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Guest Editors: Marco Bernini and Lapo Bencini

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Editorial Board

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Editorial

Multimodal Treatment of Gastric Cancer: Surgery, Chemotherapy, Radiotherapy, and Timing

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Surgery is no more alone in the scenario of gastric cancer treatment since many years. Although the encouraging results of adjuvant chemotherapy obtained in Japan with S-1 were not achieved in Western countries, a novel approach, after the results of MAGIC trial, published in 2006, has been introduced by means of perioperative, or just preoperative, chemotherapy in the West, with many trials ongoing.

Radiotherapy has been used from many years until now in the USA, with good results, and is part of many schedules in Europe as well. Chemotherapy has been proposed even in an intraperitoneal setting with hyperthermia, after extensive resection and peritonectomy (HIPEC).

Novel drugs, known as immunological, have also been introduced, following gastric cancer molecular genetics studies, which appears to be a new frontier of translational research, as for many other cancers, in order to tailor a specific treatment to each case.

Similar to that achieved with the management of colorectal and esophageal cancers, multimodal treatment is therefore mandatory, to date, for gastric cancer, but timing, type of therapies, and schedules seem to be a puzzle yet.

The present special issue tries to span the diverse and wide possibilities of gastric cancer treatments and topics, from endoscopic dissection to HIPEC, without foregoing molecular genetics aspects, which are all part of nowadays clinical practice of surgical oncologists. Prominent interest is, obviously, in results in terms of overall survival with different approaches as well as complete response to preoperative treatments. Nonetheless, the impact of multimodal treatments on surgical perioperative complications and side effects should be taken into account, considering the aging of oncologic patients.

One paper addresses a mini-invasive gastric cancer treatment, namely, submucosal dissection (ESD), which is a recent upgrade of the former mucosectomy. This technique is routine practice, when indicated, for early gastric cancers in Japan, while it is still not very well diffused in Western countries. The paper shows how this procedure is particularly helpful in elderly patients, without compromising the necessity of a subsequent gastrectomy, if needed.

Another paper shows the mechanisms of cisplatin-induced apoptosis and of cisplatin sensitivity. The authors also identify the molecular substrate called bridging integrator 1 (BIN1) to act as a potent predictor of cisplatin sensitivity in gastric cancer treatment. The importance of the translational research on clinical practice and patients selection for tailored chemotherapy is highlighted.

One of the papers sheds light on an exquisitely surgical issue, which has been debated for so long: splenectomy. This was maybe, as admitted by the authors themselves, the most important bias of Dutch and British trials, making them favouring D1 because of higher surgical complications and mortality of D2. The paper encompasses very well the problem of splenectomy, which is not necessary to obtain a D2 lymphadenectomy and is detrimental in terms of complications without achieving longer survivals.

Another study focuses on the effects of neoadjuvant intraperitoneal/systemic chemotherapy (bidirectional chemotherapy) for the treatment of patients with peritoneal metastasis from gastric cancer. By the multivariate analysis many patients had some survival benefit, but the high morbidity and mortality require stringent patients selection.

The authors of another paper present the survival benefit of adjuvant radiation therapy for gastric cancer following

gastrectomy and extended lymphadenectomy, demonstrating that the effect is not limited to patients treated with suboptimal surgery only.

The state of the art in the field of surgical treatment of peritoneal carcinomatosis from gastric cancer is reviewed by some authors. Concomitant systemic chemotherapy, cytoreductive surgery, and hyperthermic chemoperfusion showed promising results. Novel therapies, such as extensive intraperitoneal lavage and intraperitoneal targeted agents, are being applied in the management of this disease.

Lastly a paper draws our attention to genetics of gastric cancer, from a pathogenesis point of view to the clinical use of some biomarkers. HER2 is the only universally detected and used to proceed with the biological therapy of Trastuzumab, while VEGF is a promising biomarker not only for prognosis but also for therapy as well. hERG1 is, instead, one of many other biomarkers that might play a role in gastric cancer clinical practice.

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Research Article

Effects of Neoadjuvant Intraperitoneal/Systemic Chemotherapy (Bidirectional Chemotherapy) for the Treatment of Patients with Peritoneal Metastasis from Gastric Cancer

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Novel multidisciplinary treatment combined with neoadjuvant intraperitoneal-systemic chemotherapy protocol (NIPS) and peritonectomy was developed. Ninety-six patients were enrolled. Peritoneal wash cytology was performed before and after NIPS through a port system. Patients were treated with 60 mg/m^2 of oral S-1 for 21 days, followed by a 1-week rest. On days 1, 8, and 15, 30 mg/m^2 of Taxotere and 30 mg/m^2 of cisplatin with 500 mL of saline were introduced through the port. NIPS is done 2 cycles before surgery. Three weeks after NIPS, 82 patients were eligible to intend cytoreductive surgery (CRS) by gastrectomy + D2 dissection + periotnectomy to achieve complete cytoreduction. Sixty-eight patients showed positice cytology before NIPS, and the positive cytology results became negative in 47 (69%) patients after NIPS. Complete pathologic response on PC after NIPS was experienced in 30 (36.8%) patients. Stage migration was experienced in 12 patients (14.6%). Complete cytoreduction was achieved in 58 patients (70.7%). By the multivariate analysis, complete cytoreduction and pathologic response became a significantly good survival. However the high morbidity and mortality, stringent patient selection is important. The best indications of the therapy are patients with good pathologic response and PCI $\leq$ 6, which are supposed to be removed completely by peritonectomy.

1. Introduction

In the past, peritoneal carcinomatosis (PC) from gastric cancer has been regarded as a terminal stage [1], and the most oncologists regarded as a condition only to be palliated. Preusser et al. published a response rate to chemotherapy of 50% of patients with stage IV gastric cancer, but the response rate was the lowest in patients with PC [2]. Ajani et al. reported that PC was the most common indication of failure of the intensive chemotherapy [3]. Accordingly, surgery alone or chemotherapy alone is not an adequate management for gastric cancer patients with PC.

Over the past two decades, a new multimodal treatment called cytoreductive surgery (CRS) [4] plus perioperative chemotherapy (POC) was proposed. POC includes neoadjuvant chemotherapy (NAC), hyperthermic intraperitoneal chemotherapy (HIPEC), and/or early postoperative intraperitoneal chemotherapy (EPIC), which takes the advantage of surgery to reduce the visible tumor burden and POC to eradicate peritoneal micrometastasis and

peritoneal free cancer cells (PFCCs) [5]. Survival analyses after CRS plus HIPEC showed that complete cytoreduction is associated with survival improvement [5, 6]. Neoadjuvant chemotherapy is proposed to reduce tumor burden before operation, resulting in the improvement of the incidence of complete cytoreduction [5]. A new bidirectional chemotherapy (neoadjuvant intraperitoneal-systemic chemotherapy protocol (NIPS)) was developed to induce a reduction of the peritoneal cancer index of PC and to eradicate PFCCs [5]. NIPS can attack PC from both sides of peritoneum, not only from the peritoneal cavity but also from the subperitoneal blood vessels. Accordingly, NIPS is called as bidirectional chemotherapy.

In the present study, the effects of NIPS on the intraperitoneal cytological status, histological response of PC, the incidence of complete cytoreduction, and survivals in the patients with established PC form gastric cancer will be reported.

2. Patients and Methods

2.1. Patients. Ninety-six patients with primary gastric cancer with PC were enrolled in the study. Enrolled patients in the study were treated between April 2004 and December 2011. PC was diagnosed by biopsy under laparotomy, laparoscopy, or by the cytologic examination of ascites. The eligibility criteria included (1) histologically or cytologically proven PC from gastric adenocarcinoma; (2) absence of hematogenous metastasis and remote lymph node metastasis; (3) age 75 years or younger; (4) Eastern Clinical Oncology Group scale of performance status 2 or less; (5) good bone marrow, liver, cardiac, and renal function; (6) absence of other severe medical conditions or synchronous malignancy.

Informed consent according to the institutional guideline was obtained from all patients.

2.2. Methods to Introduce a Peritoneal Port System and Peritoneal Wash Cytology. A peritoneal port system (Hickman subcutaneous port; Bard, Salt Lake City, USA) was introduced into the abdominal cavity under local anesthesia, and the tip of the system was placed on the cul-de-sac of Douglas. Then, a peritoneal wash cytology was performed after 500 mL of physiological saline had been injected into the peritoneal cavity. To improve the accuracy of the cytology, an immunohistochemical examination using monoclonal antibodies for anti-human carcinoembryonic antigen (TAKARA Bio INC., Tokyo, Japan) and anti-human epithelial antigen (DAKO, Copenhagen, Denmark) were performed. A peritoneal wash cytological examination was performed before and after NIPS.

2.3. Bidirectional Chemotherapy. Patients were treated with 60 mg/m^2 of oral S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) for 21 days, followed by a 1-week rest. On days 1, 8, and 15 after the start of oral S-1 administration, 30 mg/m^2 of Taxotere and 30 mg/m^2 of cisplatinum with 500 mL of saline were introduced through the port. This regimen was repeated after a one-week rest [7]. Bidirectional chemotherapy is done 2 cycles before surgery. The aims of NIPS are to reduce the peritoneal surface involved by PC and to eradicate PFCCs. Toxicities were graded using the CTCAE v 3.0.

2.4. Selection Criteria of Patients for Cytoreductive Surgery (CRS) after NIPS. After two cycles of NIPS, patients who had the following criteria are excluded as the candidates for CRS: (1) evidence of para-aortic lymph node involvement and distant hematogenous metastasis confirmed by computed tomography (CT), or magnetic resonance imaging (MRI), (2) patients with progressive disease after NIPS, or (3) patients with severe comorbidities or poor general condition.

2.5. Quantitative Evaluation of the Volume of PC and Assessment Completeness of Cytoreduction. Intraoperatively, the tumor volume was quantified according to the Japanese general rules for gastric cancer study [8] and the Sugarbaker's peritoneal cancer index (PCI) [9]. The abdomen and pelvis were divided into nine regions and the small bowel into four each assigned a lesion size (LS) score of 0–3, representative of the largest implant visualized. LS-0 denotes the absence of implants, LS-1 indicates implants 0.25 cm, LS-2 implants between 0.25 and 5 cm, and LS-3 implants >5 cm or a confluence of disease. These figures amount to a final numerical score of 0–39 (Figure 1).

The aim of CRS was to obtain complete macroscopic cytoreduction as a precondition for the application of HIPEC. The residual disease was classified intraoperatively using the completeness of cytoreduction (CC) score [9]. CC-0 indicates complete cytoreduction with no residual macroscopic nodule; CC-1 indicates no macroscopic tumor but a positive histological margin on the esophageal, duodenal stump, or suspicious residual nodules less than 5 mm in diameter, CC-2 indicates apparent macroscopic residual tumors greater than 5 mm but upto 5 cm in diameter, and CC-3 indicates residual PC greater than 5 cm in diameter.

2.6. Methods of CRS Using Peritonectomy Techniques [6]. Laparotomy was done 3 weeks after the last day of NIPS. Under general anesthesia, midline incision was made from the xiphoid to the pubis. Just after laparotomy, peritoneal wash cytology is done, and PCI score was calculated in each case.

For the tissue dissection, electrosurgical techniques are used. In electrosurgery, a generator delivers high-frequency current greater than 200 kHz under high-power electricity (100 Watt), using the electrosurgical generator (Valleylab Inc., Boulder, CO, USA). The mainly used handpiece is the ball-tipped type. The 2 mm ball-tip electrode is used for dissecting on visceral surfaces.

After the left lobe of liver is freed from the left triangular ligament, resection of the lesser omentum along the Arantius duct is started. Gastrectomy combined with D2 dissection [8], greater omentectomy, splenectomy, and the resection of anterior leaf of mesocolon is done. Importantly, the small bowel should be intact for the safe reconstruction either by esophagojejunostomy or gasrojejunostomy. The aim of

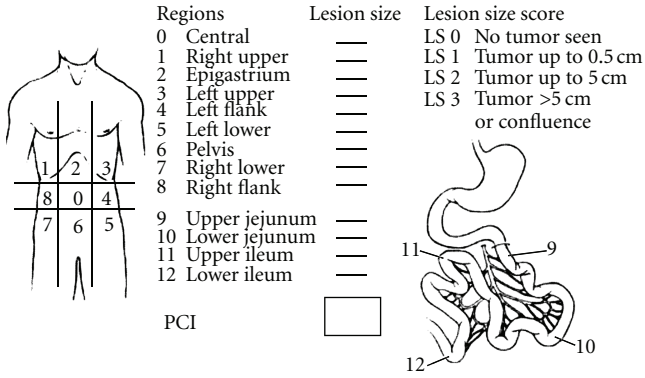

Figure 1: Peritoneal cancer index (PCI). Peritoneal cavity is divided into 13 parts, which ranges from 0 to 12. Accurate measurement of each region is scored as lesion size 0 through 3. LS 0: no implants.

peritonectomy is to remove all the macroscopic PC nodules with peritoneum. If the parietal peritoneum is involved, both sides of the parietal peritoneum are peeled off from the posterior rectus sheath to the retroperitoneal space. The dissection continues deeply and in a counterclockwise direction, starting in the right flank till reaching the peritoneum covering the left copula of the diaphragm. Then, the dissection is completed in the upper right side till reaching the anterior renal fascia, inferior vena cava, and posterior wall of the duodenum.

The peritoneum of the Morrison's pouch and paracolic gutters on both sides are completely freed from retroperitoneum and is removed with the anterior parietal peritoneum.

If the undersurface of the diaphragm is involved, stripping of peritoneum from the right and left hemidiaphragm is done. The falciform and round ligament are taken down and resected completely.

Large PC nodules attach on the transverse colon are removed in combination with extended right hemicolectomy.

The entire pelvic peritoneum is dissected from the anterior inferior abdominal wall, urinary bladder, and retroperitoneum. The peritoneum covering the urinary bladder is dissected and the rectovesical pouch is completely freed from the urinary bladder and rectum. In male, the space between seminal vesicle and peritoneum of rectovesical pouch is dissected, lifting the vas deferens off. In female, blood vessels around the uterus are dissected and cut with LigaSure (Valleylab Inc., Boulder, CO, USA). Amputation of vagina is done at a plane 1 cm below the peritoneal reflection of Douglas pouch to ensure removal of all tumor occupy the cul-de-sac.

If the tumor invades into the anterior rectal wall, rectum is cut at 1 cm below the peritoneal reflection. Reasonable length of the rectum should be preserved for the anastomosis with the colon.

The entire small bowel and its mesentery are traced from the duodenojejunal flexure to the ileocecal junction. Then, both sides of the mesentery are inspected and palpated, and the tumor nodules excised with electrosurgery. Complete cytoreduction is aimed by removing all macroscopic tumors by peritonectomy combined with electric fulguration.

2.7. Histologic Evaluation of NIPS. Histologic effects on primary tumors and PC were evaluated according to the general rules for gastric cancer treatment [8]. Histological response after chemotherapy is classified into 4 categories. Ef-0 shows no histologic response or response less than one-third of the tumor tissue. A histologic Ef-1 means that the degeneration of cancer is detected in the tumor tissue raging from one-third to less than two-thirds of the tumor tissue. EF-2 shows the degeneration of cancer tissue in wider than two-thirds of the tumor tissue, while an Ef-3 means the complete disappearance of the cancer cells.

2.8. Statistical Analyses. All patients were followed and no patients were lost to follow up. Outcome data were obtained from medical records and patients' interview. All statistical analyses had performed using SPSS software statistical computer package version 17 (SPSS Inc., Chicago, USA).

3. Results

Clinical characteristics of the 96 patients are listed in Table 1.

The average age was 51.3 years, including 42 men and 54 women. All 96 patients had primary gastric cancer and had P2 or P3 dissemination. Ascites was found in 55 (57%) patients.

Before NIPS, cytology had been positive in 68 (70.8%) of 96 patients and was positive in 21 (22.9%) after NIPS. These 68 positive cytology results before NIPS became negative in 47 (69%) patients after NIPS (Table 2).

After NIPS, 82 patients received operation, and the other 14 patients did not undergo operation due to the progression of disease or refusal of operation. At laparotomy, P status in Japanese rules became to be P0 in 7, P1 in 11, P2 in 8, and P3 in 56 patients. Mean PCI was 6.3, ranging from 0 to 33, and PCI $\leq$ 6 and PCI $\geq$ 7 were 56 and 26 patients, respectively.

Table 3 indicates the operation methods. Total gastrectomy was performed in 67 patients. A variety of supplemental procedures were performed to achieve tumor cytoreduction. The common procedures for visceral peritonectomy were transverse colectomy combined with right hemicolectomy and omentectomy ($N = 33$), pelvic peritonectomy in 38 including low anterior resection in 17, bilateral salpingo-oophorectomy in 36 of 48 female patients, segmental resection of small bowel, and small-bowel mesentery in 18 and 16 patients. Left and right subdiaphragmatic peritonectomy was performed in 25 and 22 patients, respectively. Mean operation time was 230 min (120~690 min), and mean blood loss was 1571 mL (850~4540 mL).

Complete cytoreduction (CC-0) was achieved in 58 of 82 patients (70.7%). CC-0 was achieved in 48 (78.7%) of 61 patients with negative cytological status after NIPS, but was done in 10 (47.6%) of 21 patients with positive cytology after NIPS. Regarding the PCI, CC-0 was done in 54 (96.4%) of 56 patients with PCI score $\leq$ 6, but was performed in

Table 1: Clinicopathologic characteristics of 96 primary gastric cancer patients with PC.

	Results	CRS ($N = 82$)	No operation ($N = 14$)
Age, years (median)	25–76 (51.3)	25–74 (52.2)	28–75 (46.9)
Gender (male/female)	42/54	34/48	8/6
Histologic type			
Differentiated		3	1
Poorly differentiated		79	13
Lymph node metastasis			
pN0		16	
pN1		40	
pN2		17	
pN3		9	
Macroscopic type			
Type 3		16	3
Type 4		66	11
Liver metastasis	0	0	0
Completeness of cytoreduction			
Complete cytoreduction (CC-0)		61	
Incomplete cytoreduction (CC-1~3)		21	
Hyperthermic intraoperative chemotherapy (HIPEC)			
Done		53	
Not done	29		

Table 2: Changes of peritoneal lavage cytology before and after NIPS.

Wash cytology after NIPS			
Wash cytology before NIPS	Negative	Positive	Total
Negative	27	1	28
Positive	47	21	68 (70.8%)
74	22 (22.9%)	96	

Table 3: Surgical procedures for CRS.

Surgical procedures	Patients number
Gastrectomy	
Total gastrectomy	67
Subtotal distal gastrectomy	15
Resection of right diaphragmatic copula	22
Resection of left diaphragmatic copula	25
Greater omentectomy	82
Pelvic peritonectomy	38
Hysterectomy	34/48
Salpingo-oophorectomy	36/48
Right hemicolectomy	33
Low anterior resection	17
Small-bowel resection	18
Resection of small-bowel mesentery	16

10 (38.4%) of 26 patients with PCI ≥ 7. Causes of incomplete cytoreduction were diffuse involvement of small bowel in 7 patients, PCI score higher than 20 in 6 patients, positive margin on esophageal stump in 3 patients, and local invasion into the retroperitoneal tissue in one patient.

Table 4: Adverse effects after NIPS.

Side effects	Grade 3	Grade 4	Grade 5
Hematological			
Leukopenia	1 (1.0%)	0	0
Thrombocytopenia	1 (1.0%)	1 (1.0%)	0
Nonhematological			
Stomatitis	0	0	0
Diarrhea	1 (1.0%)	0	0
Nausea, vomiting	1 (1.0%)	0	1 (1.0%)
Fatigue	2 (2.1%)	1 (1.0%)	0
Renal function (need hemodialysis)	1 (1.0%)	0	0
7 (7.3%)	2 (2.1%)	1 (1.0%)	

During NIPS, side effects of level 3, 4, and 5 were found in 7 (7.3%), 2 (2.1%), and 1 (1.0%) patients (Table 4). One patients died of aspiration pneumonia due to ileus (grade 5).

After NIPS and CRS, 8, 9, and 3 patients developed grade 3, 4, and 5 complications (Table 5). The most frequent complications are anastomosis dehiscence. The overall operative mortality rate was 3.7% (3/82), and the cause of

death was multiple organ failure with renal failure, hepatic coma, and sepsis due to anastomosis leakage. Grade 4 complications were found in 9 patients, and three patients developed renal failure were treated by hemodialisis. Six patients underwent operation for the postoperative bleeding in one patient, drainage of the abscess from anastomotic leakage in four patients, and port infection in one patient. All 8 patients developed grade 3 complications recovered well after appropriate treatments.

Twenty-six (31.7%) were alive at the time of analysis. The survival curve for all patients is shown in Figure 2. Median survival time (MST) of patients who underwent CRS was 14.4 months, with a one-, three-, and five-year survival of 61%, 16%, and 16%, respectively. MST of patients who did not receive operation was 9.0 months (Figure 2). There was a significant survival difference between the two groups ($P < 0.05$). Patients who received a complete resection (CC-0) had an MST of 21.1 months, and MST of patients who received incomplete cytoreduction (CC-1∼3) was 8.4 months ($P < 0.001$) (Figure 3). Significant survival difference was found between CC-0 and CC-1∼3 group. MST of patients with PCI ≤ 6 was 20.4 months with a 5-year survival of 21.0% and that of patients with PCI ≥ 7 was 9.6 months with no 5-year survival. There was a significant survival difference between the two groups ($P < 0.001$) (Figure 4).

Histologic effects on primary tumors were found in 71 of the 82 tumors, and Ef-1, -2, -3 response in the primary tumors were detected in 43 (52.5%), 28 (34.1%), and 0 tumor (0%), respectively (Table 6). In contrast, the complete histologic disappearance of PC was observed in 30 (36.8%) of 82 patients (Table 6). Stage migration from stage 4 to stage 1, 2, or 3 was experienced in 2, 2, and 8 patients. MST of patients with Ef-0/Ef-1 effects in PC tissue was 5.8 months with a 5-year survival of 0% and that of patients with Ef-2/Ef-3 effects was 24.0months with a 5-year survival of 28.0% (Figure 5). There was a significant survival difference between the two groups ($P < 0.001$).

As shown in Table 7, among various prognostic factors, CC score and pathologic effects were independent prognostic factors.

Recurrence was found in peritoneum, lung, liver hilum, liver, and bone in 27, 5, 3, 2, and 2 patients, respectively.

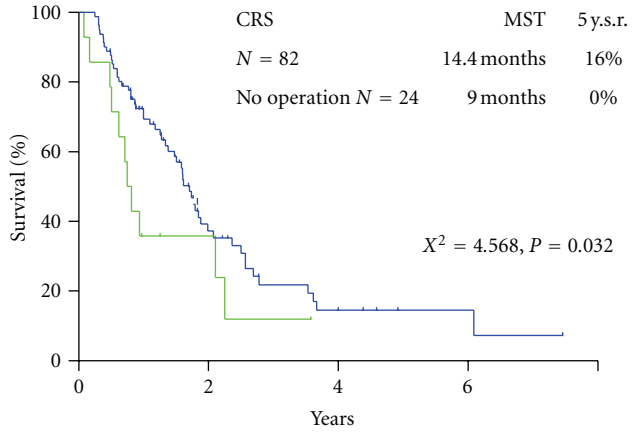

Figure 2: Survivals of 82 patients who underwent CRS, and 14 patients who did not underwent CRS.

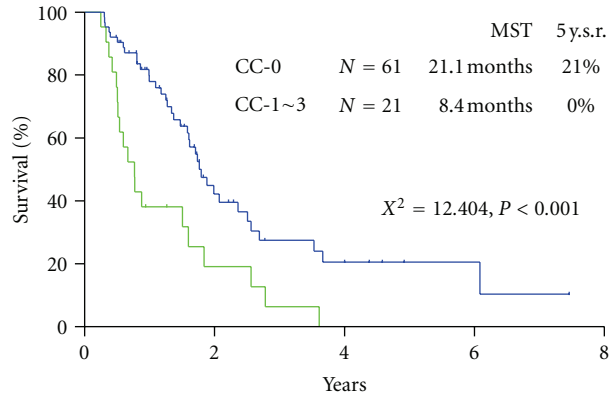

Figure 3: Survival difference after CC-0 and CC-1 resection.

4. Discussion

The current state-of-the-art treatment for colorectal peritoneal dissemination consists of a comprehensive management strategy using CRS and POC [10]. Patients with a low tumor volume, well/moderately differentiated tumors, and complete cytoreduction may potentially benefit from combined treatment. In gastric cancer patients with PC, no survival benefit has been reported by cytoreduction alone [1]. In contrast, CRS with peritonectomy plus HIPEC confers a prolonged survival period [5]. Furthermore, complete cytoreduction is an essential factor for a good outcome, and NIPS plus peritonectomy may improve the incidence of complete cytoreduction [7]. The aims of neoadjuvant chemotherapy (NAC) are stage reduction, eradication of micrometastasis outside the surgical field, and the improvement of resectability. Systemic chemotherapy usually is used for NAC. In the late 1990s, TS-1, irinotecan, taxanes, and docetaxel were introduced for gastric cancer treatment, and the response rates after monotherapy with these drugs were around 20%. Combination chemotherapy with S-1 and CDDP produced outstanding results, with a response rate of 74% [11]. Yabusaki et al. reported the results of NAC with S-1 and CDDP in 37 advanced gastric cancer patients. After 2 courses of treatment, the overall response rate was 68%, but the response rate for patients with peritoneal dissemination was only 14% (2/14) [12]. S-1 plus CPT-11 and CPT-11 plus CDDP produced a high-response rate of 42% and a long period of progression-free survival, but treatment failure as a result of toxicity was also observed [13].

These results indicated that systemic chemotherapies have minimal effects on PC. In other words, the peritoneal cavity acts as a sanctuary against systemic chemotherapy, probably because of the existence of a blood-peritoneal barrier consisting of stromal tissue between mesothelial cells and submesothelial blood capillaries [9, 14]. This barrier accounts for a total thickness of 90 μm [14]. Accordingly, only a small amount of systemic drugs are capable of

Table 5: Complications after NIPS and CRS.

Complications			
Grade 1-2	Grade 3	Grade 4	Grade 5
$N = 4$ (4.9%)	$N = 8$ (9.8%)	$N = 9$ (11.0%)	$N = 3$ (3.7%)
Minor leakage: 2	Pancreas fistula: 2	Renal failure: 3	MOF from leakage: 3
	Major leakage: 4	Major leakage: 4	
Abd. wall dehiscence: 1	Abdominal bleeding: 1	Bleeding: 1	
Abdominal abscess: 1	Port infection: 1		

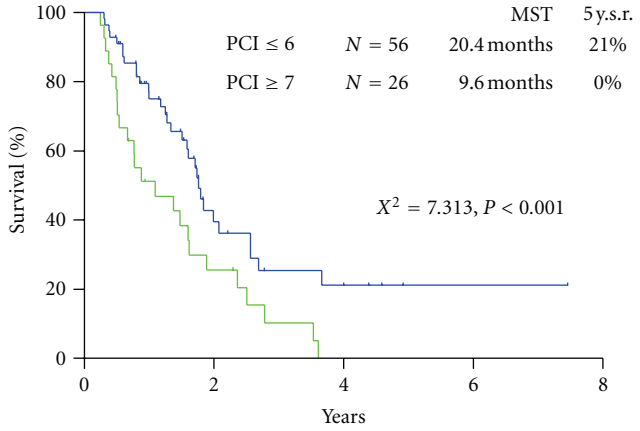

Figure 4: Survival difference of patients with PCI ≤ 6 and those with PCI ≥ 7.

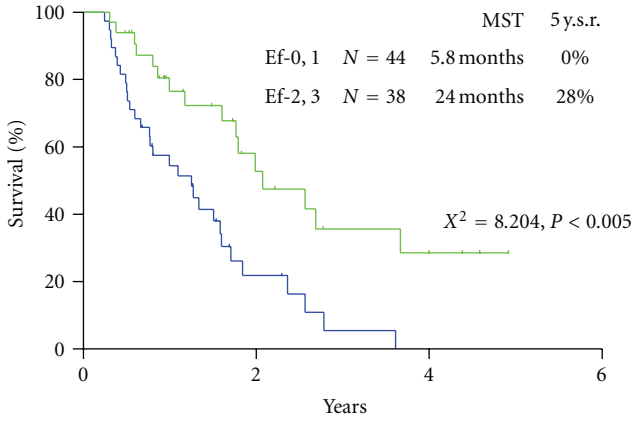

Figure 5: Survival difference of patients, according to the histological response on PC after NIPS.

Table 6: Pathological response after NIPS.

	Responder			
Ef-0	Ef-1	Ef-2	Ef-3	
Primary tumor	11 (13.4%)	43 (52.5%)	28 (34.1%)	0 (0%)
PC	25 (30.4%)	20 (24.3%)	7 (8.5%)	30 (36.8%)

penetrating this barrier and passing into the peritoneal cavity, and a higher percentage of the administered drugs instead moves to the bone marrow and vital organs other than the peritoneum, resulting in the development of adverse effects.

In contrast, IP chemotherapy offers potential therapeutic advantages over systemic chemotherapy by generating high local concentrations of chemotherapeutic drugs in the peritoneal cavity [15, 16]. This advantage of IP chemotherapy can be expressed by the area under the curve (AUC) ratios of intraperitoneal versus plasma exposure.

Relatively high AUC IP/IV ratios were obtained after the IP administration of paclitaxel, docetaxel, gemcitabine, 5-fluorouracil, and doxorubicin [16]. These drugs may be good candidates for IP chemotherapy.

Other important factors in the selection of drugs for IP chemotherapy are a high-penetration activity into the PC nodules and chemosensitivity. Cisplatin and carboplatin have the highest penetration activity and were confirmed to penetrate 1 to 2 mm from the surface of PC nodules [15]. In an experimental PC model using a highly metastatic cell line derived from human gastric cancer, docetaxel, 5-FU, carboplatin, and TS-1 plus cisplatin was highly effective for improving the survival of nude mice bearing PC, and the IP administration of these drugs is expected to become a standard therapy for gastric cancer patients [17, 18].

From these clinical and experimental results, a new bidirectional chemotherapy combined with the oral administration of S-1 and IP CDDP and docetaxel has been developed. By simultaneously administering intravenous and intraperitoneal chemotherapy, a bidirectional diffusion gradient can create a wider treatment area than single treatment.

The Cox multivariate analysis clearly demonstrated the complete cytoreduction and pathological response after NIPS were the independent prognostic factors. The factors to achieve CC-0 resection correlated with the negative cytology after NIPS and PCI score ≤ 6. Furthermore, peritonectomy techniques enable to achieve CC-0 resection even in the patients with higher PCI score [6, 7].

Preoperative evaluation of PC from gastric cancer is limited, and the sensitivity of CT for detecting PC was influenced by the lesion size. Koh et al. reported the value of preoperative CT in estimating PC in patients with colorectal carcinomatosis [19]. The depiction rate of small-bowel involvement had the lowest sensitivity, with a rate of 8–17%., and the false-negative rate significantly decreased with the lesion size. Small nodules (<0.5 cm) were visualized using CT with a sensitivity of 11%, in contrast to a sensitivity of 94% for PC with a diameter greater than 5 cm. Accordingly, preoperative assessment of PCI by CT is not recommended in gastric cancer. In contrast, cytological examination from a port

Table 7: Prognostic parameters (results of Cox proportional hazard model and logrank test).

	Cox hazard model			Logrank test		
X^2	P	Realtive sisk	95% CI	X^2	P	
Sex (male versus female)	2.158	0.141	0.652	0.3697–1.1531	4.298	0.038
Age (≤65 versus >65)	0.603	0.437	1.399	0.5991–3.2692	0.289	0.59
Histologic type (diff. versus poorly)	0.024	0.876	1.171	0.1584–8.6651	0.631	0.427
CC (CC-0 versus CC-1)	4.197	0.04	2.005	1.0306–3.9041	8.537	0.003
PCI (≥6 versus ≤7)	1.592	0.206	1.528	0.7908–2.9536	5.737	0.017
Pathologic response on PC (Ef-1, -2 versus Ef-2, -3)	4.269	0.038	0.429	0.1927–0.9575	10.303	0.001
LN status (pN0 versus pN1, 2, and 3)	2.478	0.115	2.121	0.8317–5.4108	3.739	0.053
Cytology after NIPS (class I versus class V)	0.047	0.828	1.079	0.5415–2.1513	0.365	0.546

system is very convenient and is an objective evaluation of the intraperitoneal cytologic status. In the present study, CC-0 was achieved in 48 (78.7%) of 61 patients with negative cytological status after NIPS, but was done in 10 (47.6%) of 21 patients with positive cytology after NIPS. There was a significant difference in the incidence of CC-0 resections between the two groups. Accordingly, cytologic examination through a port system may be one of the indicators for CC-0 resection. Furthermore, before NIPS, cytology had been positive in 68 (70.8%) of 96 patients and was positive in 21 (22.9%) after NIPS. These 68 positive cytology results before NIPS became negative in 47 (69%) patients after NIPS. NIPS can eradicate PFCCs before CRS and may prevent the attachment of PFCCS on the surgical wound at CRS.

After systemic neoadjuvant chemotherapy, a complete PC response is very rare. Inokuchi et al. reported that the response rate of PC after S-1 plus irinotecan was 69% 9/13), but no CR was experienced for PC [20]. Baba et al. also reported the limited effects of systemic S-1+CDDP on PC from gastric cancer [21].

After histological examination of the primary tumors and the resected peritoneum, histologic effects on primary tumors were found in 71 of the 82 tumors. However, the Ef-2 response and the complete pathologic response of Ef-3 in primary tumors were found in 28 tumors (34.1%) and 0 tumor (0%). In contrast, those in the peritoneal dissemination were observed in 30 (36.8%) of 82 patients. In addition, stage migration from stage 4 to stage 1, 2, or 3 was experienced in 12 patients (14.6%). These results indicate that NIPS can be a powerful strategy for eradicating PFCCs and for the reduction of the PCI score.

The present study demonstrated that the survival results were significantly better when the PCI was lower than 6. CC-0 resection was done significantly higher in patients with PCI ≤ 6 than PCI ≥ 7. The frequent cause of incomplete cytoreduction was the diffuse involvement of the small bowel. NIPS can reduce PCI score, and the timing of peritonectomy can be determined by the laparoscopic examination. Garofalo reported an excellent experience of laparoscopic diagnosis for PC [22]. There was a good correlation between the open-surgery data and the laparoscopic PCI scores. If the PCI score determined by the laparoscopy is larger than 7 accompanying with small-bowel involvement, NIPS is recommended to reduce PCI score on the small bowel. In contrast, patients with a PCI score >7 even after NIPS should be treated with palliative intent without peritonectomy. The PCI score is believed to be an independent prognostic factor, and a PCI score is capable of serving as a threshold for favorable versus poor prognosis.

NIPS may add to the morbidity and mortality of further surgical treatment [7]. The incidence of major side effects (grade 3, 4, and 5) after NIPS was 10.4% (10/96). Chemotherapy-related death was found in one patient, and she died of aspiration pneumonia due to bowel obstruction without relation with chemotherapy. Renal dysfunction occurred in one patients (1.0%), but the patients recovered fully by hemodialysis. Accordingly, the new bidirectional chemotherapy regimen is considered to be a safe method as compared with the results of previous reports of systemic chemotherapy [2, 3, 23, 24].

The present study demonstrated that NIPS plus peritonectomy may improve the incidence of complete cytoreduction. However, NIPS might increase the risk of a peritonectomy procedure plus a gastrectomy combined with a lymphadenectomy. Glehen reported a mean operation time of 5.2 hours (range, 1.5–9.5 hours), a 30-day mortality rate of 4% (2/49), and a major complication rate of 27% (13/49) [22]. In the present study, three hospital deaths (3.7%) occurred in patients who died of multiple organ failure (MOF) from a pancreatic fistula, anastomotic leakage, and sepsis. Postoperative major complications occurred in 20 (24.4%) patients. A second operation was necessary in 6 patients, who had complications from leakage of esophagojejunal anastomosis, bleeding, ileal and colonic fistulae, and port-site infection. Glehen reported a higher complication rate of 47% in patients who underwent extensive cytoreductive surgery (gastrectomy combined with the removal of more than 2 peritoneal zones) [22]. The magnitude of surgery, the number of resected organs, the number of anastomoses, and the operation time are considered to have contributed to the significantly higher complication rate. To avoid futile aggressive treatments, the stringent selection of patients must be emphasized preoperatively. Surgeons should have not only a large amount of surgical experiences with gastrointestinal and genitourinary diseases, but also an extensive knowledge of organ anatomy and physiology. Also, surgeons must be able to judge the balance between the postoperative risk associated with the magnitude of the peritonectomy and the survival benefit and quality of life after the aggressive treatment.

In conclusion, NIPS and complete cytoreduction are the essential treatment modalities for the improvement of survival of patients with PC from gastric cancer. Surgeons should experience a learning curve with this procedure at the specialized center and should recommend the accumulation of experience to achieve an acceptable morbidity rate.

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Review Article

Immunohistochemical Biomarkers in Gastric Cancer Research and Management

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Gastric cancer still represents a major health problem, despite a decrease in its incidence in the last years. Due to the social impact of gastric cancer (GC), there is a need for novel biomarkers in order to stratify patients into appropriate screening, surveillance, or treatment programs. Although histopathology remains the most reliable and less expensive method, numerous efforts have been made searching for novel biomarkers. In recent years, several molecules have been identified and tested for their clinical relevance in GC management. In this paper, we will focus on a well-known GC marker, whose determination is mandatory in GC, HER2, a marker whose correlation with prognosis is still controversial (VEGF-A) and a quite novel, unconventional marker, the ether-à-go-go-related gene 1 (hERG1). All these proteins can be easily detected with immunohistochemistry, a technique widely used both in diagnostic and research laboratories that represents a link between surgical and molecular pathology, basic science, and clinical medicine.

1. Gastric Cancer

Gastric cancer (GC) still represents a major health problem, despite a decrease in its incidence in the last years [1]. According to the most recent estimates, GC accounts for 8% of the total cancer cases and for 10% of the deaths for all cancers [2]. GC is characterized by a clear geographical distribution, with over 70% of the cases occurring in developing countries. This is partly due to dietary habits as well as *Helicobacter pylori* infection prevalence. Indeed, the reasons accounting for the decreased GC incidence in most countries are related to changes in dietary habits, amelioration of food preservation, reduction in *H. pylori* chronic infection [3–5] as well as reduction in smoking [1].

The majority of stomach tumors are sporadic, while only a small percentage have a familial component, with an autosomal pattern of inheritance. GC is a multifactorial disease characterized by both genetic and environmental components. In sporadic cancers of the stomach, the environmental component seems to be predominant. Conversely, the genetic component plays a major role in familial cancers. About 90% of GCs are classified as adenocarcinomas, whilst the remaining 10% is represented by non-Hodgkin lymphomas, leiomyosarcomas, squamous cell carcinomas, and undifferentiated carcinomas. In this paper, we will mainly refer to adenocarcinomas, addressing them as simply "GCs." According to the Lauren's classification, two subtypes of GC can be distinguished basing on their different histology: the intestinal (I-GC) and diffuse (D-GC) types [6]. The two GC types also display different biological and etiological characteristics. Tumor cells of I-GC form glandular-like structures, a feature which lacks in D-GC, which, on the contrary, is characterized by the infiltration and thickening of the gastric wall by tumor cells. The two histological subtypes are the result of distinct pathogenetic pathways, well described in the two models, proposed to depict the pathogenesis of I-GC [7] and D-GC [8]. As shown in Figure 1, I-GC occurrence is preceded by the development of chronic gastritis, which in turn leads to atrophy, and by the subsequent appearance of intestinal metaplasia. Intestinal metaplasia arises from the proliferation of gastric stem cells, whose progeny differentiates into "intestinal type" cells (columnar, goblet, and Paneth cells), due to the persistent irritation of the gastric mucosa, caused by *H. pylori* [9].

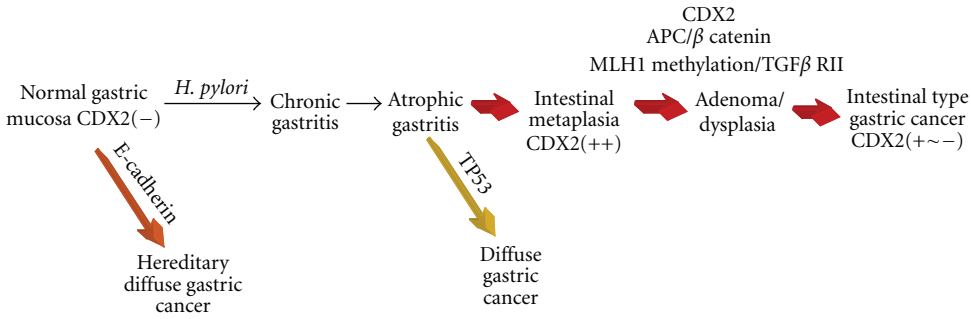

Figure 1: Correa model for intestinal type GC.

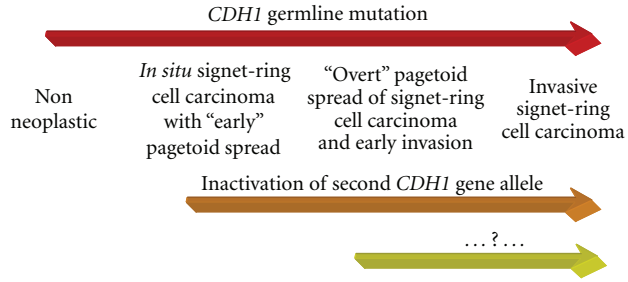

Figure 2: Carneiro's model for diffuse type GC.

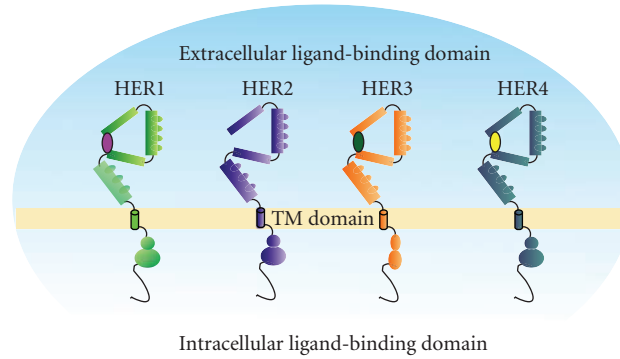

Figure 3: HER family receptors.

The Correa model is not applicable to the pathogenesis of D-GC. The latter is, however, well described by the Carneiro model [8] (Figure 2).

The diffuse type GC is characterized by reduced or abnormal E-cadherin expression [10, 11]. The inactivation of the second CDH1 allele (e.g., the gene encoding E-cadherin) leads to the appearance of an *in situ* carcinoma, with the presence of signet-ring cells with a "Pagetoid" pattern of diffusion, which is subsequently followed by the invasion of surrounding tissues. According to this model, the intraepithelial presence of signet-ring cells does not represent a secondary colonization. On the whole, E-cadherin loss/abnormality represents an early event in the cancerogenesis of D-GC [8], and the dysregulation of the gene is one of the most frequent genetic alterations in diffuse type GC [12].

Due to the social impact of GC, there is a need to stratify patients into appropriate screening, surveillance, or treatment programs. Although histopathology remains the most reliable and less expensive method, numerous efforts have been made to identify and validate novel biomarkers to accomplish the above goals. In recent years, several molecules have been identified and tested for their clinical relevance in GC management. Table 1 shows an overview of some of the biomarkers reported so far, along with the most correlated clinical parameters. With the exception of HER2, none of the biomarkers reported in the table is currently used in clinical practice, and some of them were described in single studies.

Immunohistochemistry (IHC) staining of formalin-fixed and paraffin-embedded tissues is widely used in diagnostic surgical pathology to gather additional information embracing those obtained with classical hematoxylin and eosin staining. IHC assists the pathologists in areas of tumor classification, multilineage differentiation, molecular correlates, and infectious etiologies. Moreover, IHC is commonly used to detect markers, which in turn can provide information on the biological behaviour and prognosis of a tumor. Different biomarkers detected by IHC are now a common component for many institutional review board protocols, for a more precise risk stratification and target identification. Therefore, IHC represents a link between surgical and molecular pathology, basic science and clinical medicine, surgery, and radiology [13].

As evidenced in Table 1, the number of potential biomarkers in GC is quite high and still increasing. Those which are easily detectable and quantifiable through IHC will be the object of the present review. In particular, we will focus on HER2, a well-known GC marker, whose determination is mandatory in GC, a marker whose correlation with prognosis is still controversial, VEGFs and quite novel, unconventional marker, hERG1.

1.1. HER2. The HER family comprises four different receptors: HER1 (EGFR or ErbB1), HER2 (ErbB2 or HER-2/Neu), HER3 (ErbB3), and HER4 (ErbB4) (Figure 3).

These receptors cooperate in the regulation of different processes, such as cell proliferation, differentiation, and

survival [14]. HER family members are implicated in the development of different kinds of tumors and are now recognized targets for biological therapy in breast, colorectal, lung, head and neck, gastric and gastro-oesophageal junction cancer (reviewed in [15]). Upon ligand binding, the receptors dimerize, become phosphorylated, and transduce intracellular signals, that ultimately regulate the above-mentioned cellular processes. Receptor dimerization can also occur through the process of receptor pairing, other than ligand binding [16]. Indeed, HER receptors can either homo- or hetero-dimerize with other HER family members, allowing multiple receptor combinations [16, 17].

Dimer formation leads to the phosphorylation of key intracellular proteins, that provide docking sites for a variety of subsiding signalling molecules. The latter then transmit signals to different downstream cascades, including the MAPK and the PI3K/AKT pathways [16, 18].

The HER2 gene has been recognized as a key regulator in the development of different types of tumors in particular breast cancer [60]. In GC, HER2 acts as an oncogene, since gene amplification reflects in protein overexpression, therefore giving selective advantage to malignant cells. In GC, HER2 overexpression has been correlated with poor outcome and a more aggressive disease [20] as well as with shorter survival [19–22, 61–65]. Based on data presented at the ASCO meeting in 2009, about 22% of patients with advanced GC have tumors which overexpress HER2. In a large multicentric trial carried out on GC patients (ToGA study), a survival benefit of trastuzumab (herceptin) treatment in HER2-positive patients (IHC score 3+) has been shown [23]. Therefore, HER2 represents a promising therapeutic target. However the optimal HER2 testing strategy has not been defined yet. Due to the recent approval of trastuzumab for HER2-positive GC in Europe, HER2 diagnostics is now mandatory: IHC is used as primary test, and it is followed by fluorescence *in situ* hybridization (FISH) in IHC2+ cases [27]. A more recent paper [26] showed that HER2 amplification can be detected in the two components (intestinal type and diffuse type areas of the neoplastic lesion) of Lauren mixed-type tumors. Standardization of HER2 testing procedures and interpretation is, therefore, an essential step to ensure accurate and reproducible results. This point acquires even more relevance, since it has been shown that HER2 status determination through the same protocol used for breast cancer, might lead to significant loss of patients [66] as there are important and significant differences in HER2 status determination between the two types of cancer (Table 2, see also [67]). As reported in Table 2, samples are given a score according to the intensity, degree of membrane reactivity and the percentage of immunoreactive cells. Scores 0 and 1+ are considered as negative, score 3+ is considered as positive, while 2+ samples are considered as equivocal and should be retested by fluorescence *in situ* hybridization (FISH) and chromogenic *in situ* hybridization (CISH).

If FISH is used as first screening step, only few IHC3+ cases may be missed but it might be found a high percentage of nonresponders according to ToGA results [24, 68]. In a more recent study published in 2011 [69] it was shown that HER2 testing in GC could be performed using standard breast cancer procedures and the American Society of Clinical Oncology/College of American Pathologists scoring criteria, while a group from Korea concluded that a GC-specific scoring system should be used [70].

In contrast to HER2 and despite supportive preclinical data, observed clinical success with anti-HER1 inhibitors and endocrine therapy combinations in breast cancer has been limited [71, 72]. In addition to HER1 and HER2, there is growing interest in HER3 as a potential therapeutic target [73]. Recently, HER3 and its physiologic ligand heregulin (HRG) have been implicated in the development of resistance to antiestrogen therapies in breast carcinoma [74]. Similarly, the dual HER1 and HER2 TKI lapatinib has clinical activity and is approved for the therapy of patients

Table 1: Immunohistochemical markers in GC.

IHC marker	Parameter	Reference
HER2	Prognosis	[19–23]
	Therapeutic response	[24]
	Lymph node metastasis	[25]
	Lauren histotype	[26, 27]
VEGF	Prognosis	[28–31]
	Lauren histotype	[32]
	Tumor progression	[33]
	Therapeutic response	[34]
hERG1	Prognosis	[35]
KLF5	Grading	[36]
	Stage	[36, 37]
	Lymph node status	[36, 37]
	Prognosis	[36, 37]
CA IX	Lymph node metastasis	[38]
	Prognosis	[38]
Ki67	Lymph node metastasis	[25]
PKP3	Stage	[39]
	Prognosis	[39]
MMP-2	Prognosis	[29, 31]
HDAC	Prognosis	[40–42]
Bcl-2	Lymph node metastasis	[25]
Bcl-6	Prognosis	[43]
SATB1	Lymph node metastasis	[44, 45]
	Distant metastasis	[44, 45]
	Stage	[44, 45]
c-myc2	Lymph node metastasis	[25]
TGF β	Stage	[46]
E-cadherin	Prognosis	[31, 47–52]
	Invasion	[53]
	Grading	[54, 55]
	Lauren histotype	[54]
COX-2	Prognosis	[56]
TSP-1	Prognosis	[57]
Bax	Prognosis	[58, 59]

Table 2: HER2 testing by immunohistochemistry in gastric cancer.

IHC parameters for HER2 testing protocol	IHC score	Classification
Intensity of reactivity		
Absent	0	Negative
Faint	1+	Negative
Weak to moderate	2+	Equivocal*
Moderate to strong	3+	Positive
Degree of membrane reactivity		
Complete	2+	Equivocal*
	3+	Positive
Incomplete	0	Negative
	1+	Negative
Percentage of immunoreactive cells (membrane reactivity)		
≥10%	1+	Negative
	2+	Equivocal*
	3+	Positive
<10%	0	Negative

*Samples scored IHC 2+ should be retested with fluorescence *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH).

whose disease has progressed on trastuzumab [74]. Pertuzumab is a recombinant humanized monoclonal antibody directed against the dimerization domain II of HER2 that is required for ligand-dependent dimerization with HER3 [74]. While trastuzumab prevents ligand-independent HER2 signaling, pertuzumab interferes with ligand-dependent HER3-mediated signaling.

1.2. VEGF. The vascular endothelial growth factor (VEGF) family is a multifunctional growth factors' family, involved in processes such as angiogenesis, inflammation, and vascular regeneration. The family includes different members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PlGF, characterized by the different ability to bind to three main receptors Flt1, KDR, and Flt4 (Figure 4).

It has long been known that VEGF-A is the key regulator of tumor angiogenesis [75], a complex process with a clear relevance to tumor progression and metastasis. The maximum diameter a tumor can reach without developing a new vascular network is about 1-2 mm. Hypoxia within tumor mass induces VEGF-A secretion and increased VEGFR-2 expression [76].

It has been demonstrated that VEGF-A expression is higher in I-GC compared to D-GC [32]. The expression of VEGF-C, whose main role is that of promoting lymphangiogenesis, is related to lymph node metastasis in GC [77]. VEGF expression is mirrored by microvessel density (MVD), and they are both hallmarks of enhanced angiogenesis within the tumor mass and are therefore useful tools for GC management [78]. MVD has been investigated as a promoting factor for angiogenesis with conflicting results about its relation to survival in GC. VEGF secretion promotes endothelial cell proliferation and therefore the establishment of a new vascular network. The evaluation of MVD reflects this latter process since it is evaluated by IHC with anti-CD34 or anti-CD31 antibodies which specifically indicate new formed vessels. MVD was significantly related to the T stage, as to the TNM classification, while VEGF-C expression was significantly higher in N-positive patients [79]. No relation was found between MVD and VEGF-C expression, but VEGF-C and MVD turned out to be related to clinicopathological features [79].

Although the VEGF superfamily has been identified to critically influence tumor-related angiogenesis, the prognostic significance of VEGF expression in GC is still controversial. In particular, VEGF-A expression seems to be a negative prognostic factor, at least in EGCs [80]. Moreover, VEGF-A expression has been proven to be relevant to therapeutic response in GC patients treated with fluorouracil alone or together with cisplatin [81]. We contributed to this discussion showing that the IHC expression of VEGF-A, which positively correlated with the Lauren's intestinal histotype, has a positive impact on overall survival in univariate analysis (manuscript in preparation). The reasons of the different conclusions drawn by several groups might be related to the design of the study and sample characteristics as well as geographical differences, keeping in mind that GC is a complex disease with striking differences in different countries. Furthermore, a recent paper [33], in which the impact of VEGF-A/C/D on tumor dissemination and survival in GC was evaluated, led to conclude that VEGF-D, being associated with progressive disease, could be a helpful marker of disseminated disease. The authors concluded that the targeting of VEGF-D might be therefore a potential therapeutic strategy.

Besides controversies in the interpretation of IHC data, the Avastin in Gastric Cancer (AVAGAST) trial started in 2007. It was a multinational, randomized, and placebo-controlled trial aimed to evaluate the efficacy of adding bevacizumab to capecitabine-cisplatin in the first-line treatment of advanced gastric cancer. Although AVAGAST did not reach its primary objective, adding bevacizumab to chemotherapy was associated with significant increases in progression-free survival and overall response rate in the first-line treatment of advanced gastric cancer [34].

1.3. hERG1. The *human ether-à-go-go-related gene 1* (*hERG1*) encodes for a protein, hERG1, which is functionally a voltage-dependent potassium channel (K_V), with outward rectifying characteristics. hERG1 has the typical structure of K_Vs: it is composed of four subunits, each of which formed by six transmembrane segments (S1–S6), which are assembled to form a tetramer surrounding a central aqueous pore. The S4 segment of each subunit is composed of basic aminoacids (Lys and Arg) and represents the voltage sensor [82] (Figure 5).

hERG1 constitutes the molecular basis of the cardiac rapid repolarizing current (IKr) (reviewed in [83]) and is therefore physiologically relevant to regulate the cardiac action potential [83]. In addition, hERG1 was found to be over- and mis-expressed in a wide variety of human cancers

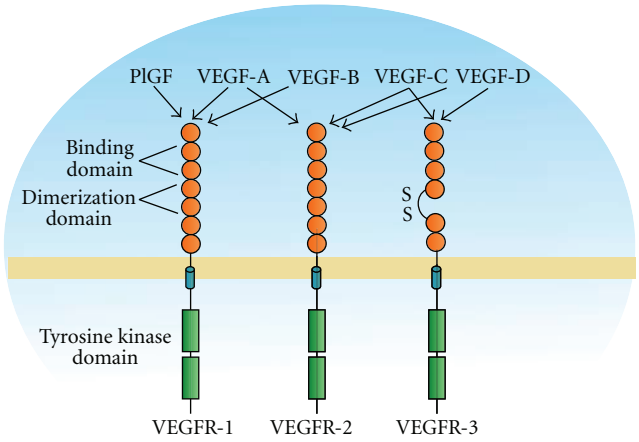

Figure 4: VEGF receptors and their ligands.

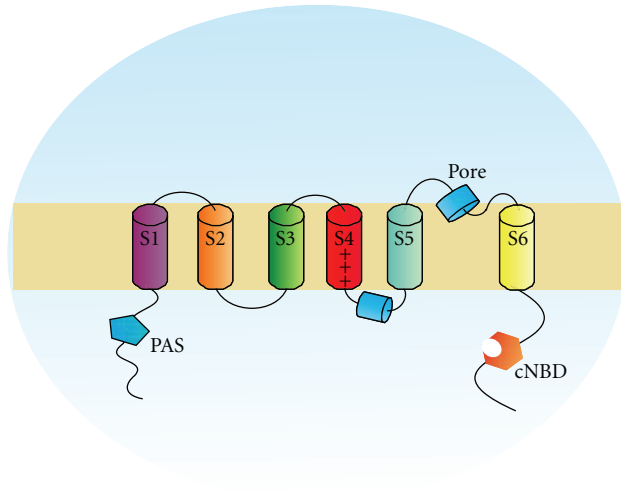

Figure 5: hERG1 potassium channel structure; PAS: Per Arnt Sim domain, cNBD: cyclic nucleotide-binding domain.

[84–93], where its activity is relevant to drive tumor progression. In particular, hERG1 activity is modulated by hypoxia [94] and regulates VEGF-A secretion in astrocytomas [84]. In addition, hERG1 channels mediate VEGF-Receptor-1 (FLT-1)-induced cell migration and signalling in acute myeloid leukemias [90]. hERG1 is also overexpressed in cancers of the gastrointestinal tract, in particular in colorectal [91, 92] and oesophageal adenocarcinomas [93]. In colorectal cancer, it has been recently demonstrated that the IHC positivity to hERG1, in conjunction to lack of expression of the glucose transporter 1 (Glut-1) is an independent negative prognostic factor in TNM stages I and II colorectal cancers [91].

A few papers addressing the expression and role of hERG1 in GC have been published so far. hERG1 channels are expressed in GC cell lines, where their activity regulates cell proliferation *in vitro* [95, 96]. Shao and colleagues [95] demonstrated that cisapride, a specific blocker of hERG1, can inhibit the growth of GC cells, by altering cell distribution within the cell cycle and inducing apoptosis. A more recent paper by the same group [97] demonstrated the correlation between hERG1 expression and tumor grading and TNM stage. Furthermore, inhibition of the channel with specific siRNAs resulted in a reduction of tumor growth and colony formation. Based on these results, hERG1 protein could be considered as a potential therapeutic target. More recently, Ding and colleagues [35] demonstrated significant differences in hERG1 protein expression, according to factors such as serosal invasion, venous invasion, and TNM stage. The mean survival time for hERG1 positive patients was significantly shorter than that of hERG1 negative ones and hERG1 expression was proven to be an independent prognostic factor [35]. To our knowledge, these are the only available data concerning hERG1 in GC addressing hERG1 as a negative prognostic factor. On the contrary when performing a study in a larger cohort of 524 GC patients (manuscript in preparation) encompassing different stages of the disease, we obtained different conclusions. In particular, our data confirmed that hERG1 is an independent prognostic factor, but we demonstrated its association with positive prognosis.

2. Concluding Remarks

Histopathology still represents the most powerful tool for gastric cancer management and, in recent years, novel biomarkers have been identified and tested for their correlations with clinical parameters as well as prognosis. In the near future new markers will be certainly validated, and the use of genomics and proteomics might help greatly clinicians in cancer management. Anyway, the possibility of validating potential tumor markers using IHC has clear advantages as it is easy and cost effective and virtually every pathology laboratory could perform it. Taking into account the importance and the usefulness of IHC markers, it will be of great importance in the next future to keep on searching for novel biomarkers as well as validating those already identified.

Authors' Contribution

E. Lastraioli and M. R. Romoli contributed equally to the work.

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Research Article

Survival Benefit of Adjuvant Radiation Therapy for Gastric Cancer following Gastrectomy and Extended Lymphadenectomy

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Purpose. Although randomized trials suggest a survival benefit of adjuvant chemotherapy and radiation therapy (XRT) for gastric adenocarcinoma, its use in patients who undergo an extended lymphadenectomy is less clear. The purpose of this study was to determine if a survival benefit exists in gastric cancer patients who receive adjuvant XRT following resection with extended lymphadenectomy. *Methods*. The SEER registry was queried for records of patients with resected gastric adenocarcinoma from 1988 to 2007. Multivariable Cox regression models were used to assess independent prognostic factors affecting overall survival (OS) and disease-specific survival (DSS). *Results*. Of 15,060 patients identified, 3,208 (21%) received adjuvant XRT. Adjuvant XRT was independently associated with improved OS (HR 0.67, CI 0.64–0.71) and DSS (HR 0.69, CI 0.65–0.73) in stages IB through IV (M0). This OS and DSS benefit persisted regardless of the extent of lymphadenectomy. Furthermore, lymphadenectomy with >25 LN resected was associated with improved OS and DSS compared with <15 LN or 15–25 LN. *Conclusion*. This population-based study shows a survival benefit of adjuvant XRT following gastrectomy that persists in patients who have an extended lymphadenectomy. Furthermore, removal of >25 LNs results in improved OS and DSS compared with patients who have fewer LNs resected.

1. Introduction

Gastric cancer is the fourth most common cancer and second leading cause of cancer-related deaths worldwide [1]. It has been estimated that there were 21,000 new cases of gastric cancer and 10,570 deaths from gastric cancer in the United States in 2010 [2]. Most patients in the U.S. present with locally advanced disease in which the tumor penetrates the muscularis propria and/or involves the perigastric lymph nodes at the time of diagnosis [3]. Surgical resection remains the only curative option for gastric adenocarcinoma. However, locoregional and systemic recurrence rates remain high, and ten-year overall survival (OS) rates after resection with curative intent range from 3–42% for advanced disease [4–6]. Given the high rates of recurrence after resection, the additional use of adjuvant chemotherapy and radiation therapy (XRT) has been investigated.

In 2001, the US Intergroup study (INT-0116) demonstrated an improvement in OS for patients with stage IB through IV (M0) gastric cancer who underwent resection followed by adjuvant 5-FU-based chemotherapy and XRT compared with patients who underwent surgical resection alone [7]. Based on these results, this adjuvant therapy regimen became the standard of care for resectable gastric cancer in the U.S. A retrospective observational study using the Surveillance, Epidemiology, and End Results (SEER)—Medicare database reproduced these findings, demonstrating an improvement in OS for patients with gastric cancer who received adjuvant chemoradiation therapy [8].

In the INT-0116 trial, a D2 lymph node dissection (resection of regional lymphatics and perigastric lymph nodes (LNs), as well as LNs along the named vessels of the celiac axis) was recommended in all patients. However, only 10% of patients actually received this level of nodal clearance. In fact, 54% of patients underwent a D0 resection (gastrectomy with incomplete resection of the N1 nodes) [7]. This calls into question whether adjuvant chemoradiation therapy was associated with improved disease-free survival (DFS) and OS in this trial by decreasing locoregional recurrence in patients who underwent an inadequate lymph node dissection (LND). In a post hoc analysis of INT-0116, there was no significant evidence that chemoradiation failed to work in the D2 subgroup; however, with only 54 patients in this group, the authors acknowledge that the power of this analysis was very low [9].

The primary aim of this study, therefore, was to evaluate whether the survival benefit of adjuvant chemoradiation therapy persists in patients undergoing gastrectomy and extended lymphadenectomy by using a large, national database that could provide significant statistical power to detect a survival difference.

2. Materials and Methods

A data set consisting of patients with gastric adenocarcinoma was created through a query of the SEER database. SEER is an authoritative source for cancer incidence, survival, and prevalence encompassing approximately 28% of the United States population [10]. The SEER program collects demographic information (e.g., age, gender, and race) and clinical information (e.g., primary tumor site, tumor histology, tumor grade, stage, treatment, and survival) from 17 cancer registries across the United States. Stage information from the SEER database was converted to the American Joint Committee on Cancer 6th edition (AJCC) tumor node metastasis (TNM) criteria.

All patient records in the SEER registry from 1988 to 2007 with surgically resected gastric adenocarcinoma were queried ($n = 78,511$). Patients in whom gastric adenocarcinoma was not the primary malignancy or patients who lacked a histologically confirmed diagnosis of gastric adenocarcinoma were excluded from analysis. Patients with gastric lymphoma, gastrointestinal stromal tumors, or other gastric malignancies were excluded. In addition, those patients who had incomplete clinicopathologic information, metastatic disease, or who had undergone preoperative or intraoperative XRT were excluded.

Statistical analysis was performed using a Cox regression model with gender, race (categorized as white, black, and other), age, number of LNs resected, AJCC stage of disease, and radiation status as covariates. The primary and secondary outcome measures were OS and DSS, respectively. Kaplan-Meier methods and the log-rank test were also used to compare survival between patients who did or did not receive adjuvant XRT according to stage and extent of LND. For purposes of statistical analysis, the number of LNs resected was subdivided into groups of <15 LNs, 15–25 LNs, and >25 LNs. Age was dichotomized to ≤60 years and >60 years of age. All P values were two-tailed tests, with alpha of 0.05.

The Vanderbilt Institutional Review Board (IRB) was contacted regarding this study; however, because the data from SEER is de-identified prior to release to our institution, no formal IRB approval was required.

3. Results

A total of 15,060 patients met the inclusion criteria. Of these, 3,208 (21%) received adjuvant XRT after gastric resection and 11,852 (79%) underwent gastric resection alone. Patient characteristics are shown in Table 1. Several statistical differences between patient populations were identified. Patients of younger age (≤60), male gender, and higher stage tumors were more likely to receive adjuvant XRT ($P < 0.001$). Patients who had ≥15 LNs removed were also more likely to receive adjuvant XRT ($P < 0.001$).

Kaplan Meier survival analysis demonstrated a significant 5 year OS benefit of adjuvant XRT when compared with surgery alone for all patients with AJCC stage IB ($P = 0.002$) and higher ($P < 0.001$). Median OS among patients with stage IB, II, IIIA, and IIIB gastric cancer who received adjuvant XRT was 65, 34, 23, and 19 months, respectively, compared with 54, 21, 14, and 11 months, respectively, for patients who underwent surgery alone. These results are summarized in Table 2. Five year DSS was also improved for patients with stage II and higher who received adjuvant XRT ($P < 0.001$). Median DSS for patients who received adjuvant XRT with stages II, IIIA, and IIIB was 41, 24, and 20 months, respectively, compared with 26, 17, and 12 months for patients who had surgery alone.

An extended LND was also associated with improved OS by Kaplan Meier analysis. Median survival was 34 months for patients who had >25 LNs resected, 27 months if 15–25 LNs were resected, and 25 months if <15 LNs resected ($P < 0.001$). Five-year OS was 38% when >25 LNs resected, 33% with 15–25 LNs resected, and 31% if <15 LNs were resected (see Figure 1). Median DSS was also improved with a more extended LND: 45 months for patients with resection of >25 LN, 34 months for 15–25 LNs, and 32 months for <15 LNs ($P < 0.001$) (Figure 2).

By Cox multivariate regression analysis, adjuvant XRT was independently associated with improved OS (HR 0.67, CI 0.64–0.71) and DSS (HR 0.69, CI 0.65–0.73) in patients with stages IB through IV (M0) undergoing resection for gastric cancer (see Tables 3 and 4). Notably, when extent of lymphadenectomy was included as a covariate in this analysis with patients stratified into groups according to the number of LNs resected (<15 LN, 15–25 LN, or >25 LN), the demonstrated OS and DSS benefit of adjuvant XRT persisted regardless of the extent of lymphadenectomy. In this model, a more extended LND (>25 LN) was also independently associated with improved OS and DSS when compared with a less extensive LND (<15 LN-OS HR 0.65, CI 0.60–0.69; DSS HR 0.62, CI 0.57–0.67 or 15–25 LN-OS HR 0.84, CI 0.78–0.91; DSS HR 0.81, CI 0.75–0.88).

Additional variables that were independently associated with improved OS and DSS included female gender

Table 1: Patient characteristics.

	No XRT		XRT		*P* value
	N	%	*N*	%	
N	11852	100	3208	100	
Age					
≤60	2344	20	1286	40	<0.001
>60	9508	80	1922	60	
Sex					
Male	7452	63	2234	70	<0.001
Female	4400	37	974	30	
Race					
White	7926	67	2187	68	
Black	1364	12	368	11	0.284
Other	2562	22	653	20	
LN dissection					
<15 nodes	7731	67	1823	58	
15–25 nodes	2633	23	912	29	<0.001
>25 nodes	1141	10	424	13	
Grade					
I	820	7	86	3	
II	4213	36	919	29	<0.001
III	6583	56	2129	66	
IV	236	2	74	2	
Stage					
IA	2207	19	31	1	
IB	2717	23	503	16	
II	3146	27	1183	37	<0.001
IIIA	2041	17	817	25	
IIIB	395	3	212	7	
IV (M0)	1346	11	462	14	

Table 2: Overall survival by stage.

Stage	No XRT		XRT		*P* value
	N	Median survival (mo)	*N*	Median survival (mo)	
All	11852	25	3208	28	<0.001
IA	2207	114	31	86	0.969
IB	2717	54	503	65	0.002
II	3146	21	1183	34	<0.001
IIIA	2041	14	817	23	<0.001
IIIB	395	11	212	19	<0.001
IV (M0)	1346	9	462	17	<0.001

($P < 0.001$), race other than black ($P < 0.001$), younger age (≤60) ($P < 0.001$), and lower tumor stage ($P < 0.001$) as shown in Tables 3 and 4.

4. Discussion

The primary aim of our study was to determine whether adjuvant radiation therapy provides a survival benefit specifically to patients who have undergone an extended lymphadenectomy. To answer this question, we used data from SEER, a large, national database statistically powered to detect differences in OS and DSS. Using a multivariate analysis of over 15,000 patients, we demonstrate that an OS and DSS advantage persists in patients receiving adjuvant XRT even in the subgroup who have undergone an extended LND (>25 LN). The strength of this analysis lies in the inclusion of a large number of patients who underwent extended lymphadenectomy ($N = 1,565$). Although a subgroup analysis of patients who underwent D2 resection in INT-0116 was performed, the analysis was underpowered due to the small number of patients studied ($N = 54$).

The extent of lymphadenectomy accompanying gastrectomy remains controversial. An extended LND is thought to decrease locoregional recurrence and provide more accurate

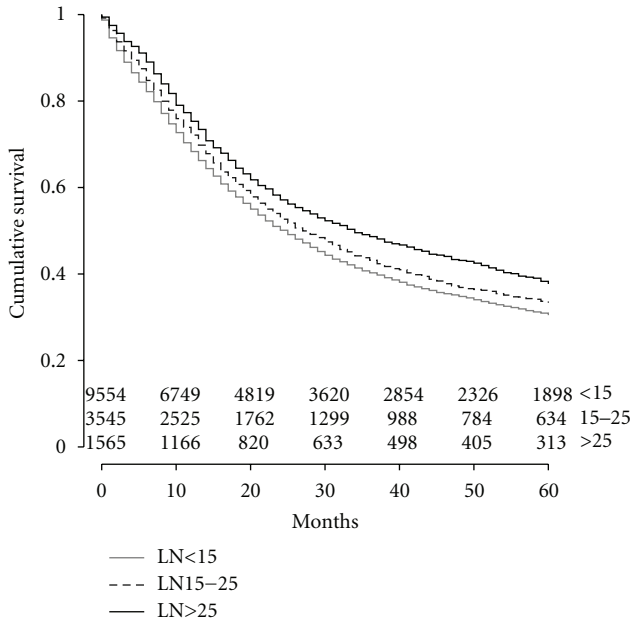

Figure 1: Kaplan Meier curve of overall survival by lymph node resection. Lymph node (LN).

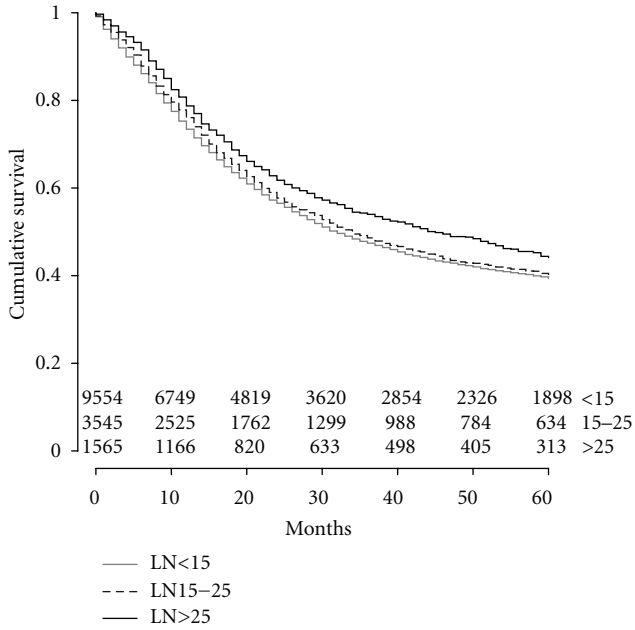

Figure 2: Kaplan Meier curve of disease specific survival by lymph node resection. Lymph node (LN).

staging information. Although Japanese series have consistently shown a significant survival benefit with D2 LND, two large European randomized controlled trials, the Dutch Gastric Cancer Trial (DGCT) and the UK Medical Research Council (MRC) trial, failed to demonstrate this benefit [11–17]. In both of these trials, the morbidity and mortality of patients who underwent a D2 LND was significantly higher than patients who underwent a limited (D1) LND [17, 18].

However, a subset analysis of DGCT found a survival benefit of D2 LND for stages II and IIIA, a finding also demonstrated by a large, prospective German trial [16, 19]. Furthermore, a subset analysis of the MRC trial demonstrated superior survival for patients who underwent a D2 LND without splenectomy or pancreatectomy, indicating that the high-operative mortality may have masked the survival benefit of a D2 LND [17]. Retrospective reviews in the USA using SEER and the National Cancer Database have similarly demonstrated an improved OS after extended lymphadenectomy for patients with stages II–IV gastric cancer [20, 21].

The long-term results of DGCT have been recently released and indicate that D2 lymphadenectomy is associated with reduced locoregional recurrence rates and improved DSS, and the authors therefore recommend a modified spleen-preserving D2 lymphadenectomy [6]. In addition, the National Comprehensive Cancer Network (NCCN) has recently modified their recommendations to include a D2 lymphadenectomy for patients with resectable gastric cancer [22].

The D level of a lymphadenectomy is defined by the Japanese Research Society for the Study of Gastric Cancer (JRSGC) based on the location of the primary tumor in the stomach and the level of regional lymph node station involvement [9]. However, the number of lymph nodes removed can also be used to approximate the extent of LND. According to the Japanese literature, a radical lymphadenectomy corresponding to a D2 resection consists of removal of 26 or more nodes [23]. In a Korean study of 990 patients who underwent curative resection with negative margins and D2 lymphadenectomy, 87% of patients had >25 nodes resected [24]. In the Dutch randomized trial comparing D1 and D2 lymphadenectomy, the median number of nodes removed was 17 and 30, respectively [16]. This suggests that a D2 lymphadenectomy correlates with resection of approximately 25 or more LNs. Thus, when the anatomic nodal location is unknown, the level of LND (D1 or D2) can be approximated using the total number of resected LNs. It was necessary to use this definition in our analysis, as SEER data consists of the number of LN removed but does not include information about anatomic nodal stations.

In this study, we also found a survival benefit of adjuvant XRT after surgical resection in patients with stage IB through stage IV (M0) gastric cancer. Median survival improvement ranged from 8 months to 13 months depending on stage. These findings correspond to and confirm the results of the INT-0116 trial, which showed a 9 month median survival advantage with the use of adjuvant XRT and 5-FU [7].

Additionally, our study shows that a more extended LND in-and-of-itself results in improved OS and DSS with the survival benefit increasing as the extent of lymphadenectomy increases. These findings suggest that patients with gastric cancer should undergo a margin negative resection with an extended (>25 nodes or D2) lymphadenectomy and confirms that adjuvant radiation therapy is an important component of their treatment.

There are several limitations to this study. First, data on the use of chemotherapy is unavailable for analysis using

Table 3: Cox multivariate regression analysis for overall survival.

Variable	HR	95% CI	*P* value
No XRT	1.00	Reference	<0.001
Adjuvant XRT	0.67	0.64–0.71	
Age			
≤60	1.00	Reference	<0.001
>60	1.49	1.42–1.57	
Gender			
Male	1.00	Reference	<0.001
Female	0.88	0.84–0.91	
Race			
White	1.00	Reference	
Black	1.06	0.99–1.13	0.075
Other	0.77	0.73–0.81	<0.001
Lymph nodes			
LN <15 : >25	0.65	0.60–0.69	<0.001
LN 15–26 : >25	0.84	0.78–0.91	<0.001
Stage			
IA	1.00	Reference	
IB	1.689	1.55–1.84	0.004
II	3.08	2.84–3.35	<0.001
IIIA	4.44	4.08–4.83	<0.001
IIIB	6.02	5.34–6.78	0.003
IV (M0)	7.14	6.52–7.82	<0.001

Table 4: Cox multivariate regression analysis for disease-specific survival.

Variable	HR	95% CI	*P* value
No XRT	1.00	Reference	<0.001
Adjuvant XRT	0.69	0.65–0.73	
Age			
≤60	1.00	Reference	<0.001
>60	1.26	1.19–1.33	
Gender			
Male	1.00	Reference	<0.001
Female	0.88	0.84–0.93	
Race			
White	1.00	Reference	
Black	1.04	0.97–1.11	0.307
Other	0.75	0.71–0.80	<0.001
Lymph nodes			
LN <15 : >25	0.62	0.57–0.67	<0.001
LN 15–26 : >25	0.81	0.75–0.88	<0.001
Stage			
IA	1.00	Reference	
IB	2.47	2.18–2.80	<0.001
II	5.38	4.78–6.05	<0.001
IIIA	8.32	7.38–9.39	<0.001
IIIB	11.31	9.74–13.12	<0.001
IV (M0)	13.78	12.16–15.61	<0.001

the SEER database. Although adjuvant 5-FU chemotherapy and XRT was the standard of care during the study period, it is certainly possible that patients may have received an alternative adjuvant regimen. Some patients may have undergone surgery/5-FU only or surgery/XRT only. As these are not standard adjuvant therapy regimens, it is likely that the number of patients falling into these categories is low. In one study using SEER-Medicare data in which chemotherapy use is known, fewer than 15% of the 2,333 patients received surgery and either chemotherapy or radiation alone [8]. Further, the MAGIC trial, which demonstrated a benefit of perioperative chemotherapy was published in 2006, so it is unlikely that a significant proportion of patients received chemotherapy alone outside of a clinical trial during the dates of this study [25].

SEER data also does not include information about local or distant recurrence; therefore, disease-free survival (DFS) cannot be determined. Additionally, there is no information about margin status after resection or about the dose or details of the radiation administered.

5. Conclusions

In summary, this study supports the use of adjuvant XRT in the treatment of gastric adenocarcinoma, as it appears to improve OS and DSS in patients with stage IB-IV (M0) gastric cancer. More importantly, our findings demonstrate that the survival benefit of XRT persists regardless of the extent of lymphadenectomy. This suggests that the benefit of XRT is not simply a mechanism to compensate for inadequate surgical clearance of disease, but is in itself critical for achieving locoregional control. Future prospective studies should include the use of adjuvant XRT and extended LND as independent variables to validate these findings.

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Review Article

Surgical Treatment of Peritoneal Carcinomatosis from Gastric Cancer

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Peritoneal carcinomatosis from gastric cancer is considered a fatal disease with limited treatment options. Recent advances in the understanding of the disease process, systemic chemotherapy, and application of cytoreductive surgery and hyperthermic chemoperfusion have shown promising results in the management of this difficult disease. Novel therapies such as extensive intraperitoneal lavage and intraperitoneal targeted agents are being applied in the management of this disease. We review the current literature in this field and describe the rationale behind some of these advances.

1. Introduction

Gastric cancer is the second leading cause for cancer-related mortality worldwide with almost 22,280 patients being diagnosed with gastric cancer annually in the United States [1, 2]. Peritoneal dissemination occurs commonly in patients with gastric cancer by means of intracoelomic dissemination or due to tumor spillage at the time of an operation [3]. High risk of peritoneal carcinomatosis from gastric cancer has led to common use of laparoscopy in the management of patients with gastric cancer. Unfortunately, systemic chemotherapy has not been shown to have a significant benefit to patients with peritoneal carcinomatosis. Despite short-duration response rates (43%) for visceral metastases with epirubicin-, cisplatin-, and 5-fluorouracil-based regimens, the response rate for peritoneal carcinomatosis is less than 14% [3, 4]. The blood peritoneal barrier which is 90 μm wide prevents a high concentration of intravenous chemotherapy from accumulating in the peritoneal surface, and this has led to increased interest in locoregional treatment for peritoneal carcinomatosis (PC) from gastric cancer [3].

Surgery has been the mainstay for treatment of gastric cancer without peritoneal dissemination, but advances in neoadjuvant chemotherapy with the MAGIC trial have led to significant survival benefits for patients [5]. This strategy demonstrated an effective use of multimodality therapy for patients with gastric cancer.

The application of hyperthermic intraperitoneal chemoperfusion or HIPEC to gastric cancer has been described by several groups over the last decade [3, 6, 7]. This technique refers to the combination of extensive cytoreductive surgery performed by an experienced team to remove all visible tumor, followed by intraoperative circulation of heated chemotherapy in the abdominal cavity during the procedure. This technique is currently considered standard of care for patients with PC from colorectal cancer, pseudomyxoma peritonei, and mesotheliomas [8].

Despite being described in the late 20th century, and its attractive method of delivery of chemotherapy to bypass the blood-peritoneal barrier, the adoption of HIPEC for the treatment of gastrointestinal malignancies was not common until 2003 when a randomized controlled trial demonstrated a doubling of survival for patients with PC from colorectal cancer [9]. The application of HIPEC to patients with gastric cancer was reported by several western groups at the same time, which also showed promising results [6].

The rationale behind cytoreductive surgery for regional spread of disease includes removal of all peritoneal surfaces bearing tumor by using traditional surgery in combination

with techniques including electrosurgery. Direct application of chemotherapy agents to the peritoneal surfaces in combination with hyperthermia (approximately 42°celsius) allows for direct tissue penetration of the chemotherapy, with limited systemic side effects.

We describe in our paper the application of HIPEC to the treatment of patients with gastric cancer and provide an evidence-based review of outcomes of patients undergoing regional therapies for PC.

2. History of HIPEC for Gastric Cancer

The use of intraperitoneal therapy for the management of malignant ascites was described as early as the 18th century when wine and Bristol water were instilled in the peritoneal cavity [10]. Since then, numerous advances have been made in the intraperitoneal management of tumors with regional spread. In fact, the NCI recognized intraperitoneal therapy as the standard of care in 2006 for ovarian cancer based on numerous randomized controlled trials [7]. Randomized data also supports the use of hyperthermic intraperitoneal chemoperfusion for patients with colorectal cancer with PC.

The application of HIPEC to patients with advanced gastric cancer was reported in 1988 by Fujimoto et al. who reported a median survival of 7.2 months in 15 patients [11]. Subsequently, several western centers adopted this technique with reports of 1-year survival of 43–45% [6, 12]. A meta-analysis reported by Yan et al. in 2007 identified randomized trials performed from 1983 to 2002, and a pooled analysis of the trials using hyperthermic intraperitoneal therapy showed a survival benefit with a pooled hazards ratio of 0.60 (95% CI 0.43–0.83, $P = 0.002$) when compared to surgery alone [7]. This survival benefit was improved significantly when early postoperative intraperitoneal chemotherapy was added to the HIPEC arm (HR 0.45 (95% CI 0.29–0.68, $P = 0.0002$)) [7].

3. Recent Randomized Trials

Recent randomized trials have shown very promising results for patients with PC from gastric cancer, and these reflect not only the advancements in surgery and anesthesia but also multimodality therapy being applied to these patients. Yang et al. reported a randomized controlled trial with 68 patients, with 34 in each arm who were randomized to get CRS+HIPEC versus CRS alone [13]. This trial was not stratified on the peritoneal carcinomatosis index (PCI) which is a marker of the burden of disease in the peritoneal cavity and consequentially complete cytoreduction was achieved only in 58.8% in both arms. Despite this, the median survival was increased by 70% in the CRS+HIPEC arm with a median survival of 11.5 months. This was in concordance with papers published in the US, and Europe previously [3].

Despite showing a significant benefit in survival, the overall survival remains short. Nevertheless, novel applications of intraperitoneal therapy seem to offer patients the most effective therapy to date. Kuramoto and colleagues reported the use of extensive intraoperative peritoneal lavage in conjunction with intraperitoneal chemotherapy for patients with cytology only positive gastric cancer who underwent surgical resection [14]. These investigators reported the use of 10 liters of lavage after surgical resection in conjunction with intraperitoneal chemotherapy for patients with gastric cancer without evidence of tumor deposits. In this randomized trial, the investigators reported a 5-year survival of 43% which exceeds previous papers for gastric cancer. In this trial, the authors did not report an increased incidence of adverse effects from the extensive lavage, although the adverse effects themselves were not reported. It is possible that the lavage can lead to dyselectrolytemia and increased GI toxicity; however, this needs to be studied further.

Papers such as those from Kuramoto et al. [14] have led investigators to believe that the application of HIPEC for advanced gastric cancer is aimed to prevent peritoneal carcinomatosis or to act when the burden of disease is extremely low. Glehen et al. have found that patients with a low burden of disease (peritoneal carcinomatosis index <9) have a significantly better survival after complete cytoreductive surgery and hyperthermic chemoperfusion [12]. This has also led Yonemura et al. to propose bidirectional neoadjuvant chemotherapy in which patients with low-volume peritoneal disease are given neoadjuvant intravenous (IV) and intraperitoneal chemotherapy and then taken to the operating room for cytoreductive surgery and hyperthermic chemoperfusion [3].

Currently, the EUNE protocol (European Union network of excellence on gastric cancer) includes patients with aggressive histology but low-volume peritoneal disease such as T3-T4 lesions with node-positive disease or patients with positive peritoneal cytology [15]. All patients will receive three cycles of IV platinum-based therapy similar to the MAGIC protocol, followed by D2 surgical resection. Patients will then be randomized to undergo HIPEC with oxaliplatin versus the surgical resection alone. The trial is aimed to demonstrate the efficacy of regional therapies in both prevention of recurrence and overall survival.

4. Approach to Advanced Unresectable Peritoneal Disease

Patients with peritoneal carcinomatosis often develop significant bowel obstructions, intractable ascites, and cachexia (Figures 1 and 2). Surgical approaches for best symptom palliation should be widely used at centers of peritoneal surface malignancy. Surgical bypasses or venting gastrostomy tubes even in the setting of gastric cancer are acceptable for palliation. The application of intraperitoneal therapy such as anti-EPCAM (epithelial cell adhesion) antibodies has shown significant benefits in puncture-free survival (survival without repeated paracentesis) for patients with malignant ascites in a phase II/III randomized trial [16]. In addition, for EPCAM+ tumors, the use of intraperitoneal catumaxomab has also shown an improved progression-free interval in phase II studies [17]. The use of catumaxomab in the United States has been restricted due to the pending FDA approval.

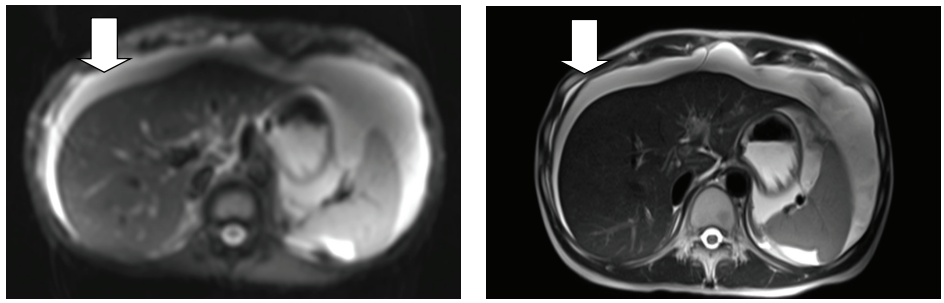

Figure 1: Diffusion-weighted MRI demonstrating ascites (arrow) in patients with gastric cancer following partial gastrectomy with chemotherapy-associated steatohepatitis.

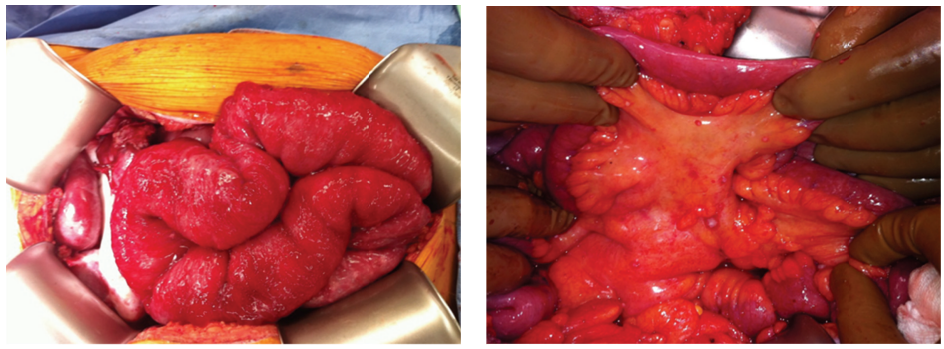

Figure 2: Mesenteric disease from gastric cancer primary leading to bowel obstruction.

Catumaxomab was fairly well tolerated in the published trial with pyrexia and abdominal pain being the most common side effects (60% and 43%, resp.). However, the incidence of individual grade III toxicities was <10%.

5. Conclusions

Patients with peritoneal carcinomatosis from gastric cancer have novel surgical options for treatment of disease. The approach is justified by data reported, and selection is of paramount importance. The application of cytoreductive surgery and hyperthermic chemoperfusion appears most favorable for patients with low-volume disease. Use of techniques such as extensive peritoneal lavage and intraperitoneal anti-EPCAM antibodies (i.e., catumaxomab) is an exciting advance in the treatment of these patients. Surgical palliation must be considered for patients who have intractable symptoms from the disease.

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Review Article

Mechanisms of Cisplatin-Induced Apoptosis and of Cisplatin Sensitivity: Potential of BIN1 to Act as a Potent Predictor of Cisplatin Sensitivity in Gastric Cancer Treatment

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Cisplatin is the most important and efficacious chemotherapeutic agent for the treatment of advanced gastric cancer. Cisplatin forms inter- and intrastrand crosslinked DNA adducts and its cytotoxicity is mediated by propagation of DNA damage recognition signals to downstream pathways involving ATR, p53, p73, and mitogen-activated protein kinases, ultimately resulting in apoptosis. Cisplatin resistance arises through a multifactorial mechanism involving reduced drug uptake, increased drug inactivation, increased DNA damage repair, and inhibition of transmission of DNA damage recognition signals to the apoptotic pathway. In addition, a new mechanism has recently been revealed, in which the oncoprotein c-Myc suppresses bridging integrator 1 (BIN1), thereby releasing poly(ADP-ribose)polymerase 1, which results in increased DNA repair activity and allows cancer cells to acquire cisplatin resistance. The present paper focuses on the molecular mechanisms of cisplatin-induced apoptosis and of cisplatin resistance, in particular on the involvement of BIN1 in the maintenance of cisplatin sensitivity.

1. Introduction

Gastric cancer is one of the most prevalent malignancies in Japan and East Asian countries [1], This cancer represents the major cause of mortality in these countries, despite great advances in diagnosis and multimodal treatments [2]. Cisplatin (cis-Diamminedichloroplatinum (II): CDDP) is an important chemotherapeutic agent in the treatment of advanced gastric cancer. Some trials of combination chemotherapy with S-1 and cisplatin as the first-line or second-line treatment for advanced and recurrent gastric cancer have yielded good responses and this treatment is well tolerated [3, 4]. However, there is diversity in the efficacy of cisplatin and in patient response to anti-cancer drugs including cisplatin, which can be of importance in terms of therapeutic outcome. Molecules and factors that are capable of predicting patient responses and resistance to anticancer agents are therefore of great interest and have been extensively studied. A recent study of the potent predictor of cisplatin sensitization Bridging integrator 1 (BIN1, also known as box-dependent MYC-interacting protein 1) demonstrated that BIN1 plays an important role in sensitization to cisplatin [5]. BIN1 is a nucleocytoplasmic adaptor protein that is involved in pleiotropic cellular functions such as suppression of oncogenic transformation. Here, we provide an overview of the molecular mechanism of cisplatin-induced apoptosis and of cisplatin resistance as well as of the mechanisms by which BIN1 sensitizes cancer cells to cisplatin.

2. Cisplatin and Its Stereoisomer Transplatin

Cisplatin was first discovered in 1965 as a strong inhibitor of bacterial cell growth and some years later was found to be a potent antitumor drug in studies using the murine leukemia

L1210 cell line [8, 9]. Cisplatin is actually one of the most widely used anticancer drugs, and the central role of this drug in human cancer chemotherapy attests to its current importance. Cisplatin-based chemotherapy is highly efficient for the treatment of patients with a variety of cancers such as lung, ovarian, head and neck, and gastric cancer [3, 10]. On the other hand, its trans analogue, transplatin, is known to be biologically inactive because of the diversity of qualitative and quantitative DNA adducts that it forms compared with cisplatin [6].

3. Molecular Mechanisms of Cisplatin-Induced Pro-Apoptotic Effects

3.1. DNA Strand-Crosslinks and DNA Damage Recognition. Cisplatin exerts its cytotoxic properties by reacting with DNA, which eventually culminates in irreversible apoptosis. Cisplatin primarily interacts with the N7-sites of purine residues in DNA to form DNA-DNA interstrand and intrastrand crosslinks [11]. The intrastrand adducts, ApG and GpG in particular, are responsible for the cytotoxic effects of cisplatin and account for 85–90% of the bound platinum [12]. These adducts block DNA replication and transcription. DNA adduct formation is followed by DNA damage recognition by over 20 proteins including hMSH2 of the mismatch repair (MMR) complex, the nonhistone chromosomal high-mobility groups 1 and 2 (HMG1 and 2) proteins and the transcriptional factor "TATA-binding protein" (TBP) [13–15]. The putative role of these DNA damage recognition proteins is to transmit DNA damage signals to downstream signaling cascades involving p53, MAPK, and p73, which ultimately induce apoptosis.

3.2. Cisplatin-Induced p53 and MAPK Activation. As mentioned above, cisplatin is believed to mediate activation of the p53 protein, a tumor suppressor, following DNA damage recognition. The transcriptional activation and stability of the p53 protein is known to be regulated by the two kinases ataxia telangiectasia-mutated protein (ATM) and ATM- and Rad3-related protein (ATR) [16, 17]. Cisplatin preferentially activates the ATR kinase which phosphorylates p53 on serine-15, resulting in its activation [18]. Of the mitogen-activated protein kinase (MAPK) signals that are involved in cisplatin-induced toxic effects, including extracellular signal-related kinases (ERKs), c-Jun N-terminal kinases (JNKs), and the p38 kinases, ERK activation appears to be the most important since activated ERK also phosphorylates p53 on serine-15 [19]. In addition, activation of JNK/p38 results in phosphorylation of the transcription factors c-Jun and activating transcription factor (ATF)-2 which, in turn, can bind to AP-1 binding sites in the promoters of multiple target genes. This cascade ultimately induces apoptosis through proapoptotic FasL gene expression.

3.3. Cisplatin Activation of p73-Dependent Apoptotic Signaling. p73 is a nuclear p53-related protein, which functions as a pro-apoptotic protein and accumulates in cisplatin-treated cells. Cisplatin-induced accumulation of p73 is dependent on MLH1 MMR proteins since it does not occur if the cells are deficient in these proteins [20]. Cisplatin-induced activation of the MMR protein-dependent p73 cell death pathway differs from its activation of p53 in that p73 activation does not involve ATR phosphorylation. The link between DNA damage recognition protein and activation of p73 may be the oncogenic tyrosine kinase c-Abl. Thus, cisplatin activates c-Abl, and accumulation of p73 following cisplatin treatment is not observed in c-Abl-defective cells. Cisplatin activation of c-Abl is regulated by MMR because MLH1-defective cells fail to activate c-Abl during cisplatin treatment. Furthermore, cisplatin cytotoxicity is reduced in c-Abl defective cells [21, 22]. The likely downstream events of both the cisplatin-induced p73 and p53 pathways are that cytochrome c is released through the mitochondrial membrane via Bax and Bak induction [23], which ultimately results in apoptosis through caspase 9 activation [6, 24] (Figure 1).

3.4. Cisplatin Modulation of Cell Cycle Checkpoints. Cisplatin-induced DNA damage induces an initial transient S-phase arrest, which is followed by inhibition of Cdc2-cyclin A or B kinases to yield a persistent G2/M arrest [25, 26]. As the inhibitory effect of cisplatin on the G1-phase cyclin-dependent kinases is a later event in the cell cycle checkpoint, accumulation of cells in the G1 phase is seldom observed and the cells remain in the G2/M phase.

4. Mechanisms of Cisplatin Resistance

To date, the mechanisms of cisplatin resistance have been suggested to involve reduced intracellular cisplatin accumulation, increased inactivation of cisplatin by thiol-containing molecules, increased DNA damage repair, and inhibition of transmitted DNA damage recognition to apoptotic pathways.

4.1. Reduced Intracellular Drug Accumulation. Reduced cisplatin accumulation in cells is caused by either inhibited drug uptake or increased drug efflux. Regarding inhibited drug uptake, active transporters such as Na^+K^+-ATPase or a gated ion channel are involved in cisplatin uptake. The inactivation and down-regulation of these uptake transporters result in cisplatin-resistance [27, 28]. Regarding increased cisplatin efflux, MRP2, which is one of the 7 known isoforms of the multidrug resistance-associated protein (MRP) family, appears to be important for cisplatin resistance. An increased level of this transporter protein was observed in resistant cells [29]. Furthermore, antisense depletion of MRP2 increased cisplatin sensitivity, supporting the involvement of MRP2 in cisplatin resistance [30]. Another transporter that is involved in cisplatin efflux is the protein encoded by the copper-transporting P-type ATPase gene, ATP7B, which mediates resistance to both copper and cisplatin. High levels of ATP7B mRNA in ovarian cancer correlated with cisplatin resistance. It has been also proposed that ATP7B expression is useful as a clinical marker of cisplatin resistance [31].

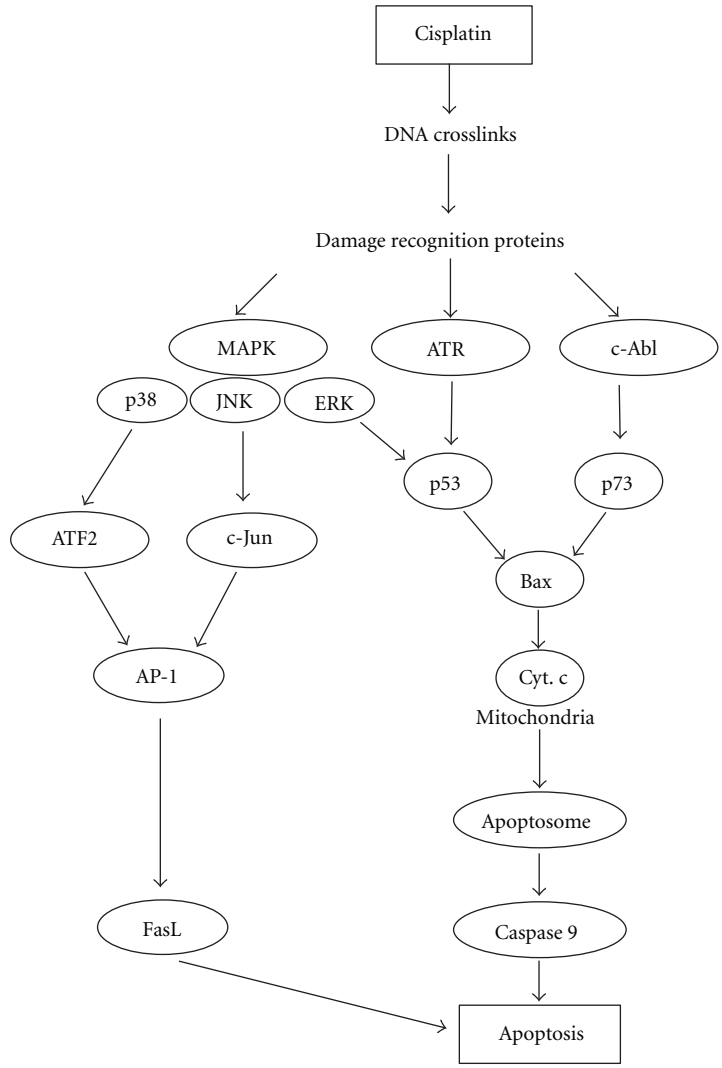


Figure 1: Signal transduction cascades mediating cisplatin-induced apoptosis. Quoted from [6] and modified.

4.2. Increased Cisplatin Inactivation by Thiol-Containing Molecules. Glutathione, (gamma-glutamylcysteinylglycine: GSH) which is the most abundant intracellular thiol, can detoxify many toxins including cisplatin. Cisplatin can be catalytically converted into cisplatin-thiol conjugates by GSH-S-transferase π, and these conjugates are ultimately inactivated [32].

4.3. Increased DNA Damage Repair. DNA damage is recognized differently depending on whether the DNA is transcriptionally active (transcription-coupled repair) or not (global nucleotide excision repair: global NER) (Figure 2).

In global NER, a complex of xeroderma pigmentosum (XP) Type C (XPC) and human homolog Rad23B (hHR23B) detects the damaged lesion and recruits transcription factor II H (TFIIH, which is composed of a number of core subunits including XPB, p34, p44, p52, and p62 as well as cyclin-dependent-kinase-(Cdk-) activating kinase subunits including Cdk 7, cyclin H, and Mat1) to the lesion together with XPG. TFIIH that contains XPB and XPD helicases creates a 10- to 25-nucleotide open DNA complex around the lesion. XPA verifies the damage in this open DNA complex. Replication protein A (RPA) then stabilizes the open DNA complex and is involved in positioning XPG and excision repair crosscomplementing (ERCC1)-XPF endonucleases that are responsible for the DNA incisions. After removal of the damage-containing nucleotides, DNA polymerase fills in the gap and ligase seals the nick.

In transcription-coupled repair, Cockayne syndrome (CS) group A, CSB, TFIIH, XPG, and possibly other cofactors displace the stalled RNA polymerase II complex from the damaged lesion, which then becomes accessible for further repair process. After this initial recognition step, the damage is repaired in a similar manner to that observed for global NER.

Since the formation and persistence of DNA adducts of cisplatin leads to the development of apoptosis, an increased level of DNA repair consequently attenuates apoptosis progression. In order to remove the DNA adducts of cisplatin, to

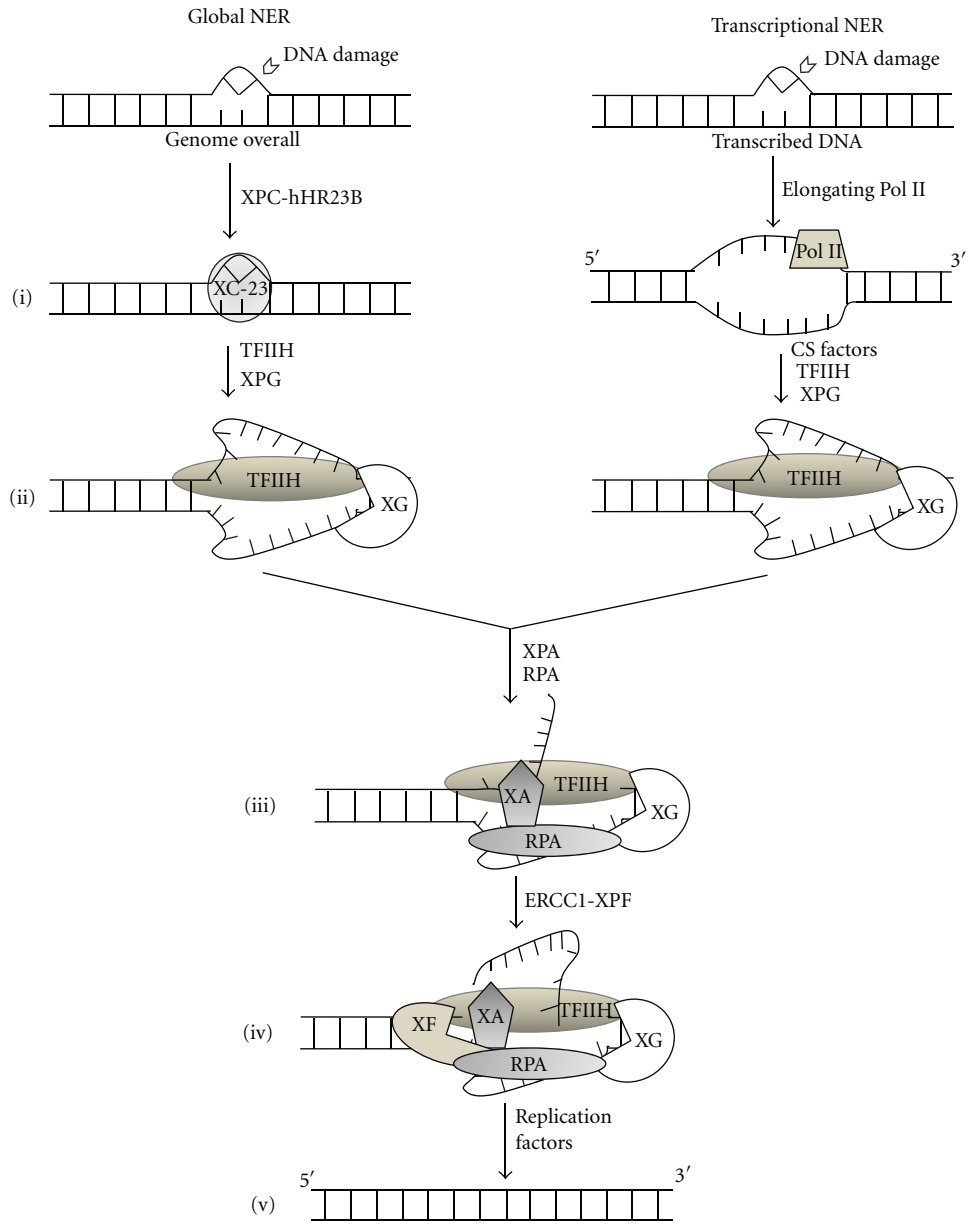


Figure 2: Molecular model of the NER system. Quoted from [7] and modified. (i) XPC-hHR23B (XC-23) binds and senses DNA distorting NER lesions in global NER, resulting in conformational alterations of the DNA. In transcriptional-coupled repair (NER), lesions are detected by elongating RNA polymerase II (Pol II). (ii) (left) XPC-hHR23B attracts TFIIH together with XPG (XG). TFIIH creates a 10- to 20-nucleotide open DNA complex. XPC-hHR23B is released. (right) CSA, CSB, TFIIH, XG, and possibly cofactors displace the stalled Pol II and then bind to the lesions. (iii) XPA (XA) and RPA bind and stabilize the open DNA complex. (iv) XG that is positioned by TFIIH and RPA cuts the damaged nucleotides at the 3′ site and ERCC-XPF (XF) that is positioned by RPA and XPA cuts them at the 5′ site. (v) DNA polymerase fills the gap and ligase seals the nick. Normal nucleotide sequence is consequently restored. Contacts drawn between molecules reflect reported protein-protein interactions.

repair the DNA damage and to promote cell survival, the cell cycle is arrested. Once this occurs, then it is followed by NER. NER processing is thus associated with cisplatin resistance. In accordance with these data, increased expression of XPA, ERCC1, ERCC1-XPF complexes, and BRACA1 has been linked to cisplatin resistance [33–35].

4.4. Inhibition of Transmission of DNA Damage Recognition to the Apoptotic Pathway. The HER-2/neu protein plays an important role in mediating transmission of the recognition of cisplatin-induced DNA damage to apoptotic pathways. HER-2/neu is a transmembrane receptor with a tyrosine kinase domain in the cytoplasm, which has homology to the epidermal growth factor receptor (EGFR). HER-2/neu activation propagates down-stream signaling through the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway. G protein-coupled receptor (GPCR) agonists including various cytokines, angiotensin II and endothelin-1, induce EGFR

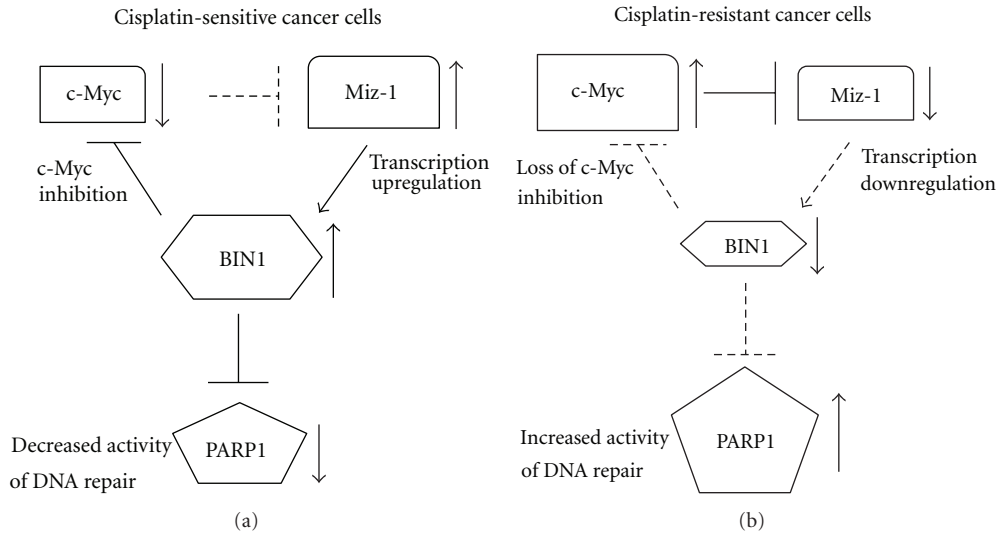


Figure 3: The molecular mechanism by which BIN1 is involved in cisplatin sensitization. Quoted from [5] and modified. The Miz-1-BIN1 interaction upregulates cellular cisplatin sensitivity by disruption of PARP1 activity. In cisplatin-sensitive cancer cells, a low level of c-Myc allows Miz-1 to stimulate BIN1 transcription, thereby maintaining a high cellular level of BIN1. The feedback inhibition of c-Myc by BIN1 perpetuates the decrease in c-Myc levels and results in decreased PARP1 activity, which consequently leads to downregulation of DNA repair activity. (b) In cisplatin-resistant cancer cells, c-Myc overexpression represses BIN1 expression by blocking the transcription activity of Miz-1. Loss of BIN1 feedback inhibition results in a robust increase in endogenous c-Myc and PARP1 activities, which consequently up-regulates DNA repair activity and cancer cell resistance to cisplatin. (a, b) Dashed lines indicate a decrease in the abundance or activity of a (positive or negative) regulator. Arrows indicate transcriptional up- or downregulation. Arrow size indicates the strength of this regulation.

and HER-2/neu transactivation [36, 37]. Cisplatin facilitates growth inhibition through activation of $p21^{Waf1/Cip1}$ in a p53-dependent manner. The PI3K/Akt that is activated by HER-2/neu induces cytoplasmic localization of the CDK inhibitor $p21^{Waf1/Cip1}$. A decrease in the nuclear level of $p21^{Waf1/Cip1}$ abrogates the p53-dependent antiproliferative effects induced by cisplatin and consequently sustains cisplatin resistance [38].

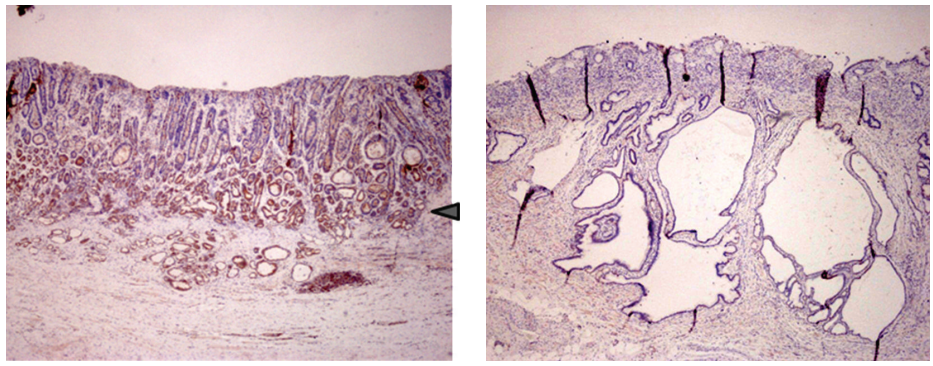
5. A New Molecular Mechanism of Cisplatin Resistance

A very recent investigation demonstrated a new mechanism by which the oncoprotein c-Myc enables cancer cells to acquire cisplatin resistance by suppressing BIN1, thereby releasing the DNA repair protein poly(ADP-ribose)polymerase (PARP) 1 [5] (Figure 3).

BIN1 was identified as a nucleocytoplasmic adaptor protein that can exert tumor suppressor properties by directly interacting with the c-Myc oncoprotein [39]. BIN1 is expressed in normal and benign cells and tissues but was undetectable in almost all estrogen receptor-positive or estrogen receptor-negative carcinoma cell lines. Complete or partial losses of BIN1 were documented in 60% of breast cancer tissue analyzed by immunohistochemistry or RT-PCR [40]. Reintroduction of BIN1 into human breast cancer and melanoma cell lines that lack its endogenous expression leads to loss of proliferation capacity and cellular death mediated by both p53- and caspase-independent pathways [40].

5.1. Involvement of BIN1 in Cisplatin-Sensitization. The molecular mechanism by which BIN1 is involved in cisplatin sensitization has become clear. Experiments involving forced depletion of the BIN1 protein using antisense or short hairpin RNA targeted toward *BIN1* in p53-positive, -null, -mutant cells resulted in increased cisplatin resistance. BIN1 interacts with c-Myc in the nucleus and inhibits its transactivation of target genes and cell transformation. Exposure of cisplatin-resistant cell lines expressing full-length BIN1 or a BIN1 deletion mutant lacking the MYC-binding domain (MBD) to cisplatin demonstrated that the MBD was essential for BIN1-mediated chromatin condensation and apoptotic cell death. However, addition of the c-Myc inhibitor 10058-F4, which disrupts the conformation of the c-Myc protein, to mimic BIN1-mediated inhibition of c-Myc was 25% less effective than full-length BIN1-expressing cells in inducing cisplatin sensitivity in an Annexin V-binding assay. These data suggest that BIN1 interacts with a non-Myc regulator protein to modulate cancer cell sensitivity to cisplatin.

5.2. BIN1 Directly Interacts with PARP1 at Its Automodification Domain and Inhibits PARP1 Activity by Blocking PARP1 Automodification Domain-Mediated Modulation of DNA Integrity. A glutathione S-transferase (GST) pull down assay with the recombinant GST-tagged full-length BIN1 protein followed by tandem mass spectrometry of coprecipitating proteins identified PARP1 as a candidate BIN1 interacting protein. Immunoprecipitation of cell lysates with an anti-BIN1 antibody followed by Western blotting with an anti-PARP1 antibody demonstrated the association of BIN1



(a) (b)

Figure 4: Immunohistochemical staining of BIN1 in a resected specimen of primary advanced gastric cancer. (a) BIN1-positive (arrowhead) and (b) BIN1-negative staining.

with PARP1. In addition, GST pull-down assays of DU145 prostate cancer cells using individual GST-fused domains of BIN1 and PARP1 showed BIN1 bound to the automodification domain of PARP1 through the BIN-amphiphysin-Rvs-related (BAR-C) domain of BIN1. The PARP1 automodification domain is known to increase in PARP1-mediated modulation of DNA integrity after DNA damage [41]. PARP1 is a key component of the base excision repair (BER) pathway and activated PARP1 recruits X-ray repair complementing defective repair in Chinese hamster cells 1(XRCC1), which acts as a scaffold for other BER-related proteins, DNA ligase III and DNA polymerase-β. Overexpression of BIN1 suppressed the poly(ADP-ribosyl)ation of histone H1 by 40%. Conversely, depletion of BIN1 increased histone H1 poly(ADP-ribosyl)ation. Immunoprecipitation of DU145 cells with an anti-PARP1 antibody followed by Western blotting with an anti-XRCC1 antibody demonstrated that BIN1 significantly abrogated PARP1-XRCC interaction. Moreover, single-cell DNA gel electrophoresis assays (comet assays) of DNA instability showed that overexpression of BIN1 increased DNA breaks and depletion of BIN1 inhibited DNA breaks, suggesting that BIN1 destabilized chromosomal DNA. Cells stably expressing the PARP1 N-terminal DNA binding domain (DBD), which disrupts the interaction between endogenous PARP1 and damaged DNA and acts as a PARP1-specific dominant negative inhibitor, attenuated PARP1 activity and the concomitant induction of cisplatin sensitivity, was sustained even in BIN1-defective cells. Thus, inhibition of PARP1 is indispensable for the induction of cisplatin sensitivity.

5.3. Overexpressed c-Myc Restores Intrinsic PARP1 Activity by Suppressing BIN1 Expression. c-Myc overexpression induces cisplatin resistance, whereas c-Myc inactivation increases cisplatin sensitivity. Using the c-Myc-estrogen receptor fused cell system, increased activity of c-Myc that was driven by 4-hydroxytamoxifen (4-OHT) robustly enhanced the poly(ADP-ribosyl)ation of histone H1, and cooverexpression of the PARP1 DBD abrogated this upregulation.

In addition, overexpression of c-Myc decreased BIN1 protein abundance and depletion of c-Myc increased BIN1 protein abundance. Thus, expressions of the *c-myc* and *BIN1* genes are inversely regulated. Furthermore, it is known that c-Myc represses the transcription of cell cycle arrest genes by a transcription initiator (Inr)-dependent repression mechanism [42]. A chromatin immunoprecipitation (ChIP) assay demonstrated that c-Myc binds to the BIN1 core promoter region through its Inr element. Miz-1, a Myc-interacting zinc-finger transcription factor, is known to bind to the Inr element of several genes that are repressed by c-Myc. Miz-1 functions as a counter partner of c-Myc and activates transcription of the genes that are repressed by c-Myc. c-Myc binds to Miz-1 at the Inr element and subsequently inhibits Miz-1-mediated transcription [42]. BIN1 promoter-driven luciferase activity was suppressed by cotransfection of Miz1 siRNA. In addition, depletion of Miz1 also decreased the expression levels of BIN1 mRNA and protein. A ChIP assay using an anti-Miz-1 antibody demonstrated that endogenous Miz-1 is recruited to the Inr-containing core promoter region of the *BIN1* gene in chromatin. Finally, depletion of Miz-1 decreased cisplatin sensitivity. These results suggested that Miz-1-induced BIN1 protein expression sustains the sensitivity of cancer cells to cisplatin.

6. Conclusion and Perspectives

Extensive knowledge regarding the molecular mechanisms of cisplatin-induced apoptosis and particularly of cisplatin resistance is indispensable for the design of therapeutic strategies using cisplatin against intractable malignancies. It has been established that the mechanism of cisplatin resistance includes reduced drug uptake, increased drug inactivation, increased DNA adduct repair, and inhibition of the propagation of DNA damage signals to the apoptotic program. A novel mechanism mediating cisplatin sensitivity has recently been proposed, in which Miz-1-induced BIN1 protein expression sustains the sensitivity of cancer cells to cisplatin. We have found patients with advanced gastric cancer who are immunohistologically positive or negative for BIN1 expression (Figure 4). Thus, BIN1 may be a new potent marker for the prediction of cisplatin sensitivity, which can be used for the design of strategies for gastric cancer

treatment. Furthermore, introduction of the BIN1 gene may also be a new therapeutic strategy for treatment of cisplatin-resistant gastric cancers. The rapid expansion in our knowledge regarding the molecular mechanisms of cisplatin resistance continues and will ensure that future anticancer treatment strategies can be devised and that the multifactorial mechanism of cisplatin resistance can be circumvented.

Abbreviations

BIN1: Bridging integrator 1
MMR: Mismatch repair
HMG: Nonhistone chromosomal high-mobility group
TBP: TATA-binding protein
ATM: Ataxia telangiectasia mutated protein
ATR: ATM- and Rad3-related protein
MAPK: Mitogen-activated protein kinase
ERK: Extracellular signal-related kinases
JNK: c-Jun N-terminal kinases
MRP: Multidrug resistance-associated protein
ATF: Activating transcription factor
GSH: Gamma-glutamylcysteinylglycine
XP: Xeroderma pigmentosum
TFIIH: Transcription factor II H
Cdk: Cyclin-dependent kinase
RPA: Replication protein A
ERCC1: Excision repair crosscomplementing
CS: Cockayne syndrome
EGFR: Epidermal growth factor receptor
GPCR: G protein-coupled receptor
PI3K: Phosphatidylinositol 3-kinase
PARP: Poly(ADP-ribose)polymerase
MBD: MYC-binding domain
GST: Glutathione S-transferase
BAR-C: BIN-amphiphysin-Rvs-related
BER: Base excision repair
XRCC1: X-ray repair complementing defective repair in Chinese hamster cells 1
DBD: N-terminal DNA binding domain
4-OHT: 4-Hydroxytamoxifen
ChIP: Chromatin immunoprecipitation
Inr: Initiator.

Conflict of Interests

The authors declare that no financial or other conflict of interests exists in relation to the content of this paper.

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Clinical Study

The Significance of Splenectomy for Advanced Proximal Gastric Cancer

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Objectives. The significance of splenectomy in advanced proximal gastric cancer is examined retrospectively. *Methods.* From 1994 to 2004, 505 patients with advanced proximal gastric cancer underwent curative total gastrectomy with preserving spleen (T) for 264 patients and total gastrectomy with splenectomy (ST) for 241 patients. *Results*. Patients who underwent splenectomy showed more advanced lesions. The metastatic rate of lymph node (LN) in the splenic hilus (No. 10) in ST was 18.3%. As for the incidence of surgical complications, there was not statistically difference except for pancreatic fistula. The index of estimated benefit of (No. 10) LN was 4.2, which was similar to that of (No. 9), (No. 11p), (No. 11d), and (No. 16) LNs. 5-year survival rate of (No. 10) positive group was 22.2%. 5-year survival rates of pSE and pN2 in T group were better than that of pSE and pN2 in ST, respectively. The superiority of ST was not confirmed even in Stage II, IIIA, and IIIB. *Conclusion*. Splenectomy was not effective for patients with (No. 10) metastasis in long-term survival. Spleen-preserving total gastrectomy will be feasible and be enough to accomplish radical surgery for locally advanced proximal gastric cancer.

1. Introduction

Although it is well known that lymph node (LN) metastasis is an important factor in the prognosis of gastric cancer, the optical extent of LN dissection remains controversial. Splenectomy has been indicated to remove the LNs surrounding the splenic artery (No. 11) and splenic hilum (No. 10). Previous reports suggested that gastrectomy with splenectomy resulted in better survival than gastrectomy alone in gastric cancer patients [1]. The Japanese retrospective studies revealed that the frequency of LN metastasis to No. 10 in proximal gastric cancer was 15–20%, and the 5-year survival rate was 20–25% [2, 3]. Total gastrectomy with splenectomy is considered to be a standard procedure for proximal advanced gastric cancer in gastric cancer treatment guidelines [4]. But two large prospective randomized trials in western countries reported that splenectomy was a risk factor for morbidity and mortality [5, 6]. Preservation of the spleen during extended lymphadenectomy decreases complications with no clear evidence of improvement or detriment to overall survival [7]. Then modified D2 lymphadenectomy avoiding splenectomy is now accepted as a standard procedure in the west countries. Our retrospective study was designed to investigate the significance of splenectomy by evaluating postoperative morbidity, frequency of the each LN metastasis, and long-term surgical outcomes of locally advanced proximal gastric cancer patients who underwent total gastrectomy with R0 resection.

2. Patients and Methods

2.1. Pathological Examination of Lymph Nodes. All regional LNs were separated immediately after gastrectomy by the operators. Node numbers were recorded using a LN map (Figure 1). Nodes were assigned to the appropriate anatomical stations according to Japanese Classification of Gastric Carcinoma (JCGC) of the 2nd English edition [8]. Nodes found at each station were labeled and immediately sent for histological examination.

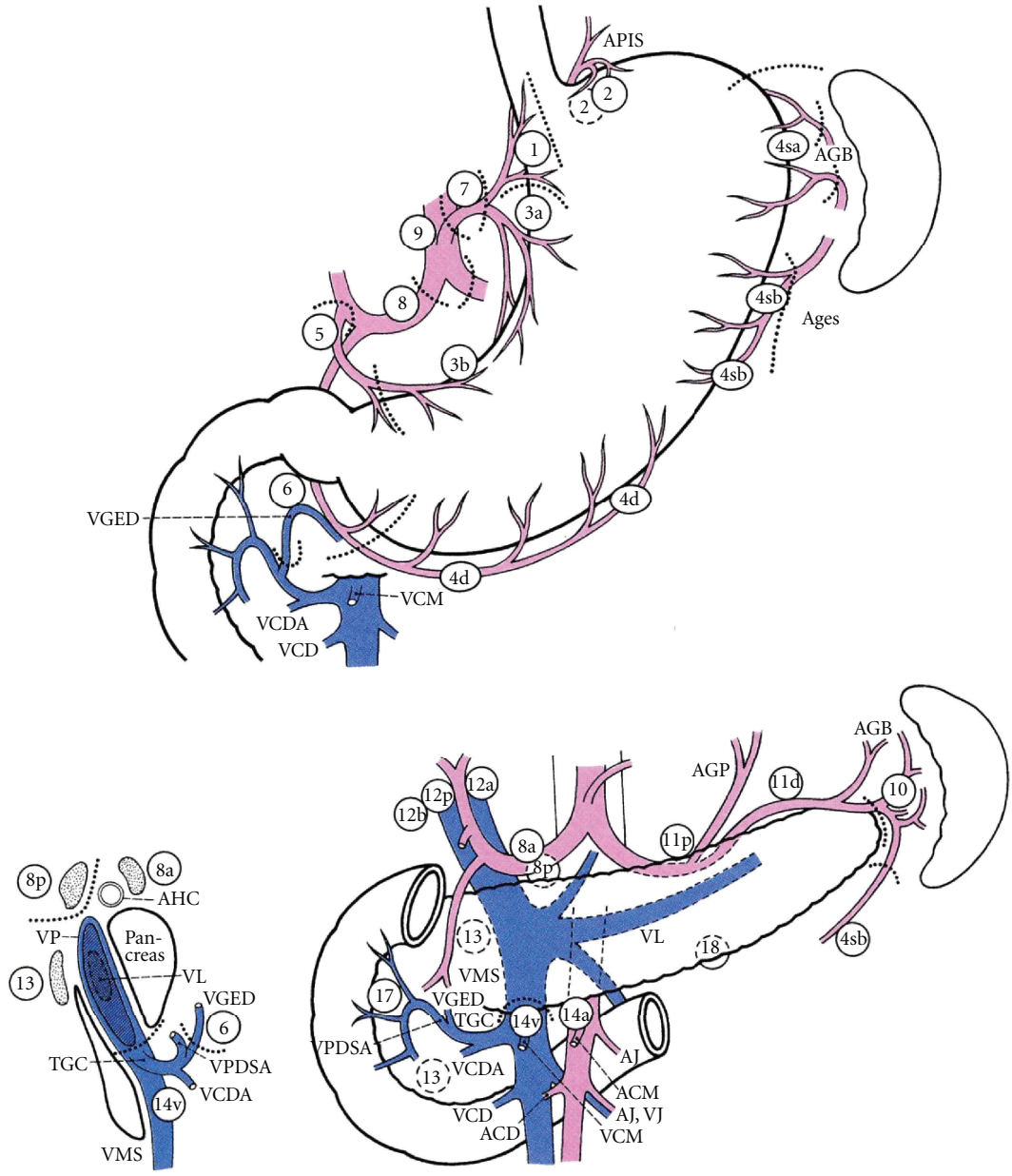

Figure 1: Lymph noses (LNs) are retrieved from the en bloc resected specimen and placed on the map exactly as they were in situ and numbered. Regional lymph node stations are defined as No. 1, right paracardial LN; No. 2, left paracardial; No. 3, LN along the lesser curvature; No. 4sa, LN along the short gastric vessels; No. 4sb, LN along the left gastroepiploic vessels; No. 4d, LN along the right gastroepiploic vessels; No. 5, suprapyloric LN; No. 6, infrapyloric LN; No. 7, LN along the left gastric artery; No. 8a LN along the common hepatic artery; No. 9, LN around the celiac artery; No. 10 LN at the splenic hilum; No. 11p, LN along the proximal splenic artery; No. 11d, LN along the distal splenic artery; No. 12a, LN in the hepatoduodenal ligament; No. 13, LN on the posterior surface of the pancreatic head; No. 14v, LN along the superior mesenteric vein; No. 16, LN around the abdominal aorta. APIS, left inferior phrenic artery; GB, short gastric artery; AGES, left gastroepiploic artery; VCM, middle colic vein; VGED, right gastroepiploic vein; VCDA, accessory right colic vein; VCD, right colic vein; AGP, posterior gastric artery; VL, splenic vein; AJ, jejunal artery; VJ, jejunal vein; ACM, middle colic artery; ACD, right colic artery; TGC, gastrocolic trunk; VMS, superior mesenteric vein; VPDIA, anterior inferior pancreaticoduodenal vein; AHC, common hepatic artery; VP, portal vein.

2.2. Patient Population. From 1994 to 2004, 505 patients with a single gastric adenocarcinoma located in the upper third portion underwent curative total gastrectomy at Niigata Cancer Center Hospital. Among them, 240 patients underwent total gastrectomy with splenectomy (ST), because the tumor involved the greater curvature or enlarged LN of No. 10 and/or No. 11. The remaining 265 patients underwent spleen-preserving lymphadenectomy (T) and remove No.

Table 1: Clinicopathological characteristics of the patients who underwent total gastrectomy with or without splenectomy (N = 505).

Characteristics	T N = 265 (%)	ST N = 240 (%)	P value
Age (year)			0.121
<70	163 (61.5)	18 (75.0)	
≥70	102 (39.5)	60 (25.0)	
Age (year)			0.481
Male	198 (74.7)	172 (71.7)	
Female	67 (25.3)	68 (28.3)	
Gross type			<0.001
Type 0, 1, 2	221 (83.4)	100 (41.7)	
Type 3, 4	44 (16.6)	140 (58.3)	
Tumor location			<0.001
U	191 (72.1)	159 (66.3)	
M, L	60 (22.6)	34 (14.2)	
UML	14 (5.3)	47 (19.6)	
Histological type			<0.001
Differentiated	151 (57.0)	97 (40.4)	
Undifferentiated	114 (43.0)	143 (59.6)	
Depth of invasion			<0.001
pT1, T2	228 (86.0)	78 (32.5)	
pT3, T4	37 (14.0)	162 (67.5)	
Lymph node metastasis			<0.001
pN0, N1	220 (83.0)	123 (51.3)	
pN2, N3	45 (17.0)	(48.8)	

* U; upper third, M; middle third, L; lower third.

11 but not No. 10. The clinicopathological features, stage and 5-year survival rates according to JCGC were compared between ST group and T group.

2.3. Procedures. Total gastrectomy with D2 and more extensive lymphadenectomy was performed according to the rules of the JCGC. The standard reconstruction was Roux-en Y method. In T group, No. 11 was dissected along the upper border of the pancreas but not No. 10 with or without mobilization of the spleen from the retroperitoneum. When the tumor involved the greater curvature and/or enlarged LN suspected metastasis at splenic hilum was found before or during operation, splenectomy was performed simultaneously as R0 resection. The index of estimated benefit from lymphadenectomy was calculated by multiplying the incidence of each nodal station by the 5-yer survival rate of patients with metastasis to that nodal station [9].

2.4. Statistical Analysis. All statistical analyses were conducted using the statistical program SPSS version 19 for Windows (SPSS, Chicago, IL, USA). Clinicopathological variables were analyzed using the chi-square test and the Student's t-test. The risk factors for No. 10 metastasis were determined using logistic regression analysis. Cumulative survival rates were calculated by the Kaplan-Meier method, and the significance of the differences in survival was determined by the log-rank test. P-value of <0.05 was considered statistically significant.

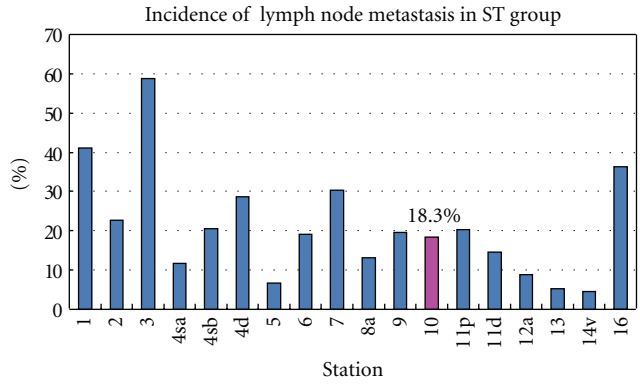

Figure 2: Incidence of each lymph node metastasis in ST group. The metastatic rate of the splenic hilar LN (No. 10) was 18.3%.

3. Results

3.1. Comparison of the Clinicopathological Features. Clinicopathological features are shown in Table 1. There was no statistical difference in age and gender between ST group and T group. But there were significant differences between two groups regarding gross type, tumor location, and histological type, depth of the tumor invasion, and status of lymph node metastasis. Namely, type 3 and type 4, UML (U; the upper, M; the middle, L; the lower), undifferentiated type, pT3 and pT4, and pN2 and pN3, are found frequently in ST group. Patients who underwent splenectomy showed more advanced lesions.

3.2. Perioperative Morbidity. Postoperative complications were listed in Table 2. There was no significant difference between two groups concerning nonsurgical complications. The incidence of surgical complications regarding anastomotic leakage, pancreatic fistula, postoperative ileus, and intra-abdominal bleeding was higher in ST group than in T group. But there was no statistical difference except for pancreatic fistula (P = 0.008).

3.3. Lymph Node Metastasis in ST Group. The lymph node metastatic rate in ST group was shown in Figure 2. No. 3 metastatic rate was highest (58.8%). The incidence of No. 10 metastasis was 18.3%, which was similar to that of No. 4sb (20.5%), No. 6 (19%), No. 9 (19.5%), and No. 11p (20.2%). No. 16 metastatic rate was 36.3% which was unexpectedly high.

The 5-year survival rate was 22.2% in patients with No. 10 metastasis and 50.8% in patients without its metastasis in ST group (Figure 3).

3.4. The Therapeutic Value of Lymph Node Dissection. The therapeutic value of extended lymph node dissection was estimated by multiplication of incidence of lymph node

Table 2: Perioperative morbidity following total gastrectomy with or without splenectomy.

Complication	T (without splenectomy)	ST (with splenectomy)	*P* value
Nonsurgical complication			
Cardiovascular	3 (1.1)	2 (0.8)	N.S.
Pulmonary	7 (2.6)	8 (3.3)	N.S.
Liver dysfunction	0	2 (0.8)	N.S.
Renal dysfunction	0	2 (0.8)	N.S.
CNS disorder	2 (0.8)	2 (0.8)	N.S.
Others	6 (2.3)	7 (2.9)	N.S.
Surgical complication			
Anastomotic leakage	1 (0.4)	4 (1.6)	N.S.
Panctreatic fistula	16 (6.0)	31 (12.9)	0.008*
Postoperative	22 (8.3)	21 (8.7)	N.S.
Bleeding	0	3 (1.3)	N.S.

N.S., not significant. *significant difference.

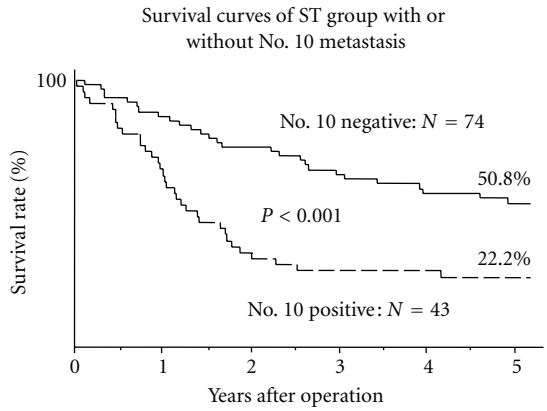

Figure 3: Comparison of cumulative survival curves of ST group between with or without No. 10 metastasis. The prognosis of the patients with No. 10 positive was poorer than that of the patients with No. 10 negative ($P < 0.001$).

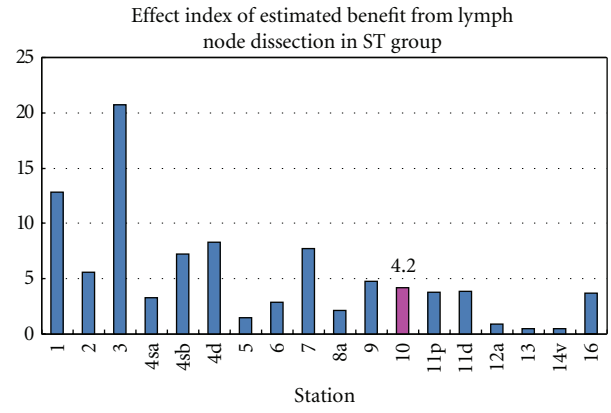

Figure 4: Effect index of estimated benefit from lymphadenectomy in ST group. The index was calculated by multiplication of the frequency of metastasis to the station and the 5-year survival rate of patients with metastasis to that station. The index of estimated benefit of No. 10 was approximately equal to that of No. 9, No. 11p, No. 11d, and No. 16.

metastasis and 5-year survival rate of patients with metastasis for each station. The index of estimated benefit of No. 10 was 4.2, which was similar to that of No. 9 (4.8), No. 11p (3.8), No. 11d (3.9), and No. 16 (3.7) (Figure 4). Almost all the regional lymph nodes of upper third portion of the stomach had high effect index of lymphadenectomy, but the treatment index of No. 4a and No. 8a was lower than that of No. 10.

3.5. Survival. In the survival rate according to depth of tumor invasion, ST group revealed lower prognosis compared with T group, but there was no significant difference between two groups in T2a and T2b (Figure 5(a)).

But the survival rate for patients with pSE (T3: tumor penetration of serosa), there was significantly difference between ST group (48.1%) and T group (67.7%). In the survival rate according to lymph node metastasis, there was no significant difference in the cumulative survival rates between two groups in pN0 and pN1 (Figure 5(b)).

But in the survival rate for patients with pN2, there was significantly difference between ST group (46.1%) and T group (66.7%). As for the survival rate according to stage, the survival of ST group was lower than that of T group in stages II, IIIA, and IIIB, but there was no significant difference (Figure 5(c)).

4. Discussion

The current standard treatment for proximal advanced gastric cancer in Japan is total gastrectomy with D2 lymphadenectomy. In order to accomplish D2 lymphadenectomy, splenectomy had been justified for complete removal of No. 10 as extended radical surgery. But extended resection which is regarded as a standard procedure in Asian countries is not effective in Western countries. The splenectomy caused

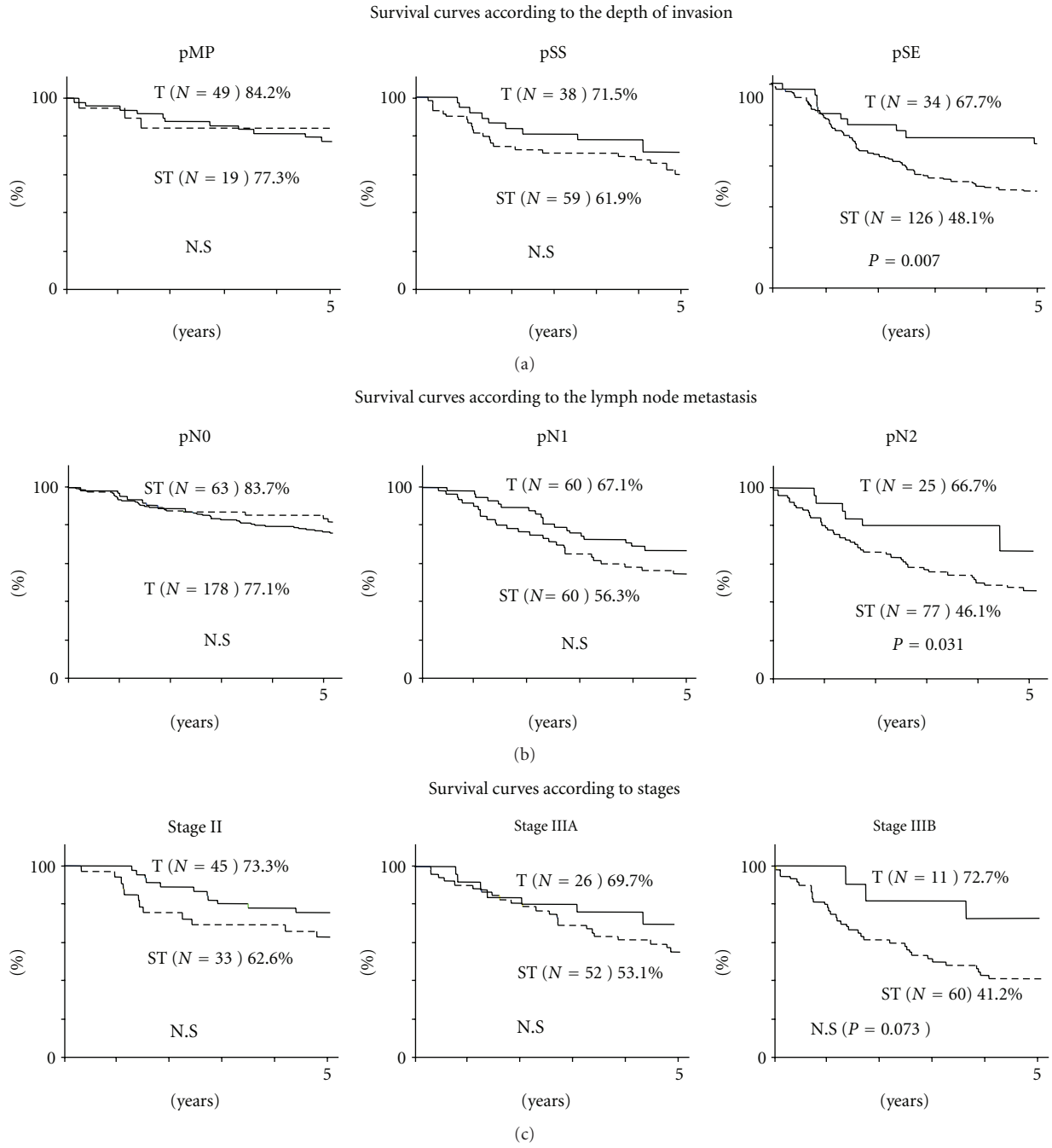

Figure 5: (a) Cumulative survival rates according to the depth of invasion (pT). As for pMP and pSS, there was no difference between T group and ST group, but the survival of the T group with pSE was better than that of ST group with pSE ($P = 0.007$). (b) Cumulative survival rates according to lymph node metastasis (pN). There was no difference in the cumulative survival rates between two groups with pN0 and pN1, but the survival of T group with pN2 was better than that of ST group with pN2 ($P = 0.031$). (c) Cumulative survival curves according to stage (pStage). There was no significant difference in the cumulative survival rates between two groups with Stage II, Stage IIIA, and Stage IIIB.

high morbidity and mortality, and it was shown to be an independent prognostic risk factor on multivariate analysis in node-negative patients in previous studies [10–15]. On the other hand, the splenectomy is considered to be a safe procedure that does not decrease surgical mortality [16]. A Korean trial has also reported that postoperative morbidity after splenectomy for D2 lymphadenectomy was not higher than simple total gastrectomy, but there was no significant difference in 5-year survival between with and without splenectomy [17]. Patients with proximal advanced gastric cancer localized on the greater curvature and type 4 might obtain relatively high survival benefits from No. 10

lymphadenectomy [18]. The splenectomy has become a safe technical procedure, but the surgical procedure of a total gastrectomy with splenectomy should be performed at a high volume hospital to avoid the postoperative complications.

The frequency of No. 10 metastasis was reported to be high in proximal advanced gastric cancer located on the greater curvature or in the posterior wall of the stomach, and lymphatic pathways along the posterior gastric artery, splenic artery, short gastric vessels, and/or gastroepiploic vessels were suggested to be important for No. 10 metastasis [19]. Lymphography has demonstrated that the lymphatic flow from the left upper region of the stomach enters the lymph node in the splenic hilum and travels to the nodes around the celiac trunk along the splenic artery [20]. In our study, the location involving the greater curvature, pN3 and No. 11d metastasis were risk factors for No. 10 metastasis, and the frequency of No. 10 metastasis was similar to that of No. 4sb, No. 9, and No. 11p metastasis. Furthermore, LN dissection effect index of No. 10 was almost as same as that of No. 9, No. 11p, and No. 11d. But the prognosis of patients with No. 10 metastasis was still poor even after its dissection. Furthermore, splenectomy does not improve survival of patients with proximal advanced gastric cancer even though curative resection was performed [21, 22]. Multivariate analysis demonstrated that nodal metastasis was independent prognostic factor, but splenectomy was not [23]. These reports suggested that the patients with No. 10 metastasis had already too extended LN metastasis to improve the prognosis. Accordingly, the splenectomy for D2 lymphadenectomy may be unnecessary in all the patients with advanced gastric cancer. On the contrary, some authors have found the survival benefit and recommended splenectomy for No. 10 lymphadenectomy. The splenectomy was one of the independent prognostic factors, and total gastrectomy with splenectomy is recommended for patients with No. 10 positive T3 proximal gastric cancer [24]. The survival of No. 10 positive patients was not to be different from that of No. 10 negative patients when curative surgery was performed [25]. The splenectomy was recommended when the tumor was located on the greater curvature or posterior wall of the stomach and had No. 4sa, No. 4sb, or No. 11 metastasis [19]. In fact, it is difficult to detect the depth of tumor invasion and No. 10 and/or No. 11 metastasis though a preoperative and intraoperative diagnostic technique. In Germany, No. 10 metastasis was observed only in advanced cancer, particularly in tumors located in the greater curvature and/or type 4 tumors [26]. Our current study showed that splenectomy adversely affected survival in pSE and pN2, while there was no significant difference in survival rates in pMP, pSS, pN0, and pN1 and among Stage II, IIIA, and IIIB. Though there were limitations of our study which was retrospectively conducted in a single institute, and there was selection bias, our study would suggest the benefit of spleen preservation on postoperative morbidity and long-term surgical outcomes. The overall survival rate stratified by stage was analyzed in a prospective randomized controlled trial [27], in which the 5-year overall survival rates of patients with stage I, stage II, and stage III were not significantly different between the 2 groups. Until 2005, our institute preferred to perform a total gastrectomy with splenectomy in advanced proximal gastric cancer for complete D2 lymphadenectomy. Recently we had a policy of splenectomy for the patients with No. 10 enlargement in the splenic hilum suggesting metastasis or tumor located in greater curvature or encircling in upper third portion of the stomach. A randomized controlled trail to evaluate total gastrectomy with splenectomy for proximal advanced gastric carcinoma with R0 resection (JCOG0110-MF) [28] has already recruited 505 patients and resulted that splenectomy was associated with higher morbidity and larger blood loss and was safely performed by specialized surgeons with low mortality. The precise impact of splenectomy on prognosis remains uncertain and the impact on long-term survival should be awaited.

In conclusion, although splenectomy for patients with proximal advanced gastric cancer was not an important risk factor for postoperative morbidity, splenectomy was not effective for patients with No. 10 metastasis in long-term survival. Spleen-preserving total gastrectomy will be feasible and be enough to accomplish radical surgery for locally advanced proximal gastric cancer.

Conflicts of Interest

There was no conflicts of interest in their submitted manuscripts.

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Clinical Study

Additional Gastrectomy after Endoscopic Submucosal Dissection for Early Gastric Cancer Patients with Comorbidities

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Purpose. We investigated the clinicopathologic features of early gastric cancer (EGC) patients who have undergone additional gastrectomy after endoscopic submucosal dissection (ESD) because of their comorbidities. *Methods.* Eighteen (7.1%) of 252 GC patients were gastrectomized after prior ESD. Reasons for further surgery, preoperative and postoperative problems, and the clinical outcome were determined. *Results.* The 18 patients had submucosal EGC and several co-morbidities. Other primary cancers were observed in 8 (44.4%). Histories of major abdominal operations were observed in 6 (33.3%). Fourteen patients (77.8%) hoped for endoscopic treatment. Due to additional gastrectomy, residual cancer was suspected in 10, and node metastasis was suspected in 11. A cancer remnant was histologically observed in one. Node metastasis was detected in 3 (16.7%). Small EGC was newly detected in 4. Consequently, additional gastrectomy was necessary for the one third. No patient showed GC recurrence. However, 9 (50%) had new diseases, and 4 (22.2%) died of other diseases. The overall survival after surgery in these patients with additional gastrectomy was poorer than those with routine gastrectomy for submucosal EGC ($P = 0.0087$). *Conclusions.* Additional gastrectomy was safely performed in EGC patients with co-morbidities. However, some issues, including presence of node metastasis and other death after surgery, remain.

1. Introduction

Endoscopic treatments, including endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD), for early gastric cancer (EGC) have markedly progressed and are widely accepted [1–3]. EMR can be safely performed for EGC conditioned by differentiated mucosal EGC smaller than 20 mm in diameter [4], and the general indications were proposed by the Japanese Gastric Cancer Association. Recently, ESD is frequently used for the treatment of EGC because ESD has the advantage of curability on achieving successful en-block resection [1–3]. Furthermore, extended indications of EMR/ESD for EGC have been clinicopathologically investigated [5–8]. The expanded criteria of EMR/ESD for EGC are still controversial [9], and the expanded criteria need to be confirmed yet. Most mucosal EGC showed low-level lymph node metastasis while approximately 15–20% of submucosal EGC showed node metastasis [10]. There are cases that underwent additional gastrectomy after EMR/ESD, because cancer invades the submucosal layer of the stomach with/without lymphovascular invasion histopathologically detected in specimens obtained by EMR/ESD. Regarding criteria for EGC treatment, patients sometimes hope for endoscopic treatment, as it is less invasive compared to surgery. Haruma et al. [11] reported that endoscopic therapy in patients who are inoperable or have a high surgical risk appears effective. There have recently been reports regarding additional treatments, including gastrectomy, after incomplete EMR/ESD [12, 13]. Patients with gastrectomy after prior EMR/ESD may have several problems regarding the surgical management and long-term outcome. Especially, the clinicopathologic issues, excluding the histopathologic features of gastric cancer, in gastrectomized patients after prior ESD have not yet been investigated.

We retrospectively investigated the clinicopathologic issues concerning gastrectomized patients after ESD for EGC, and the reasons for ESD and further surgery for EGC, and pre- and postoperative problems were determined from a surgical viewpoint.

2. Patients and Methods

2.1. Patients. We retrospectively analyzed our database of all GC patients who underwent gastrectomy. A total of 252 patients with GC were newly diagnosed and surgically treated between 2004 and 2008 at Shinshu University Hospital. Eighteen patients (15 men and 3 women, 7.1%: Group A) were additionally gastrecomized after ESD for EGC. Sixty-four patients (49 men and 15 women, 25.4%: Group B) were routinely and electively gastrectomized for EGC with submucosal invasion.

2.2. Clinicopathologic Issues. The following parameters were investigated: (1) problems before treatments, (a) comorbidities, (b) multiple cancers of other organs, and (c) history of major abdominal surgery and invasive treatment for other diseases; (2) reason for treatment of EGC, (a) reason for receiving ESD, and (b) reason for additional gastrectomy (pathologic findings of ESD); (3) pathologic findings after additional gastrectomy; (4) postoperative complications; (5) clinical outcome after additional gastrectomy. Finally the clinicopathologic features in Group A were compared with those of Group B.

2.3. Endoscopic Treatment. ESD for EGC was performed by several gastrointestinal endoscopists in Shinshu University Hospital and other hospitals in Nagano Prefecture. One hundred and fifty-three patients received endoscopic treatments in the same period, and 15 (9.8%) of the 153 patients with additional gastrectomy received prior endoscopic treatments at our hospital. The remaining 3 visited from other hospitals for endoscopic treatment of EGC in our hospital. ESD for EGC was carried out employing a few methods using hook and flex knives or an insulation-tipped diathermic knife. Usually, mucosal EGC was indicated for ESD and en-block removal. The removed tissues were formalin fixed and paraffin embedded. The obtained sections were histopathologically examined and were routinely evaluated according to the Japanese Classification of Gastric Carcinoma established by the Japanese Gastric Cancer Association [14].

2.4. Gastrectomy after ESD. When the histopathologic findings after ESD showed submucosal invasion with/without lymphatic or venous invasions, additional gastrectomy was considered. Gastrectomy with regional node dissection for EGC with/without prior ESD was performed employing an open or laparoscopy-assisted approach. When EGC was located in the lower and middle thirds of the stomach, distal gastrectomy was performed. When EGC was located in the upper third of the stomach, total or proximal gastrectomy was performed. Regional nodes were routinely dissected using the procedure of D1 (with no. 7) or (with no. 7, 8a, and 11p) in patients with EGC. In advanced GC, D2-node dissection was usually performed. These resected specimens and lymph nodes were examined according to the routine histopathologic procedures for diagnosis and staging. The clinicopathologic features of GC were described according to the Japanese Classification of Gastric Carcinoma [14]. All 18 patients with additional gastrectomy were performed D1-lymphadenectomy: 14 with node dissection surrounding left gastric arteries and 4 with additional dissection of the nodes surrounding the common hepatic and splenic and celiac arteries.

2.5. Clinical Outcome after Surgery. For 5 years after surgery, these patients were followed in the outpatient clinic of Shinshu University Hospital in order to check for recurrence/metastasis of the tumors by esophagogastroduodenoscopy every year and computed tomography (CT) of the abdomen and chest every 6 months and/or annually. Death caused by recurrence/metastasis of GC or by other diseases after surgery was investigated.

2.6. Statistical Analysis. Data are shown as the prevalence, or mean and ordinal data were compared by the Mann-Whitney U and Chi-square or Fisher's exact probability test. Five-year survival rates after surgery were calculated employing the Kaplan-Meier method. $P < 0.05$ was considered significant.

3. Results

3.1. Problems before Treatments

Comorbidities. All 18 patients had one or more comorbidities before treatments for EGC (Table 1). Additionally, two of 4 patients with diabetes mellitus had not yet been treated, and insulin treatment was started for surgery. One with chronic obstructive pulmonary disease and one with idiopathic interstitial pneumonia routinely underwent home-oxygenic treatment. Two patients with dermatomyositis were treated with predonisolone long term.

Multiple Cancers in Other Organs. Other primary cancers were observed in 8 patients (44.4%: Table 2). Nine antecedent cancers in other organs were observed in 7 patients, while one synchronous cancer was observed in the liver. In these patients, 6 tumors had undergone surgical treatment.

History of Major Surgery and Invasive Treatment. Histories of major abdominal operations were observed in 6 patients (33.3%). Gastrectomy had been carried out for previous EGC in one patient. Colectomy for advanced colon cancer had been conducted in 2 patients. Oophorectomy for ovarian cancer and benign ovarian tumor had been performed in one each. Hysterectomy for uterine cancer had been performed in one patient. Hepatectomy for hepatocellular carcinoma and the extirpation of retroperitoneal liposarcoma had been performed in one. A history of invasive treatment/operation for other disorders was observed in 3 patients (16.7%): surgical clipping of cerebral aneurysm, coronary artery bypass grafting, or percutaneous coronary intervention for ischemic heart disease. These invasive treatments with/without surgery were performed in 9 patients (50.0%).

3.2. Reason for Treatment of EGC

Reason for Receiving ESD. Regarding the patients' opinions, 14 (77.8%) of the 18 patients hoped for ESD treatment

Table 1: Preoperative comorbidities in patients with additional gastrectomy.

Comorbidities	No. of cases
Cardiovascular diseases	10 (55.6%)
Hypertension	8
Angina pectoris	2
Complete atrioventricular block	1
Pericarditis induced by radiotherapy	1
Cerebral diseases	5 (27.8%)
Infarction	4
Hemorrhage	1
Diabetes mellitus	4 (22.2%)
Pulmonary diseases	4 (22.2%)
Chronic obstructive pulmonary disease	2
Interstitial pneumonia	2
Liver cirrhosis	2 (11.1%)
Dermatomyositis	2 (11.1%)
Idiopathic thrombocytopenic purpura	1 (5.6%)
Amyloidosis	1 (5.6%)

for EGC. They gave the following reasons: their advanced age, comorbidities, history of abdominal surgery, and social convenience. Regarding the doctors' opinions, EGC was suspected as submucosal cancer, but diagnostic treatment was conducted in 4 patients (22.2%) by ESD.

Reason for Additional Gastrectomy (Pathologic Findings of ESD). Twenty-three EGC were removed by ESD in the 18 patients; double lesions patients were removed in 5 patients (27.8%). Cut-end margins of 11 lesions in 10 patients (55.6%) were positive: the positive lateral margin in 3 patients (16.7%) and the positive vertical margin in 8 patients (44.4%). In one patient, both margins were positive. Submucosal invasion was demonstrated in 19 lesions of the 18 patients. Submucosal invasion less than 500 μm from the muscularis mucosa (sm1) was shown in 5 lesions of 4 patients (22.2%), and submucosal invasion deeper than 500 μm from the muscularis mucosa (sm2) in 14 lesions of 14 patients (77.8%). Histological vessel invasions, including lymphatic and/or venous invasions, were shown in 11 lesions: 2 lesions with sm1 and 9 lesions with sm2. Based on the histopathologic findings of EGC specimens after ESD, residual cancer was suspected in 10 patients, and node metastasis was suspected in 11 patients because of presence of histologic lymphatic and/or venous invasion. The remaining 2 showed a regional node swelling (over 10 mm in diameter) on abdominal CT in the followup after ESD. Finally, the 18 patients underwent additional gastrectomy.

3.3. Pathologic Findings after Surgery. In primary lesions, cancer remnant was observed in only one patient, while no remnant was observed in 17 patients (94.4%). New cancer was detected in the resected stomach after gastrectomy in 4 patients (22.2%); 2 of the 4 patients had a third cancer. These lesions were mucosal and less than 10 mm in diameter; majority of them shows 2–4 mm in size. Node metastasis was observed in 3 patients (16.7%). Two cases pathologically showed a metastatic node, and one had 4 metastatic node. The 2 cases with preoperatively suspected node metastasis on CT revealed no metastasis histologically. In addition, *Helicobacter pylori* was observed in 17 (94.4%), but the presence was same between the patients with single and multiple gastric cancer in Group A.

3.4. Postoperative Complications. Postoperative complications were observed in 9 patients (50.0%) in Group A. Liver dysfunction was observed in 3 patients; one of them showed massive ascites, approximately 1,000-mL drainage, every day after surgery. Infection of the central catheter was observed in 2 patients with total gastrectomy. Postoperative delirium was observed in 2 patients. Other postoperative complications were acute pancreatitis, pericarditis with heart failure, angina pectoris, and atrial fibrillation with tachycardia. These complications were conservatively treated, and consequently improved. In Group B, no case with anastomotic leakage was also observed. There was no difference of postoperative morbidity between Group A and Group B. No postoperative mortality was observed. In addition, there was no difference in operative blood loss and operating time between the two groups (Table 3).

3.5. Clinical Outcome after Surgery. In the followup, there was no case of additional gastrectomy with cancer recurrence/metastasis. New disorders after surgery were observed in 9 patients (50.0%). Mental/neurological diseases, including depression and dementia, were observed in 4 patients over 75 years old. Other primary cancers were subsequently observed in 2 patients (11.1%): hepatocellular carcinoma 26 months after and lung cancer 50 months later. Cancer of the gastric remnant was newly detected 36 months after distal gastrectomy. Death due to other diseases was observed in 4 patients (22.2%) without the recurrence of EGC: respiratory failure due to amyloidosis, hepatic failure due to cirrhosis, metastatic lung cancer detected after gastrectomy, and the recurrence of esophageal cancer treated by chemoradiotherapy before ESD.

A significant difference between Group A and Group B was observed in the age, tumor size, histologic differentiation, preoperative complications, surgical risk, and death by other diseases (Table 3). New disorders after surgery were frequently observed in Group A than Group B although this was not statistically significant. No case with gastric cancer recurrence was observed in Group A as well as Group B. Group A showed a less favorable outcome after surgery in terms of overall survival than Group B ($P = 0.0087$). However, the cancer survival rate after additional gastrectomy was the same in the two groups.

4. Discussion

There are several clinicopathologic issues in patients with submucosal cancer treated by additional gastrectomy after ESD for EGC. Two major points regarding diagnostic and therapeutic issues in these patients with additional

Table 2: Multiple cancers in other organs.

Other primary cancers	No. of cases	Treatment
Metachronous cancer before ESD	7	
Squamous cell carcinoma/esophagus	2	ESD (1), CRT (1)
Adenocarcinoma/duodenum	1	ESD
Adenocarcinoma/colon	2	Surgery
Hepatocellular carcinoma/liver	1	Surgery
Adenocarcinoma/ovary	1	Surgery
Squamous cell carcinoma/uterus	1	Surgery
Liposarcoma/retroperitoneum	1	Surgery
Synchronous cancer	1	
Hepatocellular carcinoma/liver	1	Surgery

ESD, endoscopic submucosal dissection; CRT, chemoradiotherapy.

gastrectomy were considered. One is a problem regarding EGC and metastasis, including incomplete resection by ESD, the presence of node metastasis before ESD, and the oversight of small EGC and metachronous cancer after ESD. The other is a problem regarding patients involving the surgical risk, other primary cancers, and new disorders after gastrectomy. On consideration of these issues, it is important that additional gastrectomy is employed based on the histopathologic findings after ESD.

There was only one case with a cancer remnant on the removed stomach although 9 showed positive lateral and/or vertical margins after ESD. Piecemeal resections of EGC by EMR have been reported to be associated with a high risk of local recurrence [1], and ESD for EGC has the advantage of being associated with a lower frequency of recurrence than EMR [2]. Furthermore, Yokoi et al. [15] reported that ESD facilitates the curative resection of locally recurrent EGC. In the present study, 94.4% of the cases with additional gastrectomy had no cancer remnant. This finding may be explained by burn degeneration at the cut-end of EGC treated by ESD. Tanabe et al. [16] reported that the mean width of burning degeneration at the cut ends of EGC treated by ESD was 1,203 μm. Goto et al. [17] reported that preceding ESD for EGC had no negative influence on the prognosis when additional gastrectomy was performed, and it may be permissible to remove some EGC by ESD as a first step to prevent unnecessary gastrectomy. Therefore, cases without local recurrence of EGC may undergo the omission of additional gastrectomy when no node metastasis can be definitely shown.

Mutual features in the cases with node metastasis detected after additional gastrectomy were considered as follows: (1) a protruding type tumor, (2) over 25 mm in tumor size, (3) moderately differentiated type, (4) sm2, (5) positive lymphatic invasion, but (6) no cancer remnant after ESD. Furthermore, the 3 had preoperative comorbidities and a surgical history of laparotomy. Gotoda et al. [5] reported that 18.6% of submucosal EGC showed node metastasis histopathologically, while 91% of surgically treated EGC did not have node metastasis. Oda et al. [13] reported that 6.3% of noncurative patients with a possible risk of node metastasis after EMR/ESD for EGC showed regional node metastasis after gastrectomy. The present cases had to receive gastrectomy initially, because their histopathologic findings after ESD were considered to be associated with a high risk of node metastasis in EGC. However, they desired not to undergo surgery because of their advanced age, presence of physical and social complications, and history of laparotomy. It is hard to detect regional nodes with metastasis employing abdominal ultrasonography, endoscopic ultrasonography, CT, and conventional magnetic resonance imaging [18]. A new modality is necessary for an accurate diagnosis of node metastasis in patients with EGC before EMR/ESD and surgery. Additional gastrectomy based on the histopathologic findings after ESD is unnecessary in two thirds of the present cases without cancer remnants or node metastases, while the fact that additional gastrectomy is necessary in one third of the patients may be important. From the findings of the three cases with node metastasis detected after additional gastrectomy, we suggested that additional gastrectomy should be performed in EGC patients with co-morbidity showing over 25 mm in tumor size, sm2-invasion, and lymphatic invasion. However, it is possible that additional gastrectomy after ESD is avoided in the other patients, when no small cancer is detected endoscopically.

Regarding multiple gastric cancer after EMR/ESD, in the present study, 33.3% of the cases synchronously showed multiple EGC. Four lesions, detected after additional gastrectomy, were missed prior to ESD. Probably, endoscopists might overlook these lesions because of their small size. Nasu et al. [19] reported the characteristics of metachronous and synchronous EGC on initial EMR. Takenaka et al. [20] reported metachronous cancers of the gastric remnant after distal gastrectomy and the utility of ESD for EGC of the gastric remnant. Although EGC newly detected after additional gastrectomy was mucosal and small cancer, these lesions may have the potential to become metachronous cancer after ESD in the future.

Most of the patients undergoing additional gastrectomy hoped for treatment of EGC by ESD as a less-invasive procedure because they had several underlying diseases/preoperative comorbidities and surgical histories for other diseases. They were elderly and had a higher surgical risk in gastrectomy after ESD than routine gastrectomy for

Table 3: A Comparison of the clinicopathologic features of gastrectomized patients with and without ESD.

Variable		With ESD ($n = 18$)	Without ESD ($n = 64$)	P value
Age (mean ± SD: year-old)		72.5 ± 6.3	67.5 ± 10.8	0.029
Gender				0.39
	Men	15	49	
	Women	3	15	
Location				0.19
	Upper	9	19	
	Middle	6	22	
	Lower	3	23	
Tumor size (mean ± SD: mm		25.1 ± 12.1	35.1 ± 18.4	0.04
Gross type				0.08
	Protruding/elevated	11	21	
	Flat	0	2	
	Depressed/excavated	7	41	
Histologic differentiation				0.012
	Well/moderately	18	48	
	Poorly/signet ring cell	0	16	
Depth of invasion				0.71
	sm 1	4	17	
	sm 2	14	47	
Node metastasis				0.63
	Positive	3	11	
	Negative	15	53	
Lymphatic invasion				0.52
	Positive	8	34	
	Negative	10	30	
Venous invasion				0.057
	Positive	5	34	
	Negative	13	30	
Hepatic metastasis				0.78
	Positive	0	1	
	Negative	18	63	
Tumor number				0.19
	Solitary	12	52	
	Double or more	6	12	
Preoperative comorbidities				<0.001
	Positive	18	34	
	Negative	0	30	
History of major abdominal surgery				0.4
	Positive	6	15	
	Negative	12	49	
History of gastrectomy				0.7
	Positive	1	4	
	Negative	17	60	
History of major extra-abdominal surgery				0.38
	Positive	3	7	
	Negative	15	57	
Other primary cancer				0.3
	Positive	8	20	
	Negative	10	44	
Surgical risk				0.012
	Positive	18	48	
	Negative	0	16	

Table 3: Continued.

Variable		With ESD ($n = 18$)	Without ESD ($n = 64$)	P value
Operating time (mean ± SD: min)		312.5 ± 74.2	314.4 ± 82.5	0.77
	Total gastrectomy	327.2 ± 46.9	34.6 ± 65.0	0.57
	Distal gastrectomy	304.5 ± 86.7	293.8 ± 86.6	0.69
Operative blood loss (mean ± SD: mL)		190.6 ± 134.4	222.0 ± 148.0	0.43
	Total gastrectomy	206.7 ± 110.2	242.6 ± 166.4	0.79
	Distal gastrectomy	181.8 ± 150.3	208.9 ± 135.8	0.13
Postoperative complications				0.14
	Positive	9	20	
	Negative	9	44	
New disorders in followup				0.074
	Positive	9	19	
	Negative	9	45	
Death by other diseases				0.019
	Positive	4	2	
	Negative	14	62	

SD, standard deviation.

submucosal cancer. Furthermore, the nonelderly patients under 70 years old with additional gastrectomy also had several preoperative comorbidities. Hirasaki et al. [21] reported that elderly patients over 75 years old treated by ESD for EGC frequently had underlying diseases, but there were no differences in complications after ESD and the complete resection rate. Kakushima et al. [22] reported that the complete resection rate in ESD for EGC and the complication rate after ESD in elderly patients were not significantly different from those of younger patients. Because the number of elderly EGC patients with/without underlying diseases and surgical risk has been steadily increasing worldwide, we should pay attention to these EGC patients, treated by ESD and/or additional gastrectomy, regarding the clinicopathologic issues of age and comorbidities. Postoperative complications were frequently observed in the gastrectomized patients after ESD compared to those treated with routine gastrectomy; however, no mortality was observed in this series. No data from large-scale studies was found in postoperative morbidity and mortality comparing between EGC patients receiving additional and routine gastrectomy.

The disease free-survival rate after additional gastrectomy in patients with incomplete ESD for EGC was not worse [17], similar to the present study. No data were found for other primary cancers and new disorders, including mental/neurological disorders, in a long-term followup of EGC patients treated by additional gastrectomy after ESD. Approximately 1.5–5.4% of patients with subsequent cancer in other organs developing metachronously were detected after EGC treatment [23–25]. The number of patients with other subsequent cancers after surgery for EGC may not be so high, but a high frequency (16.7%) of other subsequent cancers was observed in the present study. Furthermore, no data were found regarding new disorders after additional gastrectomy for EGC, although the clinicopathologic studies of ESD in elderly patients with EGC were identified [21, 22]. The fact that the present cases with additional gastrectomy frequently had comorbidities/underlying diseases may affect the capacity to discover new disorders after additional gastrectomy. From these findings, it was considered that overall survival of cases with additional gastrectomy was poorer than in those receiving routine gastrectomy for submucosal EGC.

5. Conclusion

EGC patients with a number of comorbidities, including a surgical history and multiple cancers in other organs, may hope for less-invasive treatment by ESD. Consequently, additional gastrectomy may be recommended in one third, and we should consider several issues, including surgical problems as well as the complete resection of cancer and node metastasis before/after additional gastrectomy. Additional gastrectomy is safely performed in EGC patients with several comorbidities. It is possible that additional gastrectomy may be avoided in the other patients with comorbidities, when another small EGC may not be detected endoscopically. Regarding the followup, an issue that some of these patients died of other diseases remains. Furthermore, new modalities for diagnosis of lymph node metastasis may need for omission of additional gastrectomy.

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