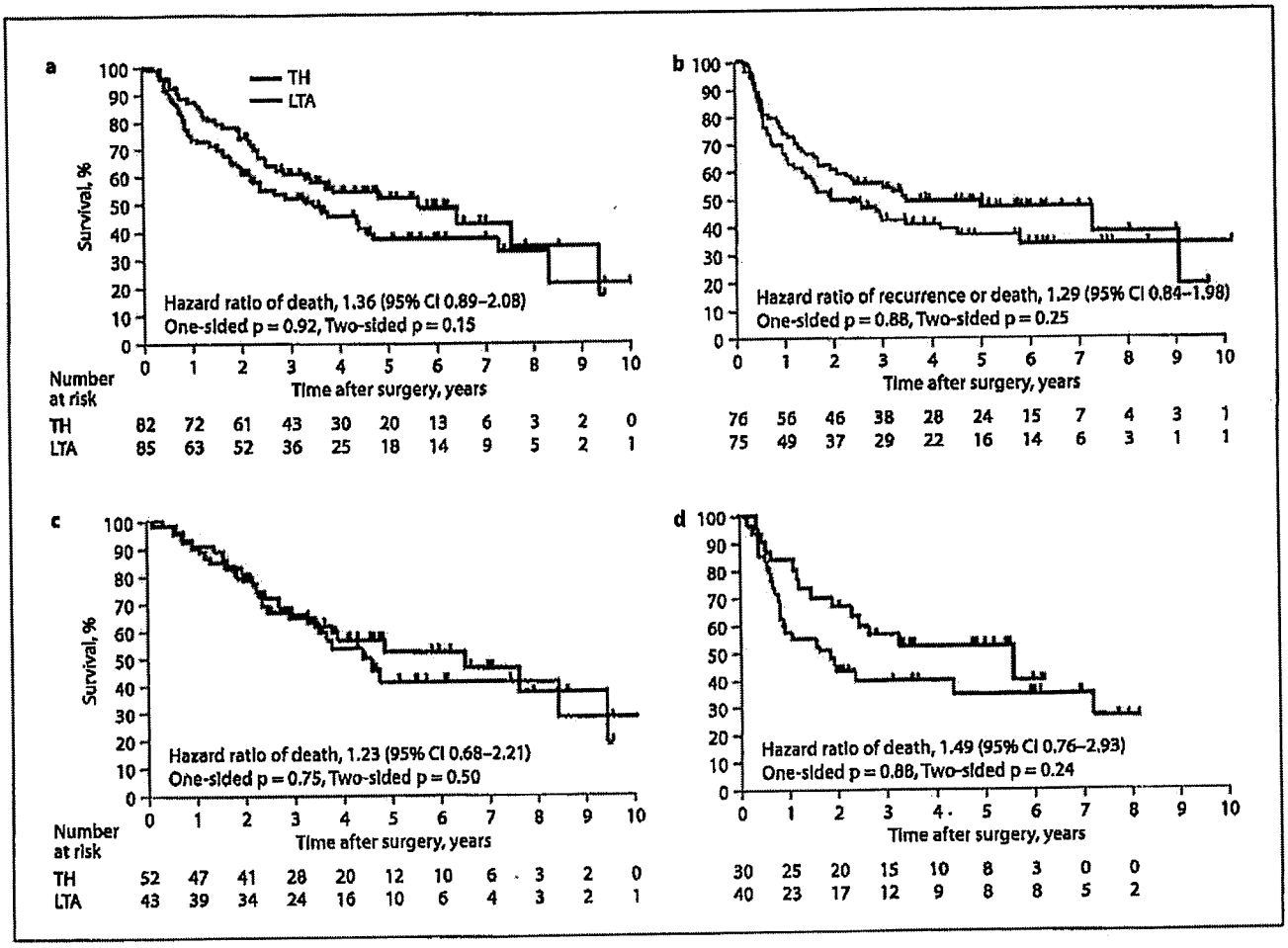

Fig. 3. Overall survival (a) and disease-free survival (b) of the entire patient population and overall survival in patients with Siewert type 2 (c) and type 3 (d) tumors by treatment groups. TH = Transhiatal; LTA = left thoraco-abdominal. Reprinted with permission from *The Lancet Oncology* [23].

Table 3. Comparison between the INT0116 study and JCOG9206-2 study

	IT0116/SWOG9008	JCOG9206-2
Surgery (D0/1/2), %	54/36/10	4/67/33
Adjuvant	Rad (45 Gy)+CX (5FU+LV)	CDDP+5FU+UFT (50%), none (50%)
Number of patients	281 (tested arm)	268 (control = 133, tested = 135)
Tumor location	A (53%), Corp (24%), cardia (21%), multifocal (2%)	L (31%), M (32%), U (28%), wide (9%)
pT (T1/T2/T3/T4)	14/74/175/18	5/87/165/11
Proportion of T3/4, %	69	66
Node positive, %	85	72
TRD	3 (1.1%)	4 (1.5%)
Overall survival (5 years), %	42	control 61, tested 62

Rad = Radiation; CX = chemotherapy; LV = leucovorin; 5FU = 5-fluorouracil; CDDP = cis-diamminedichloroplatinum; UFT = uracil-ftegafur; A = antrum; Corp = gastric body; L = distal one third; M = middle one third; U = upper one third; wide = wide spread; TRD = treatment-related death.

vasion of 3 cm or less. They clearly demonstrated that there was no survival benefit from the left thoraco-abdominal approach which was accompanied by a much higher morbidity and more remarkable deterioration of pulmonary function than the transhiatal approach. The subgroup analysis showed no survival benefit for both Siewert type 2 and 3. Especially for Siewert type 3, the transhiatal approach showed much better survival than the left thoracotomy approach (fig. 3).

From these two trials, the transhiatal approach is regarded as the standard treatment for Siewert type 2 and 3 tumors, while the transthoracic approach via right thoracotomy is recommended for Siewert type 1 tumors.

References

1 Nakajima T: Gastric cancer treatment guideline in Japan. Gastric Cancer 2002;5:1–5.
2 Bonenkamp JJ, Hermans J, Sasako M, van De Velde CJ, et al; Dutch Gastric Cancer Group: Extended lymph-node dissection for gastric cancer. N Engl J Med 1999;340:908–914.
3 Cuschieri A, Weeden S, Fielding J, Bancewicz J, Craven J, Joypaul V, Sydes M, Fayers P: Patient survival after D1 and D2 resection for gastric cancer: long-term results of the MRC randomized surgical trial. Br J Cancer 1999;79:1522–1530.
4 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.
5 Maruyama K, Sasako M, Kinoshita T, Sano T, Katai H, Okabayashi K: Pancreas-preserving total gastrectomy for proximal gastric cancer. World J Surg 1995;19:532–536.
6 Degiuli M, Sasako M, Calgaro M, Garino M, Rebecchi F, Mineccia M, Scaglione D, Andreone D, Ponti A, Calvo F: Morbidity and mortality after D1 and D2 gastrectomy for cancer: interim analysis of the Italian Gastric Cancer Study Group (IGCSG) randomised surgical trial. Eur J Surg Oncol 2004;30:303–308.
7 Robertson CS, Chung SC, Woods SD, et al: a prospective randomized trial comparing R1 subtotal gastrectomy with R3 total gastrectomy for antral cancer. Ann Surg 1994;220: 176–182.
8 Cuschieri A, Fayers P, Fielding J, Craven J, Bancewicz J, Joypaul V, Cook P; Surgical Cooperative Group: Postoperative morbidity and mortality after D1 and D2 resections for gastric cancer: preliminary results of the MRC randomised surgical trial. Lancet 1996; 347:995–999.
9 Roviello F, Marrelli D, Morgagni P, de Manzoni G, Di Leo A, Vindigni C, Saragoni L, Tomezzoli A, 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 2002;9:894–900.
10 Sasako M: Principles of surgical treatment for curable gastric cancer. J Clin Oncol 2003; 21(suppl):274s–275s.
11 Japanese Research Society for the Gastric Cancer: The general rules for the gastric cancer study in surgery and pathology. Jpn J Surg 1981;11:418–425.
12 Sasako M: Risk factors for surgical treatment in the Dutch Gastric Cancer Trial. Br J Surg 1997;84:1567–1571.
13 Hulscher JBF, van Sandick JW, de Boer AGEM, Wijnhoven BPL, Tijssen JGP, Fockens P, Stalmeier PFM, ten Kate FJW, van Dekken H, Obertop H, Tilanus HW, van Lanschot JJB: Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the esophagus. N Engl J Med 2002;347:1662–1669.
14 Bunt TMG, Bonenkamp JJ, Hermans J, van de Velde CJH, Arends JW, Fleuren G, Bruijn JA: Factors influencing noncompliance and contamination in a randomized trial of 'Western' (R1) versus 'Japanese' (R2) type surgery in gastric cancer. Cancer 1994;73: 1544–1551.
15 Degiuli M, Sasako M, Ponti A, Calvo F: Survival results of a multicenter phase II study to evaluate D2 gastrectomy for gastric cancer. Br J Cancer 2004;90:1727–1732.
16 Wu CW, Hsiung CA, Lo SS, Hsieh MC, Chen JH, Li AFY, Lui WY, Peng JW: Nodal dissection for patients with gastric cancer: a randomised controlled trial. Lancet Oncol 2006;7:309–315.
17 Japanese Gastric Cancer Association: Japanese Classification of Gastric Carcinoma, ed 1. Tokyo, Kanahara, 1995, p 15.
18 Macdonald JS, Smalley SR, Benedetti J, Este SANC, Stemmermann NG, Haller DG, Ajani JA, Gunderson LL, Jessup JM, Martenson JA: Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 2001;345:725–730.
19 Macdonald JS: Postoperative combined radiation and chemotherapy improves disease-free survival (DFS) and overall survival (OS) in resected adenocarcinoma of the stomach and gastroesophageal junction: update of the results of Intergroup Study INT-0116 (SWOG 9008). Virtual Meeting of ASCO GI Symposium.
20 Hundahl SA, Macdonald JS, Benedetti J, Fitzsimmons T: Surgical treatment variation in a prospective, randomized trial of chemoradiotherapy in gastric cancer: the effect of undertreatment. Ann Surg Oncol 2002;9: 278–286.
21 Miyashiro I, Furukawa H, Sasako M, Yamamoto S, Nashimoto A, Nakajima T, Kinoshita T, Kobayashi O, Arai K; Gastric Cancer Surgical Study Group of the Japan Clinical Oncology Group: No survival benefit with adjuvant chemotherapy for serosa-positive gastric cancer (JCOG9206-2). Proc 2005 Gastrointestinal Cancer Symp, p 84.
22 Hulscher JBF, van Lanschot JJ: Individualised surgical treatment of patients with an adenocarcinoma of the distal oesophagus or gastro-oesophageal junction. Dig Surg 2005; 22:130–134.
23 Sasako M, Sano T, Yamamoto S, Sairenji M, Arai K, Kinoshita T, Nashimoto A, Hiratsuka M: Left thoracoabdominal approach versus abdominal-transhiatal approach for gastric cancer of the cardia or subcardia: a randomised controlled trial. Lancet Oncol 2006;7:644–651.