SSAT Public Policy Committee Panel: Technology in Gastrointestinal Disease Summary Statement

PANEL MODERATOR: *Richard A. Prinz, M.D.*

Technology has and will continue to have a major impact on all aspects of our lives. This is especially true of our professional lives as medicine and surgery are being transformed by ongoing technical advances. Improved imaging and minimally invasive surgery are but harbingers of even more dramatic changes to come. The goal of this panel was to look forward at what some of these technologic developments might be and how they will affect what we do in terms of both surgical training and clinical practice. We also wanted to show what is actually being implemented and applied at the cutting edge of technology today. Finally, technology comes at a cost; the most obvious one is financial. Resources are limited, so we need to be able to evaluate the cost-benefit and cost-effectiveness of these technologies.

Our panelists are extremely well qualified as leaders in the field to address these issues. In addition to being professor of surgery at the University of Washington Medical Center, Dr. Richard Satava is the program manager of the Advanced Biomedical Technologies Program at the Defense Department's Advanced Research Projects Agency. He will describe how new technologies are emerging from the information age. Information both about our patients and about what we do will be ever more available. Robotics and telemedicine are aspects of this information explosion, because they insert a computer between our eyes and our hands. Dr. Luc Soler, an associate professor of medicine at the University of Strasbourg, has been working in computerized medical image analysis, three-dimensional modeling, and virtual reality. By using advanced imaging to create three-dimensional modeling of patients and their anatomic abnormalities, a virtual planning and simulation of the surgical procedure can be done. The preoperative planning and simulation allow for both improved care because of greater accuracy and safety and improved training because the operation can be performed and critiqued in virtual reality. Dr. Mark Talamini was the director of minimally invasive surgery at the Johns Hopkins Hospital and is now professor and chairman of the Department of Surgery at the University of California San Diego. In his new role, he will have to choose the technologies his department truly needs because he certainly cannot afford all that his faculty want. All of the technologies that are being explored have potential value for education and clinical care. However, no institution has the financial resources to purchase all of them. When we invest in one of these technologies, it is usually expensive; thus, our decision to purchase a particular one means that we cannot buy something else. An understanding of how we evaluate these different technologies in terms of their cost-effectiveness and cost-benefit is necessary if we are to make prudent investments.

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Quality of Life in GERD Patients: Medical Treatment Versus Antireflux Surgery

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Medical and surgical treatments are able to improve symptoms in patients with gastroesophageal reflux disease (GERD). The aim of this study was to evaluate the outcome in GERD patients without therapy, under continuous medical treatment, and after laparoscopic antireflux surgery. Five hundred seventy-nine consecutive patients underwent medical or surgical treatment for GERD-induced symptoms. Patients were studied in detail before and after treatment by means of a symptom questionnaire, endoscopy, esophageal manometry, 24-hour esophageal pH monitoring, and a barium esophagogram. In addition, quality of life was measured by the means of the Gastrointestinal Quality of Life Index (GIQLI) and the Health-Related Quality of Life (HRQL) questionnaire. Surgery was indicated and performed in 351 patients with persistent or recurrent GERD symptoms and/or complications, and in patients preferring surgery to medical treatment, despite the use of an adequate medication. The remaining 228 patients were treated with proton pump inhibitors (PPI) in the standard dose, or if required, the double dose. The outcome was assessed 3 and 12 months after treatment. While symptoms and quality of life were highly impaired in GERD patients without therapy compared with normal people, a significant improvement was obtained by PPI therapy. Following surgery, quality of life was normalized in all subsections and was significantly higher compared with the medically treated group. These results stayed constant in short-term and intermediate follow-up. Medical and surgical therapies are both able to improve symptoms and quality of life in GERD patients. Nevertheless, the outcome is significantly better following surgery. It can be suggested that surgical treatment may be the more successful therapy in the long-term.
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Key words: Gastroesophageal reflux disease, GERD, antireflux surgery, quality of life, medical treatment, proton pump inhibitors

Gastroesophageal reflux disease (GERD) is a common upper gastrointestinal disorder affecting up to 44% of the adult population.[1] Increasing prevalence of at least occasional reflux symptoms ranges from 12% to 54%; esophagitis varies from 30% to 79% and is frequently associated with dysphagia and respiratory symptoms found in up to 50% of the adult population.[1–6] GERD is caused by a defective antireflux barrier to gastric and duodenal contents. The impaired lower esophageal sphincter (LES) is the basic anatomical defect, and transient lower esophageal sphincter relaxations (TLESR) are thought to be the main mechanism allowing gastric acid to reflux into the esophagus and to cause damage to the esophageal mucosa.[7,8] Nevertheless, several other factors such as impaired esophageal and antroduodenal motility, the mucosal exposure time to the refluxate, delayed gastric emptying, reflux of duodenal juice, alterations of gut neuropeptides, and free radical damage contribute to the development of GERD.[2,9–11] Medical therapy mainly inhibits gastric acid production and is generally accepted as a long-term maintenance therapy. Antireflux surgery restores the function of the LES, controls reflux,

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improves impaired esophageal peristalsis, and speeds gastric emptying by reconstructing the physiology of the gastroesophageal junction.[7,8,12–16]

The aim of this study was to evaluate and compare the outcome of GERD-related symptoms in patients without treatment, under continuous medical therapy, and following laparoscopic antireflux surgery, and to assess the effect of treatment on quality of life.

MATERIAL AND METHODS

From 2000–2004, a group of 579 consecutive GERD patients underwent medical and/or surgical treatment in one expert center (foregut laboratories of the Department of Surgery). Eighty-five percent of all patients were under medical surveillance before being referred to the center; the remaining patients had no medication or contact with a physician prior to presentation. Patient evaluation included physical examination, a standardized symptom questionnaire, length of medical acid suppression, chest radiographs, upper gastrointestinal endoscopy, barium swallow esophagogram, esophageal manometry, 24-hour esophageal pH monitoring, and assessment of quality of life.

Standardized biopsies were taken from the gastroesophageal junction. Esophagitis was graded using the Savary Miller classification system.[17] Barrett's metaplasia was confirmed by the presence of columnar-type epithelium with intestinal metaplasia in the esophageal biopsy specimen.[18] In addition, laryngeal lesions were inspected carefully for presence of inflammation, edema, leukoplakia, or ulcerations.

Esophageal manometry was performed using a stationary pull-through technique with a 5-channel water perfused catheter with 5 cm spacing between the channels.[19] Twenty-four hour esophageal pH monitoring was performed as described previously.[20] A DeMeester score of more than 14.8 indicated abnormal acid reflux. Medication that might interfere with esophageal motor function (i.e., metoclopramide, cisapride, nitrates, ß-agonists, and calcium channel-blocking agents) and esomeprazole were discontinued 7 days before both studies. Preoperative barium esophagogram was completed in all patients for assessment of the anatomical situation and to clearly identify hiatal hernias.

Disease-related quality of life was evaluated using the Gastrointestinal Quality of Life Index (GIQLI) by Eypasch et al.[21,22] This questionnaire is well established, validated, and has been recommended by the European Study Group for Antireflux Surgery. Based on responses to 36 items, the general score for the GIQLI is graded on a scale from 0 to 144 points. The GIQLI is divided into five subsections: gastrointestinal symptoms (0–76 points), emotional status (0–20 points), physical functions (0–28 points), social functions (0–16 points), and a single item for stress related to medical treatment (0–4 points). The Health-Related Quality of Life (HRQL) questionnaire by Velanovich et al.[23] was the second test used to evaluate therapy effects. The responses to 10 items reflect symptoms related to GERD (1–6), physiologic measurements using 24-hour pH monitoring (1–3), dysphagia symptoms as progression of reflux esophagitis to stricture (7,8), relative annoyance of medication (9), and the patient's overall satisfaction with the present condition (10). A score from 0 to 50 points (0 = very satisfied, 50 = incapacitated) is evaluated.

Both questionnaires were handed to all patients at 3 and 12 months following treatment. Furthermore, follow-up investigations including clinical assessment, endoscopy with biopsies, esophageal manometry, 24-hour esophageal pH monitoring, and a barium esophagogram were also performed at 3 and 12 months following surgery.

All patients had medical therapy with PPIs (at least esomeprazole 40 mg daily for a minimum of 3 months). A higher dose was applied if patients still suffered from GERD symptoms on the standard dose. Antireflux surgery was indicated and performed in patients with persistent or recurrent GERD symptoms and/or complications despite maximal conservative treatment, and in patients preferring surgery to medical treatment. Based on presurgical evaluation, the Nissen fundoplication was performed in patients with normal esophageal body function, and in the case of impaired esophageal peristalsis, a partial posterior fundoplication was performed. Both procedures were completed laparoscopically as previously described.[7,14]

Statistical Analysis

Values are expressed as medians and interquartile ranges. Changes from before versus after therapy were evaluated using a Wilcoxon signed rank test for paired observations. For between-group comparisons, an unpaired Wilcoxon test was applied. A P value below 5% was considered to be statistically significant.

RESULTS

In the present study, gastroesophageal reflux disease was confirmed to be the reason for impaired quality of life in all 579 patients. There were 348 (60%) male and 231 (40%) female patients, with

a median age of 52 years (range, 16–81 years). Most patients had a positive history of heartburn, and 363 (63%) suffered from intermittent or permanent regurgitation for a median duration of 5 years (range, 2.5–10 years). Heartburn and epigastric pain were controlled in the medically treated group in 87% of the patients. On the other hand, PPI therapy had only little effect on respiratory symptoms (37%) and regurgitation (30%). The complete list of typical and general GERD-symptoms before and 3 and 12 months after surgery is shown in Table 1.

Based on endoscopic and radiological examinations, 520 patients (90%) showed a hiatal hernia with a median size of 5 cm (range, 2–15 cm). A typical Barrett's lesion was detected on endoscopy in 142 patients (25%). Four hundred sixty-four patients (80%) had a defective LES on manometry. A positive DeMeester score demonstrated on 24-hour pH monitoring was found in 429 patients (74%).

All patients were on long-term esomeprazole or pantoprazole 40 mg daily for a median period of 12 months (range, 2–120 months). If patients still suffered from GERD-induced symptoms while being on medication, a higher dose (60–80 mg) was applied. The fact that most patients were under medical surveillance before being referred to our department explains the generally lower rate of patients on permanent medical treatment.

Two hundred twenty-eight (39%) patients remained on continuous successful treatment with proton pump inhibitors (PPI). Antireflux surgery was indicated and performed in 351 (61%) patients with a long history of persistent or recurrent GERD symptoms and/or complications despite maximal conservative treatment, in patients with a relapse of GERD symptoms or esophagitis after medication was withdrawn, and in patients preferring surgery to a life-long medical treatment. The Nissen fundoplication was performed in 321 patients (91%) with normal esophageal peristalsis, and the partial posterior fundoplication was completed in 30 patients (9%) with impaired esophageal body function. The morbidity rate was 7.7%, including intraoperative bleeding, organ perforation, pneumothorax, and postoperative pneumonia. None of these patients required a reoperation. No patient died following surgery. Thirty-three patients (10%) had a positive DeMeester score 12 months following surgery. Only 64% (21/33) of patients complained of heartburn; 17 patients (5%) still require occasional PPI therapy. Fourteen patients (4%) complained of dysphagia postoperatively, 13 of which underwent successful endoscopic dilatation treatment.

Quality of life was highly impaired in all GERD patients without therapy compared with normal people (GIQLI 122 points, HRQL 0 points) and was below the quality of life index of patients with malignant or heart diseases. A significant improvement was obtained by PPI therapy (Table 2). After surgery, the quality of life was normalized in all subsections, corresponding to a mean intraindividual overall change of 33.3 points ($P < 0.0001$) and was significantly higher compared with the medically treated group (Table 2). There was no statistically difference between the normal population and the operated patients. Furthermore, a significant decrease of complaints was evident following surgery (Table 1), these findings being confirmed by a decrease of the pathophysiological parameters measured postoperatively (Table 3). These results stayed constant in the short and intermediate follow-up.

Table 1. Spectrum of symptoms before treatment, and 3 and 12 months after surgery

Symptoms	Before treatment n = 579	3 mo postoperative n = 342 (97%)	12 mo postoperative n = 326 (93%)
Heartburn	541 (93%)	16 (5%)	21 (6%)
Epigastric pain	421 (73%)	9 (3%)	5 (2%)
Regurgitation	363 (63%)	9 (3%)	5 (2%)
Dysphagia	41 (7%)	20 (6%)	9 (3%)
Respiratory symptoms	197 (34%)	14 (4%)	8 (2%)
Globus sensation	66 (11%)	8 (2%)	11 (3%)
Flatulence	136 (23%)	82 (24%)	60 (18%)
Vomiting	63 (11%)	7 (2%)	0 (0%)
Bleeding episodes	11 (2%)	0 (0%)	0 (0%)

DISCUSSION

Quality of life assessment of patients has become a major topic in recent years. The subjective

Table 2. Quality of Life tests: Health-Related Quality of Life and Gastrointestinal Quality of Life Index in gastroesophageal reflux disease patients before treatment, under medical care, and after surgical treatment

Quality of life tests	Without therapy	PPI therapy	12 mo postoperative
GIQLI	95 (79–108)	104 (89–117)*	119 (106–130)†
HRQL	20 (13–28)	12 (6–19)*	1 (0–4)†

Values expressed as medians and interquartile ranges.
*$P < 0.05$ vs. without therapy.
†$P < 0.05$ vs. without and with PPI therapy (Wilcoxon signed rank test).

Table 3. Manometric parameters of the lower esophageal sphincter and the DeMeester reflux score before and after surgery

Manometric parameters	Before surgery	3 mo postoperative	12 mo postoperative
LES resting pressure (mmHg)	5 (3–8)	17 (13–21)*	16 (13–19)*
LES intra-abdominal length (cm)	1 (0–2)	2 (2–3)*	2 (2–3)*
DeMeester score	27 (16–44)	4 (1–8)*	4 (2–8)*

Values expressed as medians and interquartile ranges.
*$P < 0.05$ vs. before surgery (Wilcoxon signed rank test).

perception of the impact of gastroesophageal reflux disease (GERD) on quality of life is related not only to the patient's specific symptoms, but also to his sense of physical well-being, the impact of the disease on social relations, and its psychological effects.[24,25] The GIQLI and the HRQL are used to measure this personal perception of the disease.[21–23]

Several studies have been published in which medical therapy has been compared with antireflux surgery, with better results demonstrated in the surgically treated group.[26,27] The results of the present study show that the quality of life in GERD patients is severely impaired in all measured items. When symptoms become severe enough to require regular PPI medication, there is a significant impact on quality of life due to the addiction to drugs.[28,29] Furthermore, patients with medical therapy may have to make significant lifestyle changes to achieve adequate control of GERD symptoms.[30] Lifestyle alterations including dietary modification, elevation of the head of the bed, and uncomfortable sleep may affect the ability of patients to live and socialize, consequently affecting their quality of life.[30] All aspects of well-being for quality of life are dramatically improved by surgery. These data agree with those of several other recent reports[24,30–32] and the results of a study comparing the outcome of antireflux surgery with medical maintenance therapy.[26]

Understanding the multifactorial etiology of GERD and the effects of PPI is important for the development of a logical treatment strategy.[33] The impaired LES is the basic anatomical defect and includes alterations in sphincter structure, position, innervation, and its hormonal control.[34] The deficiencies of the LES and TLESR enable reflux of gastric contents, duodenal juice, pepsin, and free radicals into the esophagus.[35,36] Furthermore, alterations of gut neuropeptides are also involved in the pathophysiology of GERD.[37] Conjugated bile acids can be detected in the gastric juice in 75% of reflux patients.[12] It seems that hydrogen ions and pepsin, probably acting synergistically, are the most important components of the refluxate, with the potential to cause clinically relevant esophageal mucosal damage.[12] This may lead to deterioration of esophageal peristalsis, which impairs esophageal clearance function and esophageal and antroduodenal motility.[5]

Medical treatment with PPIs is an effective therapy for GERD[33] and is generally accepted as a long-term maintenance therapy. PPIs mainly decrease acid production and reflux, neutralize acid refluxate, promote esophageal clearance, heal acute esophagitis,[38] and reduce major reflux symptoms.[12] Nevertheless, it has been argued that long-term medical maintenance therapy may abolish symptoms without healing esophagitis, and thus permit the progression of the asymptomatic esophageal lesions to the extent that severe complications may occur in the form of strictures, Barrett's esophagus, and ultimately carcinoma.[12] Furthermore, it is without doubt that medical therapy has no effect on regurgitation still occurring despite acid suppression, relief of heartburn, and healing of esophagitis.[37] Even current prokinetic drugs are inadequate to control regurgitation in a significant number of patients.[39] PPIs produce a slight to moderate hypergastrinemia during acute treatment, which remains during continued therapy. Bacterial overgrowth in the stomach and nitrosamine formation in correlation with hypergastrinemia are potential hazards, which may follow long-term acid suppression.[12]

It is important to reflect on reasons for failure of medical therapy before considering antireflux surgery. Common causes may be the use of an insufficient dosage of PPIs, administration for a too short a time period, or poor patient compliance.[38] Further causes of medical failure are obesity, continued smoking, and the simultaneous use of drugs interfering with PPI medication. PPIs do not alter the anatomical or physiological deficiencies of the LES, and reflux of large volumes may persist despite acid suppression.[34]

On the opposite, antireflux surgery inhibits the reflux of all gastric and duodenal contents and therefore prevents microaspiration and vagus nerve stimulation.[2] A major effect of fundoplication has been shown to be a substantial reduction in the number of TLESRs.[12] Furthermore, antireflux surgery reconstructs the anatomical defect in the hiatus, increases the intra-abdominal length of the LES producing a one-way mechanical flap or flutter valve, and improves impaired esophageal peristalsis and gastric emptying speed.[40] As an interesting side effect, we noticed the relatively low flatulence rate of 18% 12 months postoperatively. The flatulence rate was much higher in our patients before 2001.

At that time we started an intensive postoperative swallowing and eating training program with our patients that included dietary support, swallowing training by logopedics to avoid aerophagia, and continuous psychological support if necessary. Due to this program, we did not only notice a lower flatulence rate, but also a reduced rate of globus sensations, less vomiting, and a weight loss of a median of 8 pounds per patient. Due to the fact that GERD is a disease of multifactorial origin, we expect surgical or medical treatment to be only a part of the therapy.

In accordance with a number of previous studies,[29–32,41] the results of the present study demonstrate that quality of life returns to normal values with very high patient satisfaction following surgery. Therefore, surgery is still occupying the position as the gold standard for long-term treatment of GERD.[8,29,30,33,42] Appropriate patient selection is the key factor to success of antireflux surgery as an alternative to medical therapy.

CONCLUSION

Medical and surgical therapies are both able to improve quality of life in GERD patients. Exact diagnostic evaluation is essential to confirm the evidence of a cause-effect relationship and to predict the outcome of therapy. An important goal in the treatment of GERD should be to improve the quality of life. With increasing severity of the disease, medical therapy is more likely to fail, and the need for surgical treatment arises. According to our results, the outcome and quality of life is significantly better following surgery. It can be suggested that surgical treatment may be the more successful therapy in the long-term.

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Insulin Resistance Causes Human Gallbladder Dysmotility

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Obesity, diabetes, and hyperlipidemia are known risk factors for the development of gallstones. A growing body of animal and human data has correlated insulin resistance with organ dysfunction. The relationship among obesity, diabetes, hyperlipidemia, and abnormal gallbladder motility remains unclear. Therefore, we designed a study to investigate the association among obesity, insulin resistance, hyperlipidemia, and gallbladder dysmotility. One hundred ninety-two healthy adult nondiabetic volunteers were studied. Gallbladder ultrasounds were performed before and after a standardized fatty meal. A gallbladder ejection fraction (EF) was calculated, and an EF of $<25\%$ was considered abnormal. Serum was analyzed for cholesterol, triglycerides, cholecystokinin, leptin, glucose, and insulin. The homeostasis assessment model (HOMA) was used to determine insulin resistance. The volunteers had a mean age of 38 years (range, 18–77), and 55% were female. Thirty subjects (15%) had gallstones and were excluded from the study. Thirty subjects (19%) had abnormal gallbladder motility (EF $<25\%$). In lean subjects (n = 96) fasting glucose was significantly increased in the 16 subjects with gallbladder EF $<25\%$ versus the 80 subjects with gallbladder EF $>25\%$ (109 $\pm$ 20 mg/dl versus 78 $\pm$ 2 mg/dl, $P < 0.05$). Similarly, the HOMA index was significantly greater in subjects with gallbladder EF $<25\%$ versus gallbladder EF $>25\%$ (3.3 $\pm$ 1.2 versus 2.0 $\pm$ 0.2, $P < 0.05$). In obese subjects (n = 66), fasting glucose, insulin, and insulin resistance were not associated with a gallbladder EF $<25\%$. These data suggest that in lean, nondiabetic volunteers without gallstones, gallbladder dysmotility is associated with an elevated fasting glucose as well as a high index of insulin resistance. We conclude that insulin resistance alone may be responsible for gallbladder dysmotility that may result in acalculous cholecystitis or gallstone formation.
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KEY WORDS: Insulin resistance, gallbladder motility, obesity

Gallbladder disease represents a major health care problem in the United States. Approximately 12% of the U.S. population, or 30 million Americans, have gallstones. More then 700,000 cholecystectomies are performed each year, and the cost of caring for these patients is between $8 and $10 billion dollars annually.[1] Approximately three-fourths of the patients with gallstones in the United States have stones that are composed primarily of cholesterol. The pathogenesis of cholesterol gallstones is known to be multifactorial, with the key factors including (1) cholesterol supersaturated bile, (2) nucleation and growth of cholesterol monohydrate crystals, and (3) altered biliary motility.[2]

Obesity, diabetes, and hyperlipidemia have all been identified as significant risk factors for the development of gallstones.[3,4] However, the role that each of these factors play in this process remains unclear because many patients have two or all three of these problems. Recent studies from our laboratory in obese, diabetic leptin-deficient and leptin-resistant mice have correlated body weight, serum glucose, insulin, cholesterol, and triglycerides with poor gallbladder motility.[5,6] In addition, a study in nonobese diabetic mice has demonstrated poor gallbladder emptying in young insulin resistant animals without frank diabetes.[7] Moreover, a growing body of animal and human data have correlated insulin resistance with organ dysfunction. While obesity, diabetes, and hypertriglyceridemia are risk factors for gallstone formation, the relationship among obesity, diabetes, hyperlipidemia, and abnormal gallbladder

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motility remains unclear. Therefore, we designed a study to investigate the association among obesity, insulin resistance, hyperlipidemia, and gallbladder dysmotility.

METHODS

Study Population

Study subjects represent a subgroup of the 507 white families originally recruited for the Medical Risks and Complications of Obesity Genes (MRC-OB Genes) project at the Medical College of Wisconsin.[8] These families represent a relatively homogeneous Caucasian population of predominantly northern European ancestry who reside in Wisconsin and surrounding Midwestern states. Subjects were recruited via an obese proband from families that have large sibships and are multigenerational. Obesity is defined as a body mass index (BMI) >30 kg/m^2. Lean individuals are defined as BMI <30. Exclusion criteria included pregnancy, diabetes mellitus, history of cancer, renal or hepatic disease, severe coronary artery disease, substance abuse, corticosteroids or thyroid medications above replacement dose, history of weight loss of more than 10% of body weight in the preceding 12 months, and age <18 years.

Study Protocol

Subjects were studied in the General Clinical Research Center at the Medical College of Wisconsin after an overnight fast. BMI was determined from the measurement of body weight and height. Fasting blood samples were collected for the determination of serum lipids, glucose, insulin, leptin, and cholecystokinin. Blood was drawn 30 minutes after a standardized test meal for postprandial cholecystokinin levels. Subjects were assessed for the presence or absence of gallstones by real-time ultrasonography with a 3.5- MHz linear transducer while in a supine and left lateral position. The presence of gallstones was defined as one of the following: (1) one or more echogenic, movable, distally shadowing structures within the gallbladder; (2) high-density echoes and constant shadowing in the region of the gallbladder fossa, with poor or no visualization of the gallbladder itself; or (3) one or more echogenic structures, with or without acoustic shadowing, within the biliary tree. This method has been shown to have a 98% sensitivity and a 97% specificity for the detection of gallstones.[9] In addition, measurements were taken of the gallbladder length (L), width (W), and height (H) in order to calculate the fasting gallbladder volume (V). Volume was calculated using the formula: $V = L \times W \times H/6$[10]. Gallbladder motility (Fig. 1) was assessed by determining gallbladder contraction in response to a standardized high fat test meal.[10,11] Ultrasound measurements were taken immediately before and 30 minutes after ingestion of a standardized test meal. The gallbladder ejection fraction (EF) was calculated using the formula EF (%) $= 100 \times$ [(fasting volume $-$ residual volume)]/fasting volume. An abnormal gallbladder EF was defined as an EF $<25\%$. This cutoff represented two standard deviations below the mean gallbladder EF.

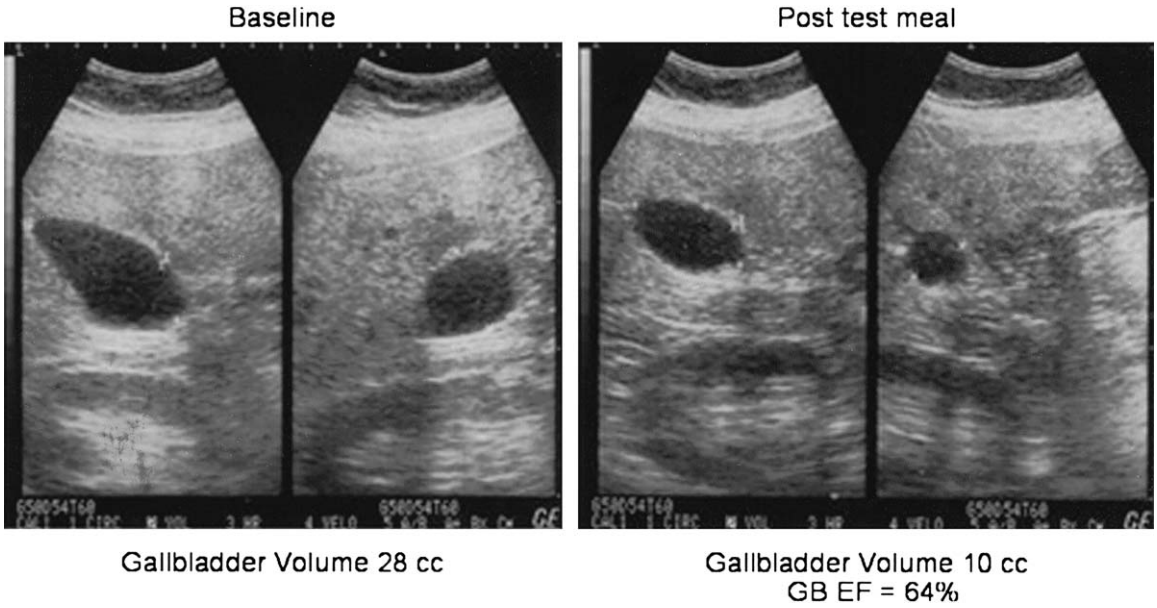

Fig. 1. Gallbladder (GB) ultrasounds and ejection fraction (EF).

Serum Analysis

Fasting plasma cholesterol and triglyceride levels were determined spectrophotometrically. Cholesterol kits were obtained from Roche-Boehringer (Indianapolis, IN). High-density lipoprotein cholesterol (HDL-C) was determined after phosphotungstic acid/$MgCl_2$ precipitation, and low-density lipoprotein cholesterol (LDL-C) was measured directly after immunoseparation (Sigma Diagnostics, St. Louis, MO). Triglyceride assay kits were obtained from Stanbio Laboratory, Inc. (San Antonio, TX). All determinations were performed in triplicate. Quality controls were performed to assure stability and reliability of the assays. Serum glucose concentrations were measured with a Glucose Analyzer II (Beckman Instruments, Brea, CA), using a glucose oxidase procedure. Replicate readings were repeated to within 3 mg/dl in triplicate. A double-antibody, equilibrium RIA (Linco Research, St. Louis) was used for the measurement of plasma insulin, using antibody specific to human insulin. Radioimmunoassay of leptin also was performed by using a specific antibody to human leptin (Linco Research). Serum cholecystokinin levels were measured using a radioimmunoassay (ALPCO Diagnostics, Windham, NH). Insulin resistance was calculated by means of the homeostasis model assessment (HOMA-R). HOMA index = fasting insulin (μU/ml) × fasting glucose (mmol/L)/22.5 with insulin resistance being defined as an HOMA index >2.5.[12]

Statistics

Statistical analysis was performed using the SPSS statistical package (SPSS Inc, Chicago, IL). The χ^2, Student's *t*-test, and analysis of variance (ANOVA) were performed as appropriate. A *P* value of <0.05 was considered significant. All data are expressed as the mean ± SEM.

RESULTS

Subject Demographics

A total of 192 subjects were studied. Asymptomatic gallstones were discovered in 30 subjects (15%), and these volunteers were excluded from further analysis. The mean age of the 162 patients without gallstones was 38 years (range, 18–77), and 55% were female. The mean BMI was 30.0 kg/m^2 (range, 19–62), and the mean weight was 87.6 kg (range, 53.0–87.6).

Gallbladder Emptying and Cholecystokinin

Ultrasound evaluation of the gallbladder revealed a baseline fasting gallbladder volume of 22.7 ml (range, 5.6–83.1), and the residual gallbladder volume after the standardized test meal decreased to 11.7 ml (range, 1.8–60.5). The mean gallbladder EF was 46.6% (range, 0.0–88.1%). Serum cholecystokinin (CCK) levels were significantly higher following the test meal as compared with baseline in subjects with normal and abnormal gallbladder motility (Fig. 2).

Gallbladder Emptying and BMI

Abnormal gallbladder emptying (gallbladder EF <25%) was found in 30 subjects (19%). In the 96 lean subjects, the mean gallbladder EF was 49.3%, and 16 subjects (17%) had evidence of abnormal gallbladder emptying. In the 66 obese subjects, the mean gallbladder EF was 43.5%, and 14 subjects (21%) had abnormal gallbladder emptying.

Obese subjects (BMI >30) had statistically significantly ($P < 0.05$) higher fasting gallbladder volumes, residual gallbladder volumes, serum total and LDL cholesterol, triglycerides, leptin, insulin, and HOMA index than did lean subjects (Table 1). No differences in gallbladder EF in response to a test meal, HDL cholesterol, or fasting glucose were observed between lean and obese subjects. Lean subjects had higher ($P < 0.05$) postmeal CCK levels.

The test meal–stimulated gallbladder EF (Fig. 3) was significantly greater in subjects with normal gallbladder motility compared with subjects with abnormal motility in both lean (56.4% versus 13.9%, $P < 0.05$) and obese subjects (53.5% versus 9.6%, $P < 0.05$). Lean subjects with gallbladder EF >25% had a significantly larger fasting gallbladder volume (20.8 ml versus 14.3 ml, $P < 0.05$) and a smaller residual gallbladder volume (8.8 ml versus 12.6 ml, $P < 0.05$) after test meal stimulation than lean subjects with gallbladder EF <25%. Obese subjects with gallbladder EF >25% had significantly smaller gallbladder residual volumes after meal stimulation (11.3 ml versus 19.7 ml, $P < 0.05$) than obese subjects with gallbladder EF <25%. Fasting gallbladder volumes were similar in the obese subjects with normal and abnormal emptying (25.3 ml versus 20.7 ml).

Gallbladder Emptying and Serum Data

Serum leptin levels were higher ($P < 0.07$) in obese patients with gallbladder EF <25% compared with obese patients with gallbladder EF >25% and lean patients (Fig. 4, Table 3). Nondiabetic lean subjects with gallbladder EF <25% had significantly

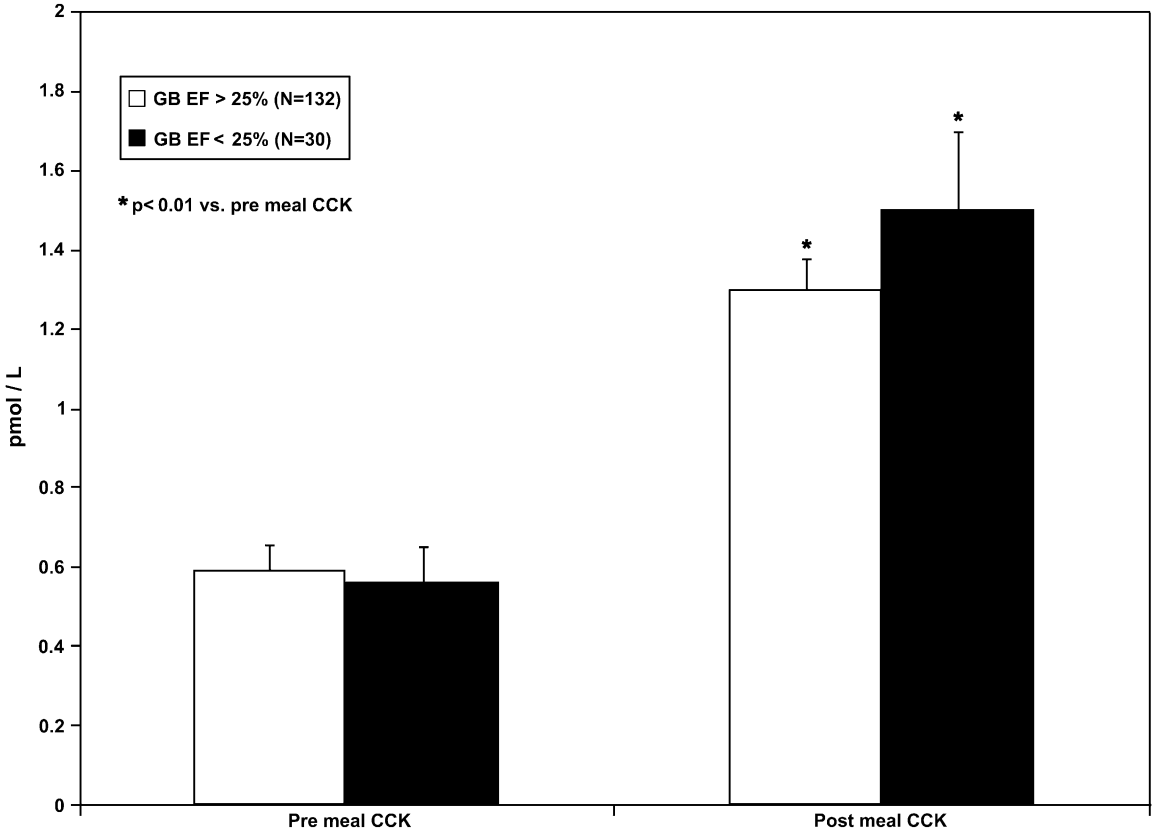

Fig. 2. Serum cholecystokinin (CCK) stratified by gallbladder ejection fraction (EF).

higher fasting serum glucose levels (109 mg/dl versus 78 mg/dl, $P < 0.01$) compared with lean subjects with gallbladder EF >25%. Serum insulin levels were not different between the two groups of obese (16.9 versus 15.0 μU/ml) or lean subjects (10.4 versus 10.5 μU/ml). The HOMA index was significantly higher in the lean nondiabetic subjects with gallbladder EF <25% compared with lean nondiabetic subjects with gallbladder EF >25% (3.3 versus 2.0, $P < 0.05$). No differences in fasting serum

Table 1. GB and serum data stratified by BMI (kg/m^2)

	N	Fasting GB volume	Residual GB volume (ml)	GB EF (%)	Premeal CCK (pmol/L)	Postmeal CCK (pmol/L)
GB and CCK data						
BMI<30	96	19.7 ± 0.9	9.4 ± 0.5	49.3 ± 2.2	0.58 ± 0.07	1.44 ± 0.11
BMI >30	66	24.3 ± 1.4*	13.1 ± 0.9*	43.4 ± 2.8	0.53 ± 0.06	1.10 ± 0.10*
	N	Total cholesterol (mg/dl)	LDL (mgl/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	
Serum lipids						
BMI <30	96	183.3 ± 4.4	125.3 ± 4.2	41.5 ± 1.3	84.5 ± 4.8	
BMI >30	66	213.3 ± 5.3*	146.3 ± 4.8*	40.7 ± 1.4	131.4 ± 19.6*	
	N	Leptin (ng/ml)	Glucose (mg/dl)	Insulin (μU/ml)	HOMA	
Serum leptin, glucose, insulin, and HOMA						
BMI <30	96	12.5 ± 1.0	82.1 ± 3.1	10.4 ± 0.7	2.2 ± 0.2	
BMI >30	66	24.2 ± 1.9*	87.7 ± 4.4	16.5 ± 1.2*	3.5 ± 0.3*	

BMI = body mass index; CCK = cholecystokinin; GB = gallbladder; EF = ejection fraction; LDL = low-density lipoprotein; HDL = high-density lipoprotein; HOMA = homeostasis m assessment model.
*$P < 0.05$ versus BMI <30.

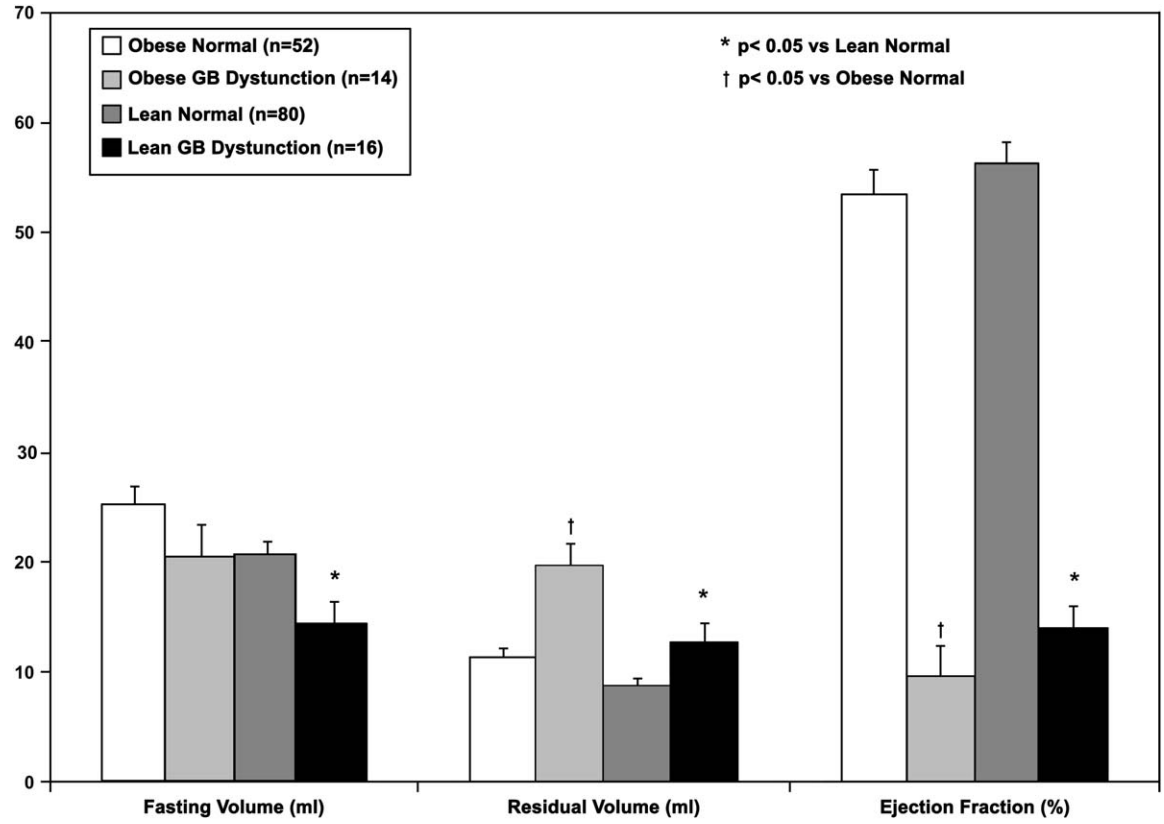

Fig. 3. Gallbladder (GB) volumes and emptying.

glucose, insulin levels, or HOMA index were observed in obese nondiabetic subjects with normal and impaired gallbladder motility (Fig. 4). In addition, serum lipids (Table 2), leptin levels, body weight, and BMI (Table 3) were not different in either lean or obese nondiabetic subjects with normal or impaired gallbladder motility.

DISCUSSION

In this study, healthy nondiabetic volunteers without gallstones had a 19% incidence of abnormal gallbladder emptying (EF <25%) in response to a standardized test meal as measured by real-time ultrasonography. Obese subjects (BMI >30) had larger gallbladder volumes, residual gallbladder volumes after meal stimulation, serum lipids, leptin, insulin, and HOMA index compared with lean (BMI <30) individuals. Obesity did not alter fasting glucose or gallbladder EF in response to a test meal. Fasting glucose and insulin resistance (increased HOMA index) were associated with a gallbladder EF <25% in lean nondiabetic subjects. However, in obese nondiabetic subjects fasting glucose, insulin, and insulin resistance were not associated with a gallbladder EF <25%.

Obesity dramatically increases the risk of gallstone formation, especially in females.[4,13–15] A well-established pathophysiologic link between obesity and gallstone formation is cholesterol-supersaturated bile.[16,17] Obese people hypersecrete biliary cholesterol, bile salts, and phospholipids, but the rate of cholesterol secretion supersedes that of the other biliary lipids, leading to cholesterol-supersaturated bile.[18] Weight reduction aggravates this phenomenon, as hepatic stores of cholesterol are mobilized, bile salt synthesis is decreased, and normal gallbladder emptying is interrupted, leading to further supersaturation of bile and rapid gallstone formation.[19,20]

In theory, increased flux of cholesterol from the bile into gallbladder muscle cells stiffens the sarcolemmal membranes, decouples signal transduction, and inhibits gallbladder muscle function.[20,21] However, ultrasound data on gallbladder volume and emptying in obese humans are conflicting.[11,22] While most studies suggest increased resting gallbladder volume in obese subjects, some reports demonstrate that these large gallbladders empty normally.[22] Our data confirm an increased resting gallbladder volume with normal gallbladder emptying in obese nondiabetic volunteers compared with lean nondiabetic volunteers without gallstones.

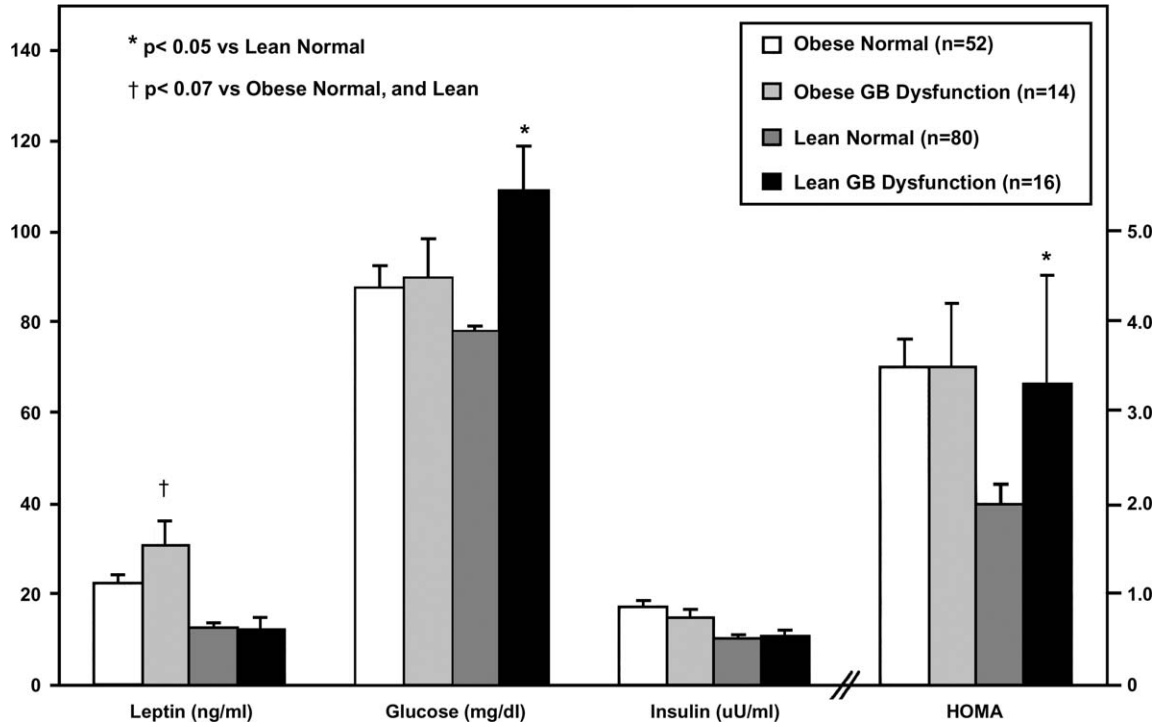

Fig. 4. Serum leptin, glucose, insulin, and homeostasis assessment model (HOMA) index. GB = gallbladder.

Multiple autopsy studies have documented a statistically significant increase in the incidence of gallstones in diabetes.[15,23,24] Epidemiologic studies in Mexican Americans[25] and in Caucasian Americans[4] have shown diabetes to be a significant risk factor for gallstones. Diabetics have been shown to have an increased cholesterol saturation index in bile compared with nondiabetics.[26] In addition, gallbladder fasting volumes have been shown to be larger, and gallbladder motility is diminished in non–insulin-dependent diabetics compared with nondiabetics.[27,28] Hyperinsulinemia is characteristically found in persons with non–insulin-dependent diabetes as a result of insulin resistance. Several authors have documented an association between hyperinsulinemia and an increased prevalence of gallbladder disease.[25,29–31]

Both hyperglycemia and euglycemic hyperinsulinemia have been shown to inhibit CCK-stimulated gallbladder motility.[32] Hyperinsulinemia may also be a key factor in these observations because insulin regulates the Na^+-K^+ pump, which may adversely affect the ionic and osmotic homeostasis of smooth muscle cells including gallbladder myocytes.[33] The Na^+-K^+ pump of presynaptic nerve terminals is also regulated by insulin.[33] Moreover, decreased Na^+-K^+ pump activity can result in increased intracellular Na^+, which in turn increases the Na^+-Ca^{++} exchange, thereby increasing intracellular calcium. Increased intracellular calcium will alter

Table 2. EF, and serum lipids stratified by BMI (kg/m^2)

	Obese (BMI >30)		Lean (BMI <30)	
	EF >25	EF <25	EF >25	EF <25
N	52	14	80	16
Triglycerides (mg/dl)	134.4 ± 24.6	120 ± 13.7	84.3 ± 5.5	85.6 ± 8.4
Total cholesterol (mg/dl)	213.4 ± 6.2	212.9 ± 10.5	184.6 ± 4.8	176.9 ± 116
LDL (mg/dl)	146.0 ± 5.7	147.5 ± 8.7	126.3 ± 4.5	120.4 ± 10.0
HDL (mg/dl)	40.5 ± 1.6	41.3 ± 3.1	41.9 ± 1.5	39.4 ± 1.6

Table 3. EF, weight, BMI, and leptin stratified by BMI (kg/m^2)

	Obese (BMI >30)		Lean (BMI <30)	
	EF >25	EF <25	EF >25	EF <25
N	52	14	80	16
Weight (kg)	105.8 ± 3.1	114.8 ± 3.2	73.7 ± 1.3	73.8 ± 2.9
BMI	36.4 ± 1.0	39.3 ± 1.5	25.2 ± 0.3	25.1 ± 0.7
Leptin (ng/ml)	22.5 ± 1.8	30.7 ± 5.9*	12.6 ± 1.1	12.1 ± 2.6

*$P \leq 0.07$ versus obese EF >25 and lean.

both smooth muscle tone and release of neurotransmitters. Moreover, we have demonstrated that gallbladder myocytes from obese, diabetic mice are foreshortened and respond poorly to CCK.[6] Thus, the abnormal gallbladder emptying seen in response to a test meal in the nondiabetic lean subjects investigated in this study may be related to a state of relative hyperinsulinemia as a result of insulin resistance.

Insulin resistance and or diabetes may also affect alterations in the density or sensitivity of acetylcholine or CCK receptors or prevent neurotransmitters from accessing their receptors. Sugars can react nonenzymatically with amino groups in proteins, lipids, and nucleic acids to form advanced glycation endproducts.[34,35] These products are thought to have many effects, including covalent cross-linking of collagen and protein matrix.[34] The cross-linking of the matrix may lead to stiffening of the gallbladder wall itself, limiting its contraction, or may impair CCK egress through blood vessel basement membranes, preventing CCK interaction with neural or myocyte receptors.

Serum insulin levels were significantly higher in the obese compared with lean subjects (16.5 ± 1.2 versus 10.4 ± 0.67 μU/ml, $P < 0.05$) with no difference in fasting glucose. As a result, the HOMA index was significantly greater in the obese subjects. Both the HOMA index and fasting insulin levels in the present study are within the range of the general U.S. population. Bravata and colleagues[36] have shown in a large U.S. epidemiological survey that HOMA index and fasting insulin levels increase as BMI increases. In their study, the median HOMA index was 3.3 for subjects with a BMI between 30 and 35 and increased to 4.7 with a BMI >40. The median HOMA index for lean subjects was between 1.6 and 2.4.

In the present study we did not see a relationship between insulin resistance and abnormal gallbladder emptying in obese nondiabetic subjects. This observation suggests that a different factor may be involved in gallbladder dysmotility in the setting of obesity. Serum leptin levels were higher in obese subjects with abnormal gallbladder motility (30.6 ± 5.9 versus 22.5 ± 1.8 ng/ml, $P < 0.07$). Recent animal studies in congenitally obese leptin-deficient (Lep^{ob}) and leptin-resistant (Lep^{db}) mice have documented impaired gallbladder motility and diminished response to acetylcholine and cholecystokinin in vitro.[37,38] Moreover, the systemic replacement of leptin to Lep^{ob} mice returns gallbladder function to normal.[39] Recently, we have demonstrated that the gallbladder is rich in leptin receptors[40] and that leptin administration upregulates gallbladder CCK-A and acetylcholine receptor genes (unpublished data). Therefore, the hyperleptinemic state that occurs in most obese subjects may counterbalance the affects of insulin resistance.

Another potential explanation for the differences in gallbladder dynamics observed between obese and lean individuals may be due to the differences in serum and, perhaps, gallbladder wall lipids.[20,41,42] Human and animal studies have correlated hypertriglyceridemia with gallbladder disease. Both prairie dogs and lean mice fed a high fat diet as well as obese leptin-deficient mice have elevated gallbladder wall free fatty acids, cholesterol, and tryglycerides.[20,41,42] As a result, the cholesterol/phospholipids ratio increases, and membrane fluidity decreases. Chen et al.[41] have reported that smooth muscle cells from human gallbladders with cholesterol stones have increased cholesterol and cholesterol/phospholipids ratios. In addition, this group has demonstrated decreased membrane fluidity in these gallbladders. Thus, our obese subjects with high serum total and LDL cholesterols as well as triglycerides may also have high gallbladder lipids, which may play a role in gallbladder function.

Both real-time ultrasonography[11,22,28] and biliary scintigraphy[43,44] have been reported to be accurate measures of gallbladder emptying in response to a test meal or CCK infusion. In this study, ultrasound was chosen to measure gallbladder EF because of the ease of its performance, absence of radioactive materials, and less expense.

CONCLUSION

This study demonstrates that lean, healthy, adult, nondiabetic volunteers with impaired gallbladder emptying and dysmotility is associated with higher fasting plasma glucose levels and normal plasma insulin levels compared with volunteers with normal gallbladder emptying. This relative insulin resistance seen in lean subjects with gallbladder dysmotility and no gallstones may be responsible for the development of acalculous cholecystitis or later gallstone formation.

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Discussion

Dr. B. Joar Svanvik (Linkoping, Sweden): I have one comment and three questions. I don't think really that you measure the ejection fraction from the gallbladder by using ultrasonography. What you are measuring is the volume of the gallbladder and how it contracts. If you do a study comparing contraction using ultrasound and HIDA scan at the same time, you will often find that the emptying of the gallbladder exceeds the contraction. There is an explanation. There is a postprandial secretion in the gallbladder after a test meal, as we published in a study from Dr. Lawrence Way's lab in 1984. So I think what you really are measuring is the volume reduction of the gallbladder. The question then is why does the gallbladder contract? Is it due to cholecystokinin released from the duodenum? So I wonder if you compared the cholecystokinin levels in the subjects with insulin resistance and compared that to the other groups?

The second question is—could it be that patients with insulin resistance have a slow gastric emptying, so they will release CCK later, perhaps later than 30 minutes when you measured. That could possibly be one explanation.

My third question is—did you consider giving a shot of CCK to see if the gallbladder contracted more sluggishly in the patients with insulin resistance?

Dr. Nakeeb: Addressing the first question in terms of the CCK levels, we did measure the CCK levels and compared them in the group of patients with normal and abnormal gallbladder ejection and emptying and there was no difference in those levels at all. I do agree with you that a better test is the HIDA scan. There is also the phenomenon of refilling of the gallbladder during this procedure. I think due to cost and technical limitations of our study, we elected to use the ultrasound evaluation. We did not have any measure of delayed gastric emptying in these patients. They were all healthy, nondiabetic controls without clinical evidence of gastroparesis.

Dr. Svanvik: Did you also inject cholecystokinin and measure the gallbladder volume?

Dr. Nakeeb: No, we did not do that.

Dr. Frank Moody (Houston, Texas): These are interesting observations, and your conclusion, however, depends on your confidence in the HOMA calculation, which, as you know, has some problems. So have you done any patients with a euglycemic clamp to be more confident in that particular number?

Now, I was surprised that age was not a discriminating variable, because you had somebody I think who was 79. I thought, as I was reading the abstract, that older people in this cohort, gender representation, and lipid profiles did not seem to have a correlation. So I would have to ask you, what was the power of your study to be able to conclude that those variables weren't involved?

Dr. Nakeeb: Both excellent questions. Our study may be underpowered. This is an interim analysis, and we have examined only half of the patients we ultimately plan to study.

Dr. Moody: Have you used the euglycemic clamp?

Dr. Nakeeb: We have collected clamp data on all of these patients. However, we have not analyzed it yet.

Dr. Richard Thirlby (Seattle, Washington): I have one question and a comment. The question relates to the reproducibility of your test, which is the ultrasound. Do you have any information on the reproducibility of the test, or have you studied any patients multiple times? Your patients with abnormal emptying actually had contracted gallbladders, not decreased emptying. In my experience, an obese patient with a contracted gallbladder more likely has eaten on their way to the clinic rather than having a contracted gallbladder all the time. I really think it is important that you have multiple studies on multiple patients to show that the test is reproducible.

Dr. Nakeeb: Yes, when we first started the study, we did do that. There were several patients in the study who we were not able to visualize the gallbladder on initially for several reasons, and so they were excluded from the study. But that is a valid point.

Dr. Thirlby: And my comment relates to your title. You say "insulin resistance causes human gallbladder dysmotility." I would contend that you really have just shown an "*association*." As you know, insulin resistance is associated with metabolic syndrome or syndrome X. These patients may also have NASH, they may have elevated CRP levels and multiple other metabolic abnormalities. You have not shown a causal relationship between diabetes and gallbladder function.

Dr. Dana Andersen (Worcester, Massachusetts): There is a lot of interest now in examining insulin resistance as a cause of many of the diseases we treat rather than a consequence of them. My question pertains to your obese groups.

The obese patients in your study seem to be, as you would predict, somewhat more insulin resistant than the lean subjects, but there seems to be no difference in terms of their functional behavior on your gallbladder studies. Have you analyzed the data the other way, which is to say to look at the spectrum of HOMA-resistant scores and then to look at gallbladder ejection fractions to see if there is a relationship that relates gallbladder emptying to insulin resistance?

Dr. Nakeeb: We did not do that, but it is something we will go back to look at in the data.

Bile-Pancreatic Juice Exclusion Promotes Akt/NF-κB Activation and Chemokine Production in Ligation-Induced Acute Pancreatitis

Isaac Samuel, M.D., F.R.C.S., F.A.C.S., Mark A. Yorek, Ph.D., Asgar Zaheer, Ph.D., Rory A. Fisher, Ph.D.

Using a unique surgical model (the donor rat model), we showed previously that duodenal replacement of bile-pancreatic juice, obtained fresh from a donor rat, ameliorates ligation-induced acute pancreatitis. We hypothesize that bile-pancreatic juice exclusion from gut exacerbates Akt/nuclear factor-κB (NF-κB) pathway activation and induces chemokine production in ligation-induced acute pancreatitis. We compared rats with bile-pancreatic duct ligation to those with duodenal bile-pancreatic juice replacement fresh from a donor rat beginning immediately before duct ligation. Sham control rats had ducts dissected but not ligated. Rats were killed 1 or 3 hours after operation (n = 7/group). Akt activation (immunoblotting, immune-complex kinase assay, and ELISA), inhibitory protein I-κB (IκB) activation (immunoblotting), and production of chemokines MCP-1 and RANTES (ELISA) were measured in pancreatic homogenates. NF-κB was quantitated in nuclear fractions using electrophoretic mobility shift assay. Duct ligation produced significant increases in pancreatic Akt, IκB, and NF-κB activation and production of MCP-1 and RANTES. Activation of the Akt/NF-κB pathway and increased MCP-1 and RANTES production in response to duct ligation were significantly reduced by bile-pancreatic juice replacement (ANOVA, $P < 0.05$). Bile-pancreatic juice exclusion stimulates Akt/NF-κB pathway activation and increases chemokine production in ligation-induced acute pancreatitis.
(J GASTROINTEST SURG 2006;10:950–959)

KEY WORDS: Acute pancreatitis, Akt, NF-κB, chemokines, bile

Using a unique surgical model (the donor rat model), we previously showed that duodenal replacement of bile-pancreatic juice, obtained fresh from a donor rat, ameliorates pancreatic morphologic changes and hypercholecystokininemia in ligation-induced acute pancreatitis.[1,2] We concluded that bile-pancreatic juice exclusion from gut exacerbates ligation-induced acute pancreatitis.[1,2] Recently, we also showed that pancreatic production of TNF-α and activation of the stress kinase $p38^{MAPK}$ are increased by bile-pancreatic juice exclusion after duct ligation.[3] Considerable interest recently has focused on the possible role of the Akt-mediated signal transduction pathway in modulating cellular production of acute inflammatory mediators.[4–6]

The transcription factor nuclear factor-κB (NF-κB) is located predominantly in the cytoplasm in unstimulated cells and is complexed with its inhibitory protein I-κB (IκB).[7,8] The cytosolic IκB/NF-κB complex dissociates when IκB is phosphorylated by activated Akt, allowing NF-κB to translocate to the nucleus and promote transcriptional upregulation of various inflammatory mediators including chemokines[5,7–9] (Fig. 1). In the present study, we examined the hypothesis that bile-pancreatic juice exclusion from gut promotes activation of the Akt/NF-κB pathway and induces chemokine production in ligation-induced acute pancreatitis. To test this hypothesis, we examined pancreatic Akt/NF-κB activation and chemokine production in response to duct

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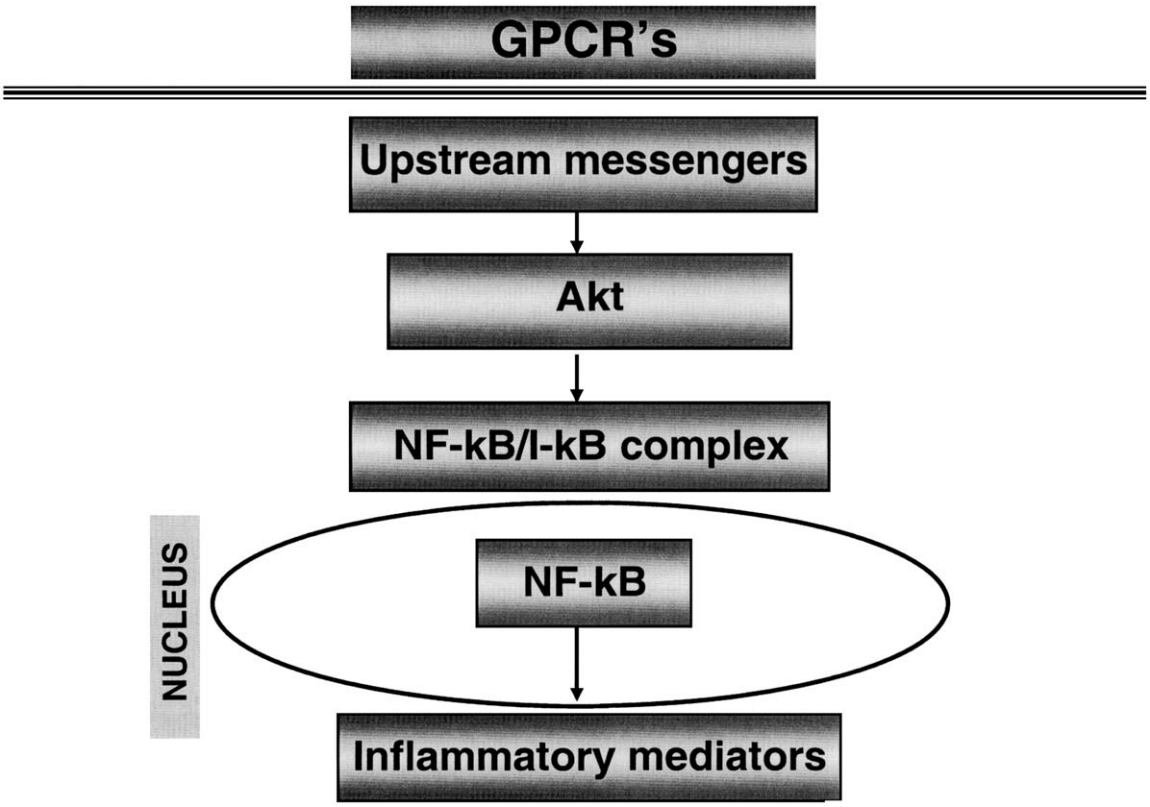

Fig. 1. Schematic representation of the Akt/NF-κB pathway in the acinar cell leading to overproduction of inflammatory mediators. Duct ligation excludes bile-pancreatic juice from the duodenum and induces acute pancreatitis. Bile-pancreatic juice exclusion induces acinar cell hyperstimulation via neurohormonal pathways. Hyperstimulation of cell surface G protein–coupled receptors (CCK-A receptor, cholinergic receptor) activates pro-inflammatory pathways such as the Akt/NF-κB pathway. The cytosolic IκB/NF-κB complex dissociates when IκB is phosphorylated by activated Akt, allowing NF-κB to translocate to the nucleus and promote transcriptional upregulation of various inflammatory mediators including chemokines.

ligation in rats with and without duodenal bile-pancreatic juice replacement from a donor rat.[3]

MATERIAL AND METHODS

Animal Surgery and Experimental Groups

Experimental protocols were approved by the University of Iowa Institutional Animal Care and Use Committee satisfying the guidelines of the U.S. Public Health Service. Male Sprague-Dawley rats weighing 250–325 g were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Midline laparotomy was performed under general anesthesia induced with ketamine hydrochloride (87 mg/kg) and xylazine hydrochloride (13 mg/kg), and the rats were studied in the following groups: (1) sham-operated control group: the distal bile-pancreatic duct was dissected but not ligated; (2) diseased-control group: the distal bile-pancreatic duct was ligated at its junction with the duodenum; and (3) diseased-treated group: duodenal replacement of bile-pancreatic juice was begun via a duodenal fistula beginning immediately prior to duct ligation. Bile-pancreatic juice for donation to experimental rats was obtained fresh from a donor rat that was prepared with a bile-pancreatic fistula.[3] The duodenal fistula and the bile-pancreatic fistula were prepared using a polyethylene tube (PE-50, I.D. 0.58 mm, O.D. 0.965 mm; Clay Adams, Parsippany, NJ). As described previously, a liquid-level photodetector and peristaltic pump were used to donate the bile-pancreatic juice collected from donor rats into the duodenum of diseased-treated rats.[1–3,10] Rats were killed after 1 or 3 hours (n = 7 rats per experimental group at each time point). Pancreata were quickly excised and snap-frozen in liquid nitrogen and stored at −80° C. The frozen pancreatic tissue specimens were then processed for individual assays, as described later.

Materials

Antibodies to total Akt, phospho-Akt, and phospho-IκB (catalog Nos. 9272, 9275, and 2681, respectively) and recombinant glycogen synthase kinase-3 protein (GSK-3; catalog No. 9278) were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Horseradish peroxidase (HRP)-conjugated secondary antibody for immunoblotting was purchased from New England Biolabs (Beverly, MA). [^{32}P]ATP (3000 Ci/mmol) was purchased from Perkin Elmer Life Sciences/NEN (Woodbridge, Ontario). Enhanced chemiluminescence (ECL) immunoblot detection reagents were from Amersham Pharmacia Biotech (Piscataway, NJ). Specific antibody to β-actin was from Sigma Chemical Co. (St. Louis, MO). The commercial ELISA kits for determination of MCP-1 (monocyte chemoattractant protein-1), RANTES (regulated on activation, normal T-cell expressed and secreted), and phospho-Akt concentrations (catalog Nos. KRC 1011, KRC 1031, and KHO 0111, respectively) were purchased from Biosource, Inc. (Camarillo, CA).

Immunoblotting

Immunoblots were prepared as described earlier.[11] In brief, portions of pancreas were homogenized in 10 mM HEPES buffer (pH 7.5) and centrifuged at 500*g* for 5 minutes to pellet cell debris. The resulting supernatant was centrifuged at 15,000*g* for 15 minutes to obtain a soluble fraction that was quantitated for protein using the Bradford assay. From each sample, 40 μg of total protein in SDS-sample buffer (62.5 mM Tris, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.1% w/v bromophenol blue) were separated on 4–20% gradient gels by SDS-polyacrylamide gel electrophoresis. This was followed by electroblot transfer of proteins onto nitrocellulose membranes that were probed with specific primary antibodies (1:1000 v/v). The blots were then incubated with the appropriate secondary antibody conjugated to HRP (1:2000 v/v) and the image was developed using the ECL method, as recommended by the manufacturer. Additional control immunoblots were performed using β-actin antibody as the primary antibody to compare protein loading.

Immunoprecipitation

Immunoprecipitation was carried out essentially as described earlier.[12] In brief, tissues were extracted using lysis buffer (1% Triton X-100, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 50 mM NaF, 0.1 mM sodium vanadate, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml concentration each of aprotinin, leupeptin, chymostatin, pepstatin A, and antipain). Protein concentration was determined with a commercial kit for the modified Lowry's method (Pierce Biotechnology, Rockford, IL). Tissue extracts were centrifuged at 12,000 rpm for 15 minutes to collect the supernatant soluble fraction. Aliquots of clear supernatants containing 500 μg total protein in 1 ml of lysis buffer were incubated with 2 μg of specific antibody to total Akt overnight at 4° C. This was followed by an additional incubation with 20 μl of a 50% suspension of protein G-agarose for 1 hour at room temperature. The insoluble immune-complex was collected and washed three times by brief centrifugations prior to immune-complex kinase assay.

Immune-Complex Kinase Assay

Akt was immunoprecipitated from tissue extracts as described earlier and followed by two additional washes with a kinase assay buffer prior to immune-complex kinase assay. Assays were carried out as described earlier,[12] in a total reaction volume of 20 μl containing 100 mM Tris-HCl, pH 7.0, 0.4 mM sodium orthovanadate, 40 mM magnesium acetate, 1 mM dithiothreitol, 30 μM calmidazolium, 10 μl of Akt immune-complex, 2 μg of kinase substrate (GSK-3), and 100 μM [γ-^{32}P]ATP (200 cpm/pmol) and incubated at 30° C for 10 minutes. Reactions were stopped by addition of SDS-PAGE sample buffer and then boiled for 2 minutes and centrifuged. Supernatants were subjected to SDS-PAGE followed by autoradiography.

Elisa

Portions of pancreas were homogenized, and the protein concentrations of soluble fractions were determined as described earlier for immunoblotting experiments. The concentration of the chemokines MCP-1 and RANTES and of phospho-Akt was measured in the soluble fraction of pancreatic homogenates using commercial kits according to the manufacturer's instructions.

Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assay (EMSA) for NF-κB was performed essentially as described earlier.[13] In brief, pancreatic nuclear extracts were prepared and 15 μg of nuclear protein was subjected to EMSA by probing with ^{32}P-labeled double-stranded oligonucleotide containing the consensus sequence for NF-κB (5′-TTT CGC GGG GAC TTT CCC GCG C-3′ and 5′-TTT GCG CGG GAA AGT

CCC CGC G-3′) or mutant NF-κB (5′- TTT CGC GCG GAC ATT CCC GCG C-3′ and 5′-TTT GCG CGG GAA TGT CCG CGC G-3′). As a control, the same extracts were probed with an unrelated oligonucleotide having the binding sequence of E-box, a constitutively expressed transcription factor (5′- ATA GGT GTA GGC CAC GTG ACC GGG TGT-3′ and 5′-ACA CCC GGT CAC GTG-3′). The products were analyzed on 5% non-denaturing polyacrylamide gels for EMSA.

Statistical Analysis

SigmaStat software (Version 2.03; www.spss.com) was used for statistical analysis. One-way ANOVA was used for analysis of pancreatic chemokine concentrations. Seven rats were studied in each experimental group at each time point and results expressed as mean ± SEM. A *P*-value below 0.05 was considered statistically significant.

RESULTS

Immunoblots of pancreatic homogenates using phospho-specific antibodies show that 1 and 3 hours of bile-pancreatic duct ligation produced dramatic increases in phosphorylated Akt and IκB (Fig. 2). Additionally, the immunoblots show that duodenal replacement of bile-pancreatic juice attenuates duct ligation-induced increases in Akt and IκB phosphorylation at both the time points. Control immunoblots using β-actin antibody showed equivalent protein loading of these samples.

Immune-complex kinase assay of Akt in pancreatic homogenates using GSK-3 as substrate demonstrated progressive Akt activation after 1 and 3 hours of duct ligation that was inhibited nearly completely in rats in which bile-pancreatic juice was replaced (Fig. 3). ELISA of phospho-Akt in pancreatic homogenates confirmed our immunoblot and immune-complex kinase assay findings, demonstrating significant Akt activation in response to duct ligation and nearly complete inhibition of this response in rats receiving duodenal bile-pancreatic juice replacement (ANOVA, $P < 0.05$) (Fig. 4).

We then evaluated NF-κB activation in nuclear extracts of pancreatic homogenates from each experimental group because it is well known that nuclear translocation and activation of NF-κB regulates the transcription of inflammatory mediators such as chemokines and that NF-κB can be activated by phospho-Akt via IκB activation. Figure 5 shows the results of EMSA to measure NF-κB in nuclear extracts from rat pancreas. As shown, duct ligation induced nuclear translocation of NF-κB at both 1 and 3 hours compared with sham-operated controls. Importantly, this progressive increase in nuclear translocation and activation of NF-κB after 1 and 3 hours of duct ligation was completely prevented by duodenal bile-pancreatic juice replacement (Fig. 5). Confirming previous observations,[14] no changes were found when the extracts were probed with an oligonucleotide that is recognized by a constitutively expressed E-box transcription factor, and negative controls using probe for mutant NF-κB did not show a band (data not shown).

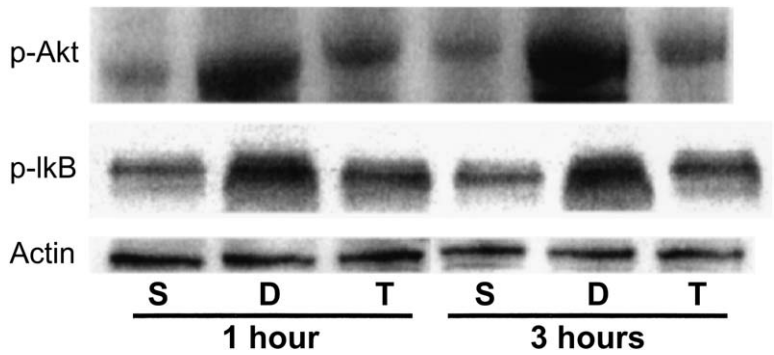

Fig. 2. Bile-pancreatic juice exclusion increases phosphorylation of pancreatic Akt and IκB following duct ligation. Representative immunoblots using specific primary antibodies to phospho-Akt and phospho-IκB show increased phosphorylation of Akt and IκB following 1 or 3 hours of bile-pancreatic duct ligation that is attenuated by duodenal bile-pancreatic juice replacement (obtained fresh from a donor rat). Immunoblot of β-Actin confirmed equivalent protein loading of samples. The position of the protein band of interest was verified with molecular size standards. S = Sham-operated controls had the duct dissected but not ligated; D = diseased-controls had duct ligation; T = treated group, diseased-treated rats had duct ligation with duodenal bile-pancreatic juice replacement fresh from a donor rat.

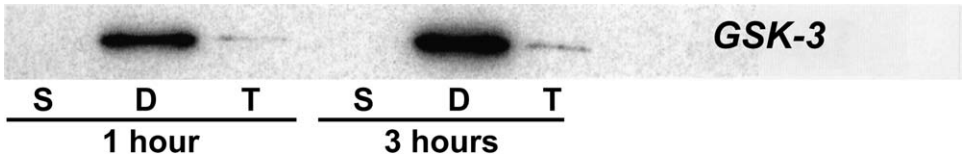

Fig. 3. Effect of bile-pancreatic juice exclusion on Akt activation following duct ligation determined by immunecomplex assay. Autoradiogram of immune-complex kinase assay of pancreatic homogenates using specific antibody to Akt with GSK-3 as substrate showed a dramatic increase in Akt activation after 1 or 3 hours of duct ligation that was markedly attenuated by duodenal bile-pancreatic juice replacement. The position of GSK-3 was verified with molecular size standards. S = Sham-operated controls had the duct dissected but not ligated; D = diseased-controls had duct ligation; T = treated group, diseased-treated rats had duct ligation with duodenal bile-pancreatic juice replacement fresh from a donor rat.

Due to the lack of marked histopathologic changes in the pancreas at the early time points selected in our study, we used pancreatic MCP-1 and RANTES production as an indicator of pancreatic inflammation. Pancreatic concentrations of the chemokines MCP-1 and RANTES (ELISA, ANOVA, $P < 0.05$) were increased significantly following 1 or 3 hours of duct ligation and these increases were prevented nearly completely by duodenal bile-pancreaic juice replacement (Fig. 6).

In summary, our results show that duct ligation is associated with significant increases in pancreatic Akt activation, IκB activation, MCP-1 and RANTES production, and NF-κB activation with nuclear translocation. Our results also show that activation of the Akt/NF-κB pathway and increased MCP-1

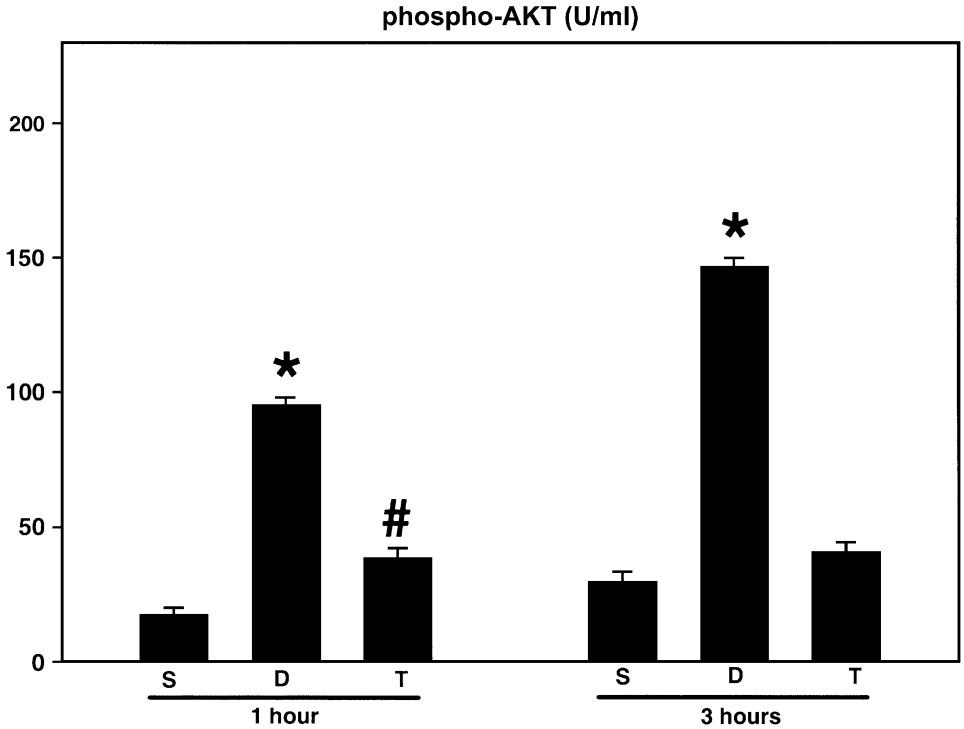

Fig. 4. Bile-pancreatic juice exclusion increases phosphorylation of pancreatic Akt following duct ligation. ELISA of pancreatic homogenates showed a statistically significant increase in pancreatic phospho-Akt concentrations after duct ligation that was significantly ameliorated by duodenal bile-pancreatic juice replacement. Results are mean ± SEM; n = 7 rats per group. *Significant difference from the sham-operated control group at the same time-point. #Significant difference from sham-operated control group and diseased-control group at the same time-point (one-way ANOVA, $P < 0.05$). S = Sham-operated controls had the duct dissected but not ligated; D = diseased-controls had duct ligation; T = treated group, diseased-treated rats had duct ligation with duodenal bile-pancreatic juice replacement fresh from a donor rat.

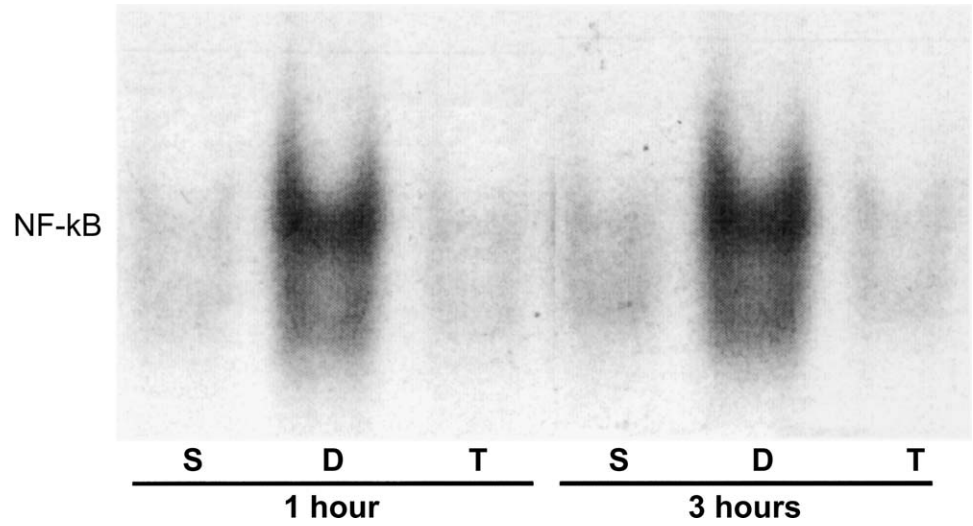

Fig. 5. Bile-pancreatic juice exclusion promotes nuclear translocation of NF-κB following bile-pancreatic duct ligation. Autoradiogram of EMSA of nuclear fractions of pancreatic tissue shows nuclear translocation of transcription factor NF-κB following duct ligation, while duodenal replacement of bile-pancreatic juice largely prevents the nuclear translocation. S = Sham-operated controls had the duct dissected but not ligated; D = diseased-controls had duct ligation; T = treated group, diseased-treated rats had duct ligation with duodenal bile-pancreatic juice replacement fresh from a donor rat.

and RANTES production after duct ligation are largely, if not completely, prevented by duodenal bile-pancreatic juice replacement. These results indicate that increased activation of the Akt/NF-κB pathway and increased chemokine (MCP-1, RANTES) production during the early phase after bile-pancreatic duct ligation probably result from the enteral response to bile-pancreatic juice exclusion rather than from the mere mechanical effects of duct obstruction.

DISCUSSION

The results of the present study provide new evidence that duodenal bile-pancreatic juice replacement attenuates duct ligation-induced Akt/NF-κB pathway activation and chemokine production in rats. These findings indicate that bile-pancreatic juice exclusion from gut promotes Akt/NF-κB pathway activation and overproduction of chemokines MCP-1 and RANTES in this experimental corollary of gallstone-induced acute pancreatitis. As the Akt/NF-κB pathway has been implicated as a proinflammatory pathway,[4–6] these findings may have importance during the early stages of disease pathogenesis in our experimental model. As the pancreatic histopathologic changes at the early time points selected for our study are too subtle to discern differences between experimental groups, we used pancreatic concentrations of the chemokines MCP-1 and RANTES as indicators of the severity of pancreatic inflammation.[15,16] Our results show that bile-pancreatic duct ligation produced significant increases in pancreatic MCP-1 and RANTES concentrations at 1- and 3-hour time points, consistent with development of acute pancreatitis in our model. Of note, these increases were largely abolished by duodenal bile-pancreatic juice replacement. These findings demonstrate that bile-pancreatic juice exclusion from gut contributes to the pathogenesis of chemokine production and acute inflammation during the early stages of ligation-induced acute pancreatitis in rats.

Recent studies have established the critical role played by cytokines and chemokines in the pathogenesis of acute pancreatitis.[15–18] Although one study failed to demonstrate increases in plasma MCP-1 concentrations in rodent acute pancreatitis induced by intraductal bile salt injection,[19] there is evidence from other studies that MCP-1 contributes to the development of acute pancreatic inflammation. Acute pancreatitis induced by intraductal bile salt injection in rabbits[17] or by supramaximal caerulein stimulation in mice showed significant increases in pancreatic MCP-1 levels.[15] Bindarit, an inhibitor of MCP-1 synthesis, administered prophylactically or therapeutically in caerulein-induced acute pancreatitis in mice was associated with significant attenuation of pancreatic MCP-1 levels, pancreatic myeloperoxidase activity and pancreatic morphologic changes.[15] In a clinical study, serum and lesser sac aspirates

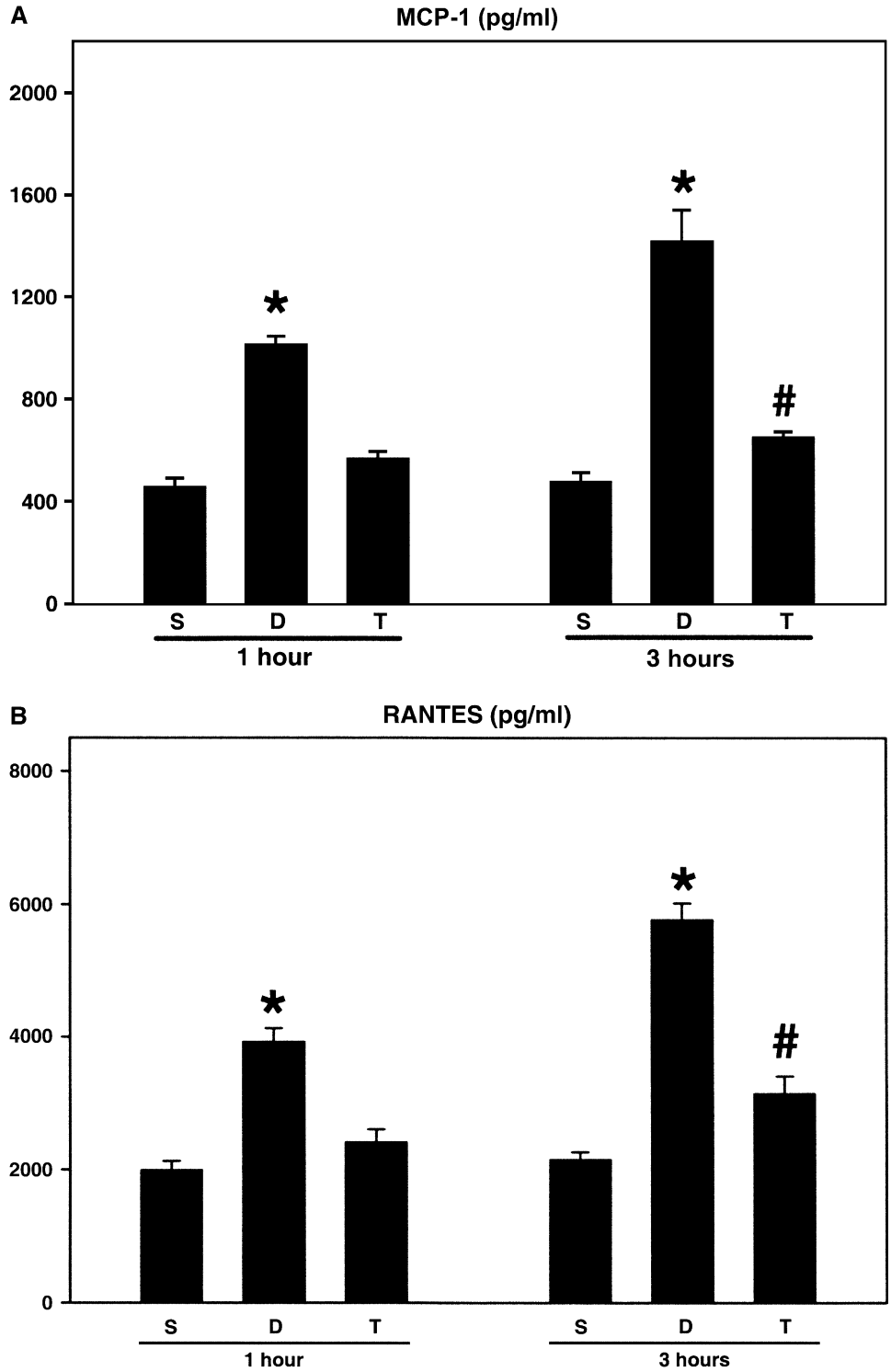

Fig. 6. Bile-pancreatic juice exclusion increases pancreatic chemokine production following duct ligation. ELISA of pancreatic homogenates showed significant increases in pancreatic production of chemokines MCP-1 and RANTES (*A* and *B*, respectively) after duct ligation that was significantly diminished by bile-pancreatic juice replacement. Results are mean ± SEM; n = 7 rats per group. *Significant difference from the sham-operated control group. #Significant difference from sham controls and diseased-control group (one-way ANOVA, $P < 0.05$). S = Sham controls had the duct dissected but not ligated; D = diseased-controls had ductligation; T = treated group, diseased-treated rats had duct ligation with duodenal bile-pancreatic juice replacement fresh from a donor rat.

from patients with acute pancreatitis showed elevated MCP-1 concentrations that correlated with severity of remote organ failure.[18] Taken together, these studies provide corroborative evidence that bile-pancreatic juice exclusion-induced increases in pancreatic MCP-1 concentrations in our experimental model contribute to exacerbation of ligation-induced acute pancreatitis.

Acute pancreatitis is a common disease associated with several etiologies and severe cases can result in significant morbidity and mortality.[20–22] Gallstones are the most common etiologic factor for acute pancreatitis worldwide.[22] Bile-pancreatic duct ligation-induced acute pancreatitis in rats is a useful experimental corollary of gallstone pancreatitis to investigate early events in disease pathogenesis.[1,23,24] As the early events in the pathogenesis of acute pancreatitis continue to elude us, therapeutic strategies remain merely nonspecific and supportive, consisting of fasting the patient, administration of intravenous fluids, and analgesia.[1,22] As the elucidation of early events in the pathogenesis of acute pancreatitis in humans is not practical, there is a reliance on experimental models of acute pancreatitis to investigate disease pathogenesis.[1,25] Ligation of the distal bile-pancreatic duct in rats obstructs the duct, excludes bile-pancreatic juice from the gut, and induces acute pancreatitis.[1,2] Bile-pancreatic juice exclusion from the gut results in feedback hyperstimulation of the exocrine pancreas via neurohormonal pathways.[10] Using our donor rat model, we previously showed that duodenal replacement of bile-pancreatic juice, obtained fresh from a donor rat, achieves substantial amelioration of pancreatic morphologic changes, hyperamylasemia, and hypercholecystokininemia in early ligation-induced acute pancreatitis.[1] We concluded that bile-pancreatic juice exclusion from gut exacerbates ligation-induced acute pancreatitis during the early stages of disease pathogenesis.[1,10] In additional studies we showed that trypsin and Na-taurocholate are the key components of pancreatic juice and bile, respectively, that exacerbate ligation-induced acute pancreatitis when excluded.[2] We also showed that a synergistic interaction between stimulation of the CCK-A receptor and the cholinergic receptor on acinar cells mediates the neurohormonal hyperstimulation of acinar cells that exacerbates ligation-induced acute pancreatitis.[2,10]

Cell surface receptors such as the CCK-A receptor and the cholinergic receptor are G protein–coupled receptors that, when bound by ligand, transduce intracellular signals via G proteins.[26,27] Stimulation of G protein–coupled receptors activates several intracellular signal transduction pathways including proinflammatory pathways such as stress-activated protein kinase pathways and the Akt/NF-κB pathway.[8] We have previously shown that the stress-activated protein kinase $p38^{MAPK}$ is activated in the pancreas in ligation-induced acute pancreatitis in rats, along with a parallel increase in pancreatic production of TNF-α.[3] We have also shown that increased $p38^{MAPK}$ activation and TNF-α production in the pancreas after duct ligation are markedly diminished by duodenal bile-pancreatic juice replacement from a donor rat.[3]

Akt is a serine-threonine protein kinase, so named because it is a cellular homolog of the product of the retroviral oncogene v-Akt (viral-Akt).[8,28] Akt is also called PKB (protein kinase B) or RAC (related to A and C protein kinase) due to its structural homology with protein kinase A and protein kinase C.[8,28] Akt regulates cell cycle events, cell growth and differentiation, and cell survival.[8] Recent evidence has implicated Akt in signaling pathways mediating inflammation.[4–6] Akt is activated by growth factors, cytokines, and other G protein–coupled receptor agonists and by various forms of cellular stress including heat shock, hyperosmotic stress, and oxidative stress.[8,29] Activated Akt is capable of activating various transcription factors including NF-κB.[7,30] NF-κB induces the nuclear transcription of proinflammatory messengers such as cytokines (interleukin 1, TNF-α), chemokines (MCP-1, RANTES), adhesion molecules (ICAM, E-selectin), and inducible effector enzymes (iNOS, COX-2), and it also regulates genes encoding apoptosis and cell proliferation.[7,31] Transcriptional regulation via Akt/NF-κB pathway activation involves a complex series of events. Activated Akt phosphorylates IκB (via IκB kinases IKK-α and IKK-β), causing IκB degradation.[7,30] This results in dissociation of the cytosolic IκB/NF-κB complex, allowing the transcription factor NF-κB to translocate to the nucleus and promote transcriptional upregulation of various inflammatory mediators including MCP-1 and RANTES.[7,30] Our evidence for increased Akt activation, increased NF-κB activation and nuclear translocation, and increased MCP-1 and RANTES production in pancreata of rats during the early phase after duct ligation raises the possibility that the Akt/NF-κB pathway may contribute to the pathogenesis of acute pancreatitis in our experimental model. The attenuation of these changes with duodenal bile-pancreatic juice replacement in rats with duct ligation indicates that bile-pancreatic juice exclusion from gut promotes activation of the Akt/NF-κB pathway. These findings are consistent with our central hypothesis that bile-pancreatic juice exclusion from gut exacerbates ligation-induced acute pancreatitis in rats during the early stages of disease pathogenesis.

However, to determine whether Akt/NF-κB pathway activation after duct ligation is predominantly a proinflammatory event with relevance to disease pathogenesis—or merely a parallel event mediating anti-apoptotic cell survival signals—is a question that will need to be the focus of further investigation.

Recent studies have demonstrated that nuclear translocation of NF-κB is not sufficient to activate NF-κB–dependent transcription. Instead, phosphorylation of the p65 subunit of NF-κB is required for effective transcriptional activity of NF-κB.[5] We performed gel supershift assays for NF-κB in previous studies where the nuclear extracts were incubated with antibody against p50 and p65 subunits of NF-κB prior to the addition of ^{32}P-labeled oligonucleotide.[14] These previous studies showed progressive NF-κB activation after 1, 3, and 24 hours of duct ligation, whereas sham-operation was not associated with NF-κB activation. Additionally, the antibody to the p65 subunit shifted the NF-κB band, whereas the anti-p50 antibody did not, suggesting a role for the p65 subunit in NF-κB activation in ligation-induced acute pancreatitis in rats. Of note, transfection of the rat pancreas in vivo with intraductal injection of the active p65 subunit of NF-κB delivered by adenoviral-mediated transfer activated NF-κB in acinar cells and was associated with an inflammatory response that was not observed when adenoviral controls were used.[31]

An immediate question raised by our study is what is the mechanism underlying activation of Akt following bile-pancreatic duct ligation. Phosphatidylinositol 3-kinase (PI3K) is the best recognized upstream activator of Akt in mammalian cells.[4,32] Following stimulation of G protein–coupled receptors by agonists such as cytokines (TNF-α, interleukin-1β) and chemokines (interleukin 8, MCP-1, RANTES), activation of the PI3K/Akt axis modulates neutrophil activation, chemotaxis, and apoptosis.[5] However, others have shown that PI3K inhibitors such as wortmannin or LY294002 do not attenuate Akt activation after hyperstimlation with CCK-A receptor analog caerulein and suggest that PI3K-mediated production of inflammatory mediators does not involve Akt in the caerulein model.[32] On the other hand, upstream activators of Akt other than PI3K have recently been demonstrated, which include cAMP and β-adrenergic stimulation, and Akt activation by cAMP and β-adrenergic agonists was found to be PI3K independent (insensitive to PI3K inhibitor wortmannin).[28,33]

Our current findings that bile-pancreatic juice replacement attenuates increased Akt/NF-κB pathway activation and chemokine production in the pancreas after duct ligation indicates that activation of this proinflammatory pathway is probably a response to acinar hyperstimulation, providing new insights into the mechanism of activation of proinflammatory pathways in this experimental model. Our original donor rat model provides a unique opportunity to study the effects of the enteral response to bile-pancreatic juice exclusion in disease pathogenesis. As the morbidity and mortality of acute pancreatitis is in large part related to exocrine pancreatic overproduction of inflammatory mediators, elucidation of the mechanisms of acinar cell production of acute inflammatory mediators is crucial for a better understanding of the mechanisms of disease pathogenesis.

CONCLUSION

Bile-pancreatic juice exclusion from gut promotes Akt/NF-κB pathway activation and increases chemokine production in ligation-induced acute pancreatitis.

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Radiofrequency Ablation in Patients With Primary and Secondary Hepatic Malignancies

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The aims of this study were to assess the technical effectiveness of radiofrequency (RF) ablation in patients with primary or secondary hepatic malignancies and to determine survival and complication rates. This was a retrospective analysis of prospectively collected data of patients treated with RF ablation and controlled for recurrence every 3 months by contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI). The outcome is compared with a comprehensive review of data published in recent literature. Forty-seven patients underwent 50 RF sessions for the ablation of 73 tumors. Local tumor progression was observed in 11 patients (23%). A tumor sized larger than 30 mm, a tumor load larger than 14 cm^3, and a percutaneous approach were associated with a faster time to local tumor progression. At the end of a mean (± SD) follow-up period of 11.4 ± 7.5 months, 39 patients (83%) were alive, including eight patients with recurrent disease. The overall cumulative survival rates at 12 and 24 months were 87% and 70%, respectively. In our center, RF ablation can be safely performed to achieve adequate local control and survival rates. Time to local tumor progression was significantly related to initial size of the tumor and tumor load. (J GASTROINTEST SURG 2006;10:960–973)

KEY WORDS: Liver cancer, radiofrequency ablation, local tumor progression

Colorectal cancer (CRC) is a major cause of cancer-related death in Europe. The cumulative lifetime risk in the Western world is approximately 5%, the incidence rate is 50/100,000. Nearly 50% of patients with colorectal carcinoma either have liver metastases at presentation (15%–25%) or will subsequently develop them (20%).[1,2] Without any treatment, the median survival after the detection of liver metastases is less than a year, depending on the extent of the disease at the time of diagnosis.[3,4] In contrast, resection of liver metastases from colorectal origin is associated with a 5-year survival rate of 30%–50%, depending on the extent of liver involvement and provided that all disease can be removed safely.[4] Unfortunately, only 10%–25% of patients with colorectal liver metastases are amenable for liver resection, either because of tumor location, comorbidity, or insufficient hepatic reserve.[5,6]

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer mortality.[1] In North America and in several European countries including the Netherlands, HCC is uncommon, with an incidence rate of less than 5/100,000 and a mortality rate of less than 5/100,000.[1,2] An increase of the incidence in low-endemic areas has been reported for the United Kingdom, France, and the United States.[7–9] In contrast, Verhoef et al.[10] recently did not find any rising trend for the Netherlands. In the last 10 years, screening programs have resulted in a relative increase in the number of resectable cases, and the absolute number of resectable cases has increased as well. Surgical resection is the golden standard of therapy and seems to be the only effective way, aside from liver transplantation, to alter survival. However, due to advanced or decompensated liver

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cirrhosis, comorbidity, and multifocality of the tumors, only 10%–37% of patients with HCC are considered to be candidates for surgical resection.[11,12]

Thus, in approximately 80% of patients with both CRC and HCC, a partial liver resection is not possible, and interest in other treatment modalities is growing. In our center, several locoregional treatment modalities have been studied, including isolated liver perfusion,[13] interstitial laser coagulation,[14] and radiofrequency (RF) ablation. RF ablation has several potential advantages over the other therapies, offering more comfort, and less morbidity and severity due to its potential for minimal invasive application. Other advantages are reduced hospital stays (1–2 days), reduced costs, and the possibility of repeated treatment. To evaluate the outcome of RF ablation in the treatment of primary and secondary hepatic malignancies at our department, we reviewed our records.

PATIENTS AND METHODS

We performed a retrospective analysis of data collected prospectively at the Erasmus MC, University Medical Center Rotterdam, The Netherlands, a tertiary referral hospital with staff experienced in the field of interventional radiology. From July 2002 to December 2005, RF ablation was used to treat a total of 73 tumors in 47 consecutive patients with a primary or secondary hepatic malignancy. Assignment to RF ablation and choice of treatment approach were determined by participants in our institution's weekly multidisciplinary liver meeting; the intervention radiologist, the surgeon, the oncologist, and the hepatologist were included. In our center, RF ablation was performed percutaneously or as part of an open surgical procedure. A percutaneous approach was used whenever possible, but when the tumor was poorly visualized on ultrasound, or when the tumor was located in the high dome of the liver or near the liver capsule where percutaneous treatment could produce thermal injury to an adjacent visceral organ, ablation was performed via laparotomy. Also, some patients underwent a combined surgical hepatic resection and RF ablation to treat multiple and bilobar disease.

All patients treated with RF ablation were deemed to have unresectable hepatic disease based on tumor multifocality, the presence of advanced stage of cirrhosis with inadequate functional parenchymal reserve, high surgical risk, or surgical refusal. Tumors should be detectable by (intraoperative) ultrasound and contrast-enhanced spiral computed tomography (CT) or magnetic resonance imaging (MRI), and informed consent from the patient should be obtained. Patients were excluded if they had extrahepatic disease, uncontrollable ascites, tumor invasion of—or a position too close to—larger vessels or bile ducts, a life expectancy below 6 months, Child-Pugh C liver cirrhosis, a thromboplastin time below 40%, or a blood platelet count less than 50,000/μL. Baseline evaluation included a history and physical examination, serum laboratory tests, and imaging with ultrasound and contrast-enhanced CT or MRI.

Technique

RF ablation was performed primarily by an interventional radiologist under general anesthetics. A 17-gauge internally cooled single or cluster RF electrode (Radionics, Burlington, MA) was introduced into the hepatic malignancies by ultrasound guidance. The RF electrodes were attached to a 480 kHz RF generator (Cool-tip RF system, Radionics) capable of producing 200 W of power. During the procedure, the applied current, power output, and tissue impedance were monitored constantly. After RF exposure, the cooling system was stopped to measure the local tissue temperature with the electrode tip. When temperature exceeded 60° C, the ablation was considered adequate. At the end of the procedure, the generator was reactivated while the RF electrode was withdrawn to ablate the needle track and prevent tumor seeding.

Follow-up

Within 24 hours, a contrast-enhanced CT was performed to assess any possible complications that might necessitate longer hospitalization. The effectiveness in terms of local control was evaluated by means of triphasic contrast-enhanced CT, carried out 6 weeks after the RF procedure. Follow-up included imaging with contrast-enhanced CT, or MRI when treated lesions were not easily characterized, as well as monitoring of the tumor-specific tumor markers α-fetoprotein (AFP) or carcinoembryonic antigen (CEA), every 3 months.

Definitions and Statistical Analysis

To assess the treatment effectiveness, local tumor progression was scored, comprising both incompletely ablated tumor tissue (local failure) as well as progression of completely ablated tumors confirmed by contrast-enhanced CT or MRI. The local failures were included to avoid bias because of the impossibility of differentiating between incompletely ablated tumor tissue that continued to grow and new tumor foci growing at the original ablated site. Tumor size was scored in 3 dimensions to calculate an estimate

of the tumor volume by using the equation for an ellipsoid (volume = $4/3\pi$ (x/2) (y/2) (z/2)). The 30 mm cutoff point for tumor size corresponds to a 14 cm^3 limit for tumor load per patient. Tumor load was defined as the sum of tumor volume per patient.

Time to first local tumor progression or time to death for each patient was modeled with a Kaplan-Meier survival analysis.[15] To avoid bias, the date of last imaging was used as the cutoff point for censoring patients. When a patient received radiotherapy at the initial ablation site, or chemotherapy in addition to an earlier ablation procedure, we considered the patient censored. Censoring was assumed independent to patient prognosis. If a patient had more than one tumor, only the largest tumor in diameter was included in the analysis so that each patient contributed only one observation to the data, and the sample size did not become incorrectly inflated due to repeated measurements within patients. If an ablated tumor showed local tumor progression, all other tumors of the patient were considered censored in this measure at that time.

Distribution of survival time and time to local tumor progression or death were analyzed in relation to the different variables collected. Univariate tests (log-rank) were used to test for differences in these distributions by any single factor. The factors that solely appeared to have a significant impact were selected for entrance into a Cox proportional hazards model to analyze their effect on survival while adjusting for each other.[16] A backward elimination procedure was used for further covariate selection in the Cox proportional hazards model.

Student's *t* test was used to perform pair-wise comparisons between continuous variables. Categorical variables were tested using the Fisher's exact test or the Pearson chi-square test. Significance was determined at the 95% confidence interval (95% CI, $P < 0.05$). All data were collected in a computerized Microsoft Excel database (Microsoft Inc., Redmond, WA). The analysis was performed with SPSS version 11.5 (SPSS Inc., Chicago, IL) statistical software.

Review

To compare our data with state of the art data, a review was performed by searches in Pub Med by using the search terms "radiofrequency ablation," "colorectal liver metastases," "hepatocellular carcinoma," and "liver cancer." Reviews, letters, case reports, editorials, and articles not written in English were excluded. Because research and improvements on RF ablation are rapidly evolving, we excluded papers published before January 2000 to make a fair comparison. Manual cross-referencing was done based on the bibliography of studies identified in the original searches. Papers were excluded if they were duplicate publications or involved the treatment of 24 or fewer patients.

RESULTS

Patient and Tumor Characteristics

Between July 2002 and October 2005, 47 consecutive patients underwent ablation for 73 primary and secondary hepatic malignancies. Of these, 30 (64%) were men and 17 (36%) were women, with a mean (± SD) age of 60.7 ± 12.0 years (range, 32.8–81.2 years). HCC was diagnosed in 22 patients and colorectal liver metastases in 21 patients, 17 of which received chemotherapy before the RF treatment (90% were responder). Four patients were diagnosed with a gastrinoma, medullar thyroid carcinoma, carcinoid cancer, or adrenal cell carcinoma. Fifty ablations were performed via 27 ultrasound-guided percutaneous procedures and 23 intraoperative procedures. Fifteen patients were treated in combination with simultaneous partial liver resection, cholecystectomy to prevent gallbladder damage, or both. Three patients underwent a sequential ablation for either remnant tumor tissue (two patients) or intrahepatic progressive liver disease (one patient).

Of the 73 treated tumors, 31 (42%) were HCC and 32 (44%) were metastases from malignancies of the colon and rectum. Other metastatic tumors included four adrenal cell carcinomas, three gastrinomas, two medullar thyroid carcinomas, and one carcinoid tumor. RF ablation was used to treat an average of 1.6 tumors per patient (range, 1–4), with a mean (± SD) size of 22 mm ± 12 mm (range, 6–80 mm). The average (± SD) hospital stay was 1.3 ± 0.8 days (median, 1 day; range, 0–4 days) for patients treated percutaneously and 12.4 ± 9.1 days (median, 11 days; range, 5–36 days) for patients treated in combination with resection.

Local Tumor Progression

Post-treatment contrast-enhanced spiral CT at 6 weeks showed complete ablation in 44 of 47 patients (94%) and in 70 of 73 tumors (96%). In two of three patients with residual viable tumor tissue, a sequential ablation was performed, showing complete response on post-treatment imaging.

During a mean (± SD) follow-up period of 11.4 ± 7.5 months (range, 4–35 months), 11 of 47 patients (23%) developed local tumor progression, confirmed by contrast-enhanced CT or MRI. Demographics of patients in which local control

Table 1. Comparison of demographics and baseline characteristics of patients with or without local tumor progression

	Local control (n = 36)	Local tumor progression (n = 11)	*P* value
Age in years (mean ± SD)	59.5 ± 12.9	64.5 ± 7.9	0.133* (NS)
Sex ratio (male:female)	24:14	6:3	0.486* (NS)
Number of tumors	62	11	
Number of tumors per patient (mean ± SD)	1.6 ± 1.0	1.5 ± 1.2	0.753* (NS)
Tumor histology of patients (tumors)			0.495† (NS)
HCC	16 (25)	6 (6)	
CRC	16 (27)	5 (5)	
Other	4 (10)	0 (0)	
RF approach			0.036‡
Percutaneous	15	9	
Open	21	2	
Index tumor size in mm (mean ± SD)	20 ± 9.1	32 ± 19	0.001*
Mean (± SD) tumor volume in cm^3	8.2 ± 11	26 ± 43	0.029*
Index AFP count in μg/L (mean ± SD)	433 ± 830	2112 ± 3452	0.084* (NS)
Index CEA count in μg/L (mean ± SD)	27.4 ± 39.3	10.7 ± 10.5	0.181* (NS)

AFP = α-fetoprotein; CEA = carcinoembryonic antigen; NS = not significant.
*Student's *t*-test.
†Pearson chi-square test.
‡Fisher's exact test.

was achieved or who developed a local tumor progression are shown in Table 1. Mean (± SD) actuarial survival time until local tumor progression was 26 ± 2.3 months (95% CI, 22–31 months). The median time until local tumor progression occurred was not measurable with the Kaplan-Meier method due to the small number of events observed. However, the Kaplan-Meier estimated local tumor progression rate at 6 months for all patients was 24%. Only one local tumor progression was observed after 6 months (10 months). Time to local tumor progression stratified by tumor histology showed no significant differences (Fig. 1).

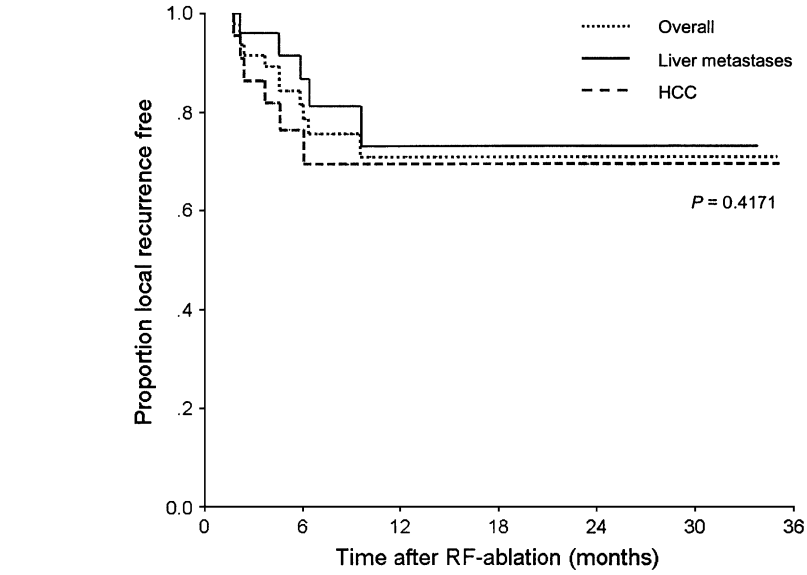
When tumor size was treated as a continuous variable, Cox regression showed that time to local tumor progression was significantly related to size of the tumor, with a hazard ratio (HR) of 1.071 and a 95% CI of 1.024–1.120 ($P = 0.002$). Stratified by HCC and metastatic tumors, Cox regression showed a significant relationship between time to local tumor progression and tumor size, with a HR of 1.065 and a 95% CI of 1.016–1.116 ($P = 0.009$). Having a tumor sized larger than 30 mm was significantly associated ($P = 0.0284$) with a faster time to local tumor progression, with a HR of 3.831 and a 95% CI of 1.153–12.736. Survival curves for patients until local tumor progression occurred, stratified by tumor diameter, are shown in Fig. 2. Both curves are statistically significant from each other, with a log-rank statistic of 5.53 (1 *df*) and a *P* value of 0.0187.

Tumor load per patient was also significantly related to local tumor progression, with a HR of 1.040 and a 95% CI of 1.012–1.069 ($P = 0.0052$). Again, after stratification by HCC and metastatic tumors, Cox regression showed a significant relationship between tumor load per patient and local tumor progression, with a HR of 1.040 and a 95% CI of 1.012–1.069 ($P = 0.0052$). Patients with a tumor load larger than 14 cm^3 significantly developed ($P = 0.0459$) an earlier local tumor progression, with a HR of 3.366 and a 95% CI of 1.022–11.079. The survival curves that are not shown were significantly different, with a log-rank statistic of 4.49 (1 *df*) and a *P* value of 0.0341.

Increasing AFP and CEA levels during follow-up were significantly associated with local tumor progression in patients with HCC (OR 15.000; $P = 0.023$) and CRC (OR 13.333; $P = 0.047$), respectively (Table 2). Tumor markers were obtained in all patients diagnosed with HCC; however, in three patients with CRC, the CEA level was not measured at baseline. None of these three patients developed a local recurrence.

Survival

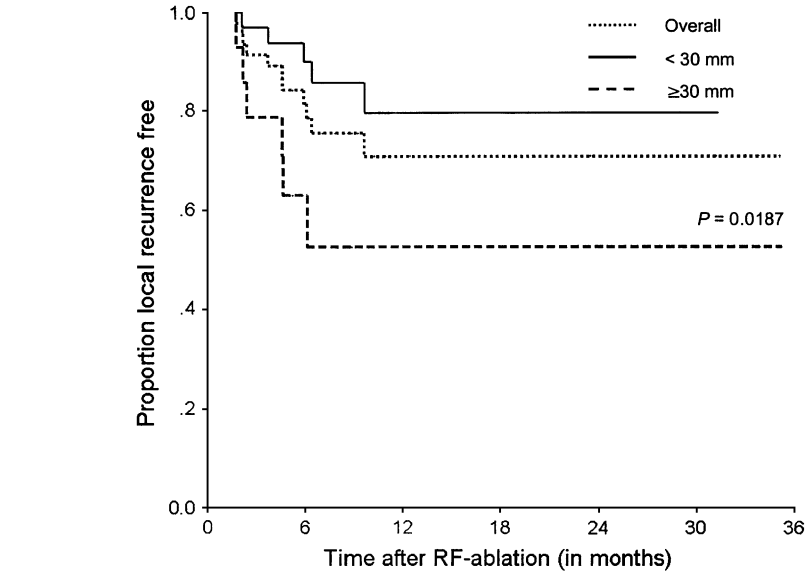
At the end of follow-up, six patients (13%) had died of progressive disease and 41 patients (87%)



No. at risk							
Overall	47	28	12	7	3	3	0
Liver metastases	25	17	7	2	1	1	0
HCC	22	11	5	5	2	2	0

Fig. 1. Time to local tumor progression by number of patients, stratified by tumor histology.

were alive, including eight patients in which local tumor progression was observed. Mean (± SD) actuarial survival time until death was 28 ± 2.8 months, with a 95% CI of 23–34 months, and is shown in Fig. 3. Again, the median time was not yet reached. The overall cumulative survival rates at 12 and 24 months were 87% and 70%, respectively. Fig. 3 also shows the overall survival stratified by tumor histology. Differences in survival were not statistically significant, with a *P* value of 0.0831 (log-rank = 3.00, 1 *df*).



No. at risk							
Overall	47	28	12	7	3	3	0
< 30 mm	33	22	10	5	1	1	0
≥30 mm	14	6	2	2	2	2	0

Fig. 2. Time to local tumor progression by number of patients, stratified by tumor diameter.

Table 2. Correlation between increasing tumor markers and onset of local recurrence

		Local tumor progression				
		No.	Yes	Odds Ratio	*P* value	95% CI
HCC	AFP decreased	12	1	(ref)	0.023*	1.325–169.870
	AFP increased	4	5	15.000		
CRC	CEA decreased	10	1	(ref)	0.047*	1.048–169.557
	CEA increased	3	4	13.333		
Total	Marker decreased	23	2	(ref)	0.001*	2.569–85.107
	Marker increased	7	9	14.786		

Ref = reference.
*Fisher's exact test.

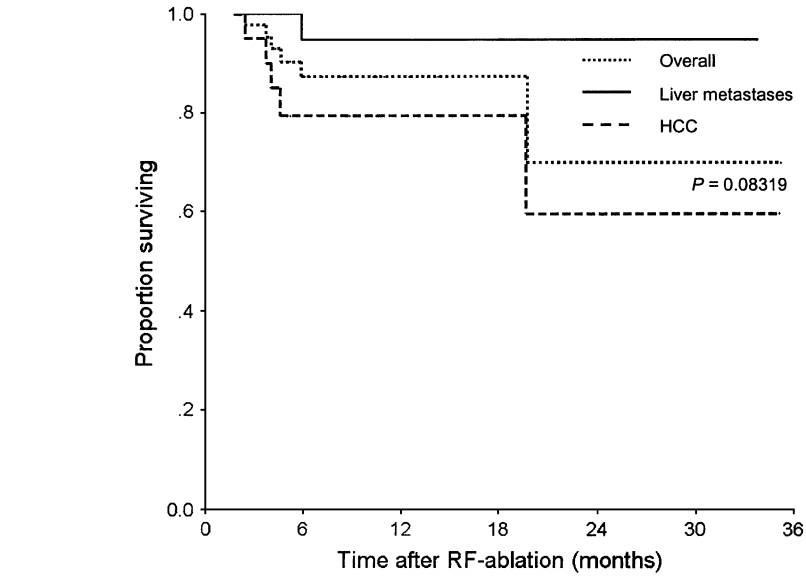
Complications

In six patients (13%), a minor complication occurred, requiring no intervention or extension of hospital stay. Three patients developed some ascites, and in three other patients a small intrahepatic hematoma was found at the postintervention spiral CT. No major complications were observed, nor was any mortality related to the RF procedure.

DISCUSSION

Reports in the literature on the use of RF ablation are increasing. Although many favorable reports have encouraged the use of RF by both surgeons and radiologists, we do not advocate RF ablation as an alternative but rather as an adjunct to hepatic resection, which remains the golden standard for the treatment of hepatic malignancies. In our study, RF ablation was used as an adjunct to resection in 15 procedures. Eight patients were ablated during laparotomy, and percutaneous RF ablation was the primary procedure in the remaining patients, who were poor candidates for surgery. It is essential that the technique of RF ablation should be optimized before proper comparison with surgical resection can be initiated. One of the main issues to be addressed is the completeness of ablation, which was the primary endpoint in our study.

We observed a patient-based local tumor progression rate of 23% and a tumor-based local tumor progression rate of 15%. Compared with studies in patients with both primary and secondary hepatic malignancies in prior literature, our results showed a few



No. at risk							
Overall	47	28	12	7	3	3	0
Liver metastases	25	17	7	2	1	1	0
HCC	22	11	5	5	2	2	0

Fig. 3. Overall survival by number of patients, stratified by tumor histology.

Table 3. Results of studies using RF as a treatment modality for both hepatocellular carcinoma and liver metastases

First author	Approach Origin HCC	CRC	OTH	Tumor (pts)	Tumor size (mm)	Follow-up time (mo)	Local tumor progression (%)	Survival rates	Complications (%)
De Baere[18,*] AJR Am J Roentgenol 2000 France	0 Perc: 33 Lap: 0 Open: 21	—	—	100 (54) Mean: 1.9 Range: —	Mean: 21 ± 11 Range: 5–42	Mean: 13.7 Range: 4–23	Pts: 9 (16) Tum: 0 (9.0)	1 yr: — 2 yr: — 3 yr: —	Minor: 4 (7.4) Major: 3 (5.6) Death: 0 (0) Total: 7(13)
Siperstein[21] Ann Surg Oncol 2000 USA	4 Lap: all	18	20	181 (43) Mean: 4.2 Range: 1–14	Mean volume: 8.7 ± 1.1 cm^3 Size Range: 10–100	Mean: 13.9 Range: 5–38	Pts: 12 (29) Tum: 22 (12)	1 yr: — 2 yr: — 3 yr —	Minor: 0 (0) Major: 0 (0) Death: 0 (0) Total: 0 (0)
Wood[24] Ann Surg Oncol 2000 USA	11 Perc: 18 Lap: 27 Open: 39	37	36	231 (84) Mean: 2.8 Range: —	Median: 20 Range: 3–90	Median: 9 Range: 1–27	Pts: 15 (18) Tum: —	1 yr: — 2 yr: — 3 yr: —	Minor: 4 (4.8) Major: 3 (3.6) Death: 1 (1.3) Total: 8 (9.5)
Bowles[25] Arch Surg 2001 USA	25 Perc: 44 Lap: 6 Open: 26	39	12	328 (76) Mean: 4.3 Range: 1–14	Median: 30 Range: 10–180	Mean: 15 Range: —	Pts: — Tum: 30 (9.1)	1 yr: 80 2 yr: 50 3 yr: —	Major: 10 (13) Major: 7 (9.2) Death: 1 (1.3) Total: 18 (24)
Wong[23] Am J Surg 2001 USA	2 Perc: 1 Open: 39	31	7	122 (40) Mean: 3.1 Range: 1–10	Mean: — Range: —	Median: 9.5 Range: —	Pts: 6 (15) Tum: —	1 yr: — 2 yr: — 3 yr: — Median: 9.5 mo	Minor/major: 8 (20) Death: 0 (0) Total: 8 (20)
Kosari[26] J Gastrointest Surg 2002 USA	12 Perc: — Lap: — Open: —	18	15	143 (45) Mean: 3.2 Range: —	Mean: 20 Range: —	Median: 19.5 Range: 6–34	Pts: — Tum: 11 (7.7)	1 yr: — 2 yr: — 3 yr: —	Minor: 3 (6.7) Major: 8 (18) Death: 0 (0) Total: 12 (27)
Jiang[19] World J Gastroent 2002 China	21 Perc: 20 Open: 16	12	3	48 (36) Mean: 1.3 Range: —	Mean: 25 Range: 5–90	Mean: 10 Range: 1–24	Pts: 6 (17) Tum: —	1 yr: — 2 yr: — 3 yr: —	Minor: 3 (8.3) Major: 1 (2.8) Death: 0 (0) Total: 4 (11)
Bleicher[17] Ann Surg Oncol 2003 USA	21 Perc: — Lap: — Open: —	59	73	447 (153) Mean: 2.9 Range: 1–13	Mean: 29 ± 16 Range: 5–135	Mean: 11 Range: —	Pts: 32 (21) Tum: 52 (12)	1 yr:— 2 yr: — 3 yr: —	Minor/major: 36 (24) Death: 0 (0) Total: 36 (24)
Pawlik[20] Ann Surg Oncol 2003 USA	5 Open: all	124	43	350 (172) Mean: 2.0 Range: —	Mean: 18 Range: 3–55	Median: 21.3 Range: —	Pts: 8 (4.7) Tum: 8 (2.3)	1 yr:— 2 yr: — 3 yr: — Median: 45.5 mo	Minor: 0 (0) Major: 1 (0.6) Death: 0 (0) Total: 1 (0.6)

Curley[50]	206	258	144	1225 (608)	Mean: 27	Mean: —	Pts: —	1 yr: —	Minor/major: 58 (9.5)
Ann Surg 2004 USA & Italy	Perc: 226			Mean: 2.0	Range:4–120	Range: —	Tum: —	2 yr: —	Death: 3 (0.5)
	Open: 382			Range: 1–12				3 yr: —	Total: 61 (10)
Teper[22]	4	18	4	56 (26)	Mean: 39 ± 26	Mean: 14.6 ± 9.2	Pts: 3 (12)	1 yr: 79	Minor: 0 (0)
Eur J Surg Oncol 2004 Germany	Open: all			Mean: 2.5	Range: —	Range: 2–36	Tum: —	2 yr: —	Major: 7 (27)
				Range: —				3 yr: —	Death: 0 (0)
								Median: 18	Total: 7 (27)
De Meijer	31	32	10	73 (47)	Mean: 22 ± 12	Mean: 11.5 ± 17.5	Pts: 11 (23)	1 yr: 87	Minor: 6 (13)
Current study 2005	Perc: 24			Mean: 1.6	Range: 6–80	Range: 4–35	Tum: 11 (15)	2 yr: 70	Major: 0 (0)
The Netherlands	Open: 23			Range: 1–4				3 yr: —	Death: 0 (0)
								Mean: 28 ± 2.8	Total: 6 (13)

— = not given; tum = no. of tumors; pts = no. of patients; perc = percutaneous; / open = intraoperative; lap = laparoscopic; OTH = other tumor origin.
*Only those patients with a follow-up of at least 4 mo were included in the analysis.

more recurrences than the reported patient-based local tumor progression rates of 4.7%–21%,[17–24] and also more than the tumor-based local tumor progression rates of 2.3%–12% (Table 3).[17,18,20,21,25,26] Compared with more homogeneous patient populations, we observed similar local tumor progression rates of 2.4%–36%,[27–35] patient-based, and 6.2%–52%,[34–39] tumor-based, for HCC (Table 4), and local tumor progression rates of 8.8%–55%,[40–43] patient-based, and 37%–39%,[42–44] tumor-based, for CRC (Table 5).

We realize that it is difficult to compare studies on RF ablation because of differences in patient selection, adjuvant treatment, and approach. Nevertheless, this comparison raises the question whether it is justified to apply a treatment modality with these local tumor progression rates to all patients. Given our results, we believe that RF should be used with caution for tumors larger than 30 mm in diameter. When applied, extra care should be taken to increase the local technical success. As local tumor progression usually occurs at the radial margins of the ablated tumor, it is essential to have a reliable monitoring of the ablation, especially when overlapping ablations are required to encompass both the tumor and an ablation margin. Currently, the main problem in monitoring is the absence of reliable real-time peroperative imaging techniques. This is also well illustrated by the statistically significant higher local failure rate in patients treated percutaneously compared with patients who were treated intraoperatively, because the latter benefited by the availability of more accurate intraoperative ultrasound (Table 1).

A way to achieve adequate local control is the method presented by Tateishi et al.[32] in patients diagnosed with HCC. They inserted the RF electrode under real-time ultrasound guidance and performed a dynamic CT scan directly at the end of the session to evaluate the ablation effect. When the result was judged as incomplete, additional sessions were performed until complete ablation was achieved. They also performed transcatheter arterial embolization at least 7 days before the RF treatment to occlude the arterial flow. This combination resulted in a very low local tumor progression rate of 2.4% during a median follow-up of 19 months, indicating that adequate local control can be achieved.

With respect to the follow-up of patients treated with RF ablation, we found a statistically significant relationship between the elevation of tumor markers and the onset of local tumor progression. Although there are novel biomarkers coming up, and more advanced markers already in use, monitoring the tumor markers AFP and CEA still plays a significant role besides routinely scanning for local, intrahepatic, or extrahepatic recurrences.[45,46]

Table 4. Results of studies using RF as a treatment modality for hepatocellular carcinoma

First author	Origin approach	Tumors (pts)	tumor size (mm)	Follow-up time (mo)	Local tumor progression	Survival rates	Complications (%)
Livraghi[38] Radiology 2000 Italy	HCC, perc: all	126 (114) Mean: 1.1 Range: 1–3	Mean: 54 Range: 31–95	Mean: 10.2 Range: 5–30	Pts: — Tum: 66 (52)	1 yr: — 2 yr: — 3yr:—	Minor: 5 (4.4) Major: 2 (1.8) Death: 1 (0.9) Total: 8 (7.0)
Curley[28] Ann Surg 2000 Italy & USA	HCC, perc: 76 lap: 31 open: 3	149 (110) Mean: 1.4 Range: 1–4	Perc: Mean: 28 ± 8 Open: Mean: 46 ± 7	Median: 19 Range: —	Pts: 4 (3.6) Tum: —	1 yr: — 2 yr: — 3 yr: —	Minor: 7 (6.4) Major: 7 (6.4) Death: 0 (0) Total: 14 (13)
Buscarini[27] Eur Radiol 2001 Italy	HCC, perc: all	101 (88) Mean: 1.1 Range: 1–3	All ⩽ 5	Mean: 34 Range: 2–73	Pts: 12 (14) Tum: —	1 yr: 89 3 yr: 62 5 yr: 33 Median: 48 mo	Minor: 14 (16) Major: 2 (2.3) Death: 0 (0) Total: 16 (18)
Guglielmi[37] Hepalogastroenterology 2003 Italy	HCC, perc: all	65 (53) Mean: 1.2 Range: —	Mean: 40 ± 13 Range: 10–70	Mean: 18 Range: 8–41	Pts: — Tum: 4 (6.2)	1 yr: 87 2 yr: 63 3 yr: 45	Minor: 11 (21) Major: 0 (0) Death: 0 (0) Total: 11 (21)
Harrison[30] J Am Coll Surg 2003 USA	HCC, perc: 46 open: 4	54 (50) Mean: 1.1 Range: 1–2	Median: 35 Range: 10–120	Median: 16 Range: 1–28	Pts: 18 (36) Tum: —	1 yr: — 2 yr: — 3 yr: —	Minor: — Major: — Death: — Total: —
Giovannini[29] J Gastrointest Surg 2003 France	HCC, perc: all	71 (56) Mean: 1.3 Range: 1–3	Mean: 41 Range:8–60	Mean: 20 Range: 6–36	Pts: 8 (14) Tum: —	1 yr: 96 2 yr: 94 3 yr: 94 mean: 36 mo	Minor: 2 (3.6) Major: — Death: — Total: 2 (3.6)
Choi[36] Radiology 2004 Korea	HCC, perc: all	53 (45) Mean: 1.2 Range: 1–2	Mean: 21 Range: 8–40	Mean: 23 Range: 10–40	Pts: — Tum: 11 (21)	1 yr: 82 2 yr: 72 3 yr: 54	Minor: — Major: — Death: — Total: —
Lam[31] Br J Surg 2004 China	HCC, perc: 18 open/lap: 33	70 (51) Mean: 1.4 Range: —	<30: 25 pts 30–50: 17 pts >50.9 pts	Mean: — Range: —	Pts: 18 (35) Tum: —	1 yr: 73 1½ yr: 61 2 yr: —	Minor: — Major: — Death: 1 (2.0) Total: —
Vivarelli[33] Ann Surg 2004 Italy	HCC, perc: all	— (79) Mean: — Range: —	⩽30:22 pts >30:57 pts	Mean: 15.0 ± 11.7 Range: —	Pts: 12 (15) Tum: —	1 yr: 78 2 yr: — 3 yr: 33	Minor: — Major: — Death: 0 (0) Total: —

(*continued*)

Table 4 (*continued*)

First author	Origin approach	Tumors (pts)	tumor size (mm)	Follow-up time (mo)	Local tumor progression	Survival rates	Complications (%)
Xu[39] Clin Radiol 2004 China	HCC, perc: 43 microwave: 54	190 (97) Mean: 2 Range: 1–5	Mean: 29 ± 12 Range: 9–88	Mean: 27.4 Range: 2–53	Pts: — Tum: 18 (9.5)	1 yr: 76 2 yr: 59 3 yr: 50 Mean: 32 mo	Minor/major: 9 (9.0) Death: 1 (1.0) Total: 10 (10)
Lin[35] Gastroenterology 2004 Taiwan	HCC, perc: all	69 (52) Mean: 1.3 Range: 1–3	Mean: 29 ± 8 Range: 10–40	Mean: 24.5 ± 11.3 Range: —	Pts: 7(14) Tum: 8(12)	1 yr: 90 2 yr: 82 3 yr: 74	Minor/major: 4 (7.7) Death: 0 (0) Total: 4 (7.7)
Tateishi[32,*] Cancer 2005 Japan	HCC, perc:	2140 (664) Mean: 3.2 Range: —	Mean: 26 Range: 8–97	Median: 27.6 Range: 2.0—61	2.4% at a median follow-up of 19 months	1 yr: 95 2 yr: 86 3 yr: 78 4 yr: 67 5 yr: 54	Minor: 17 (2.6) Major: 40 (6.0) Death: 0 (0) Total: 57 (8.6)
Lencioni[34] Radiology 2005 Italy	HCC, perc: all	240 (187) Mean: 1.3 Range: 1–3	Mean: 28 ± 7 Range: 15–50	Mean: 24 ± 21 Range: 3–78	Pts: 38(20) Tum: 41(17)	1 yr: 97 2 yr: 89 3 yr: 71 4 yr: 57 5 yr: 84 Median: 57	Minor: 9 (4.8) Major: 3 (1.6) Death: 0 (0) Total: 12 (6.4)
De Meijer Current study 2005 The Netherlands	HCC, perc: 15 open: 7	31 (22) Mean: 1.4 Range: 1–4	Mean: 31 ± 16 Range: 14–80	Mean: 10.1 ± 9.5 Range: 2–35	Pts: 6 (27) Tum: 6 (19)	1 yr: 79 2 yr: — 3 yr: —	Minor: 3 (14) Major: 0 (0) Death: 0 (0) Total: 3 (14)

— = not given; tum = no. of tumor(s); pts = no. of patients; perc = percutaneous; open = intraoperative; lap = laparoscopic; microwave = microwave ablation.
*For the analysis of local recurrence and survival rates, only patients who received RF ablation as the initial treatment for HCC were included.

Table 5. Results of studies using RF as a treatment modality for colorectal liver metastases

First author	Origin approach	Tumor (pts)	Tumor size (mm)	Follow-up time (mo)	Local tumor progression	Survival rates	Complications (%)
Solbiati[43] Radiology 2001 Italy	CRC, perc: all chemo: 84	179 (117) Mean: 1.5 Range: 1–4	Mean: 28 ± 12 Range: 6–96	Mean: — Range: 6–52	Pts: 64 (55) Tum: 70 (39)	1 yr: 93 2 yr: 69 3 yr: 46 Median: 36 mo	Minor: 1 (0.9) Major: 1 (0.9) Death: 0 (0) Total: 2 (1.7)
Cheng[49] Surg Endosc 2003 USA	CRC, lap: all chemo: 15	— (20) Mean: 2.1 ± 1.2 Range: —	≤40: 14 pts >40: 6 pts	Mean: 11.5 ± 7.8 Range: 1–38	Pts: — Tum: —	1 yr: — 2 yr: — 3 yr: — Mean: 25 ± 3.4 mo	Minor: — Major: 0 (0) Death: 0 (0) Total: 0 (0)
Livraghi[42] Cancer 2003 Italy	CRC, perc: all chemo: 70	134 (88) Mean: 1.5 Range: 1–3	Mean: 21 Range: 6–40	Median: 28 Range: —	Pts: 35 (40) Tum: 49 (37)	1 yr: — 2 yr: — 3 yr: —	Minor: 2 (2.3) Major: 1 (1.1) Death: 0 (0) Total: 3 (3.4)
Oshowo[48] Br J Surg 2003 UK	CRC, perc: all chemo: 22	25 (25) Mean: 1 Range: —	Mean: 30 Range: 10–100	Mean: 37 Range: 9–67	Pts: — Tum: —	1 yr: — 2 yr: — 3 yr: 37 4 yr: 22 Median: 25 mo	Minor: 0 (0) Major: 1 (4.0) Death: 0 (0) Total: 1 (4.0)
Abdalla[40] Ann Surg 2004 USA	CRC, open: all chemo: —	110 (578) Mean: 1.9 Range: 1–8	Median: 25 Range: —	Median: 21 Range: 4–112	Pts: 5 (8.8) Tum: —	1 yr: 92 2 yr: 60 3 yr: 53 Median: 37 mo	Minor: — Major: — Death: — Total: —
Gillams[41] Eur Radiol 2004 UK	CRC, perc: all chemo: 134	— (167) Mean: 4.1 Range: 1–27	Mean: 39 Range: 10–120	Mean: 17 Range: 0–89	Pts: 72 (43) Tum: —	1 yr: 71 3 yr: 21 5 yr: 14 Median: 22 mo	Minor: 22 (13) Major: 14 (8.4) Death: 0 (0) Total: 36 (22)
White[44] Dig Surg 2004 UK	CRC, perc: all chemo: 15	56 (30) Mean: 1.9 Range: —	Median: 30 Range: 8–70	Median: 17 Range: 3–37	Pts: — Tum: 22 (39)	1 yr: 75 2 yr: 45 3 yr: — Median: 22 mo	Minor: 2 (6.7) Major: 1 (3.3) Death: 0 (0) Total: 3 (10)
De Meijer Current study 2005 The Netherlands	CRC, perc: 7 open: 14 chemo: 12	32 (21) Mean: 1.5 Range: 1–4	Mean: 20 ± 6.9 Range: 6–35	Mean: 8.3 ± 4.4 Range: 2–18	Pts: 5 (24) Tum: 5 (16)	1 yr: 95 2 yr: — 3 yr: —	Minor: 0 (0) Major: 0 (0) Death: 0 (0) Total: 0 (0)

— = not given; tum = no. of tunors; pts = no. of patients; perc = percutaneous; open = intraoperative; lap = laparoscopic; chemo = prior chemotherapy.

At the end of follow-up, six patients had died of progressive disease, including three patients in which local tumor progression was observed. The first patient in which the virus recurred had a history of hepatitis C and liver transplantation. The second patient died of progression of chronic obstructive pulmonary disease, and the third patient died of pulmonary metastases. Thus, in all three patients local tumor progression did not directly influence survival.

In our study, we observed 1- and 2-year survival rates of 87% and 70%, respectively. Although there was no significant difference in overall survival between patients with a primary or secondary hepatic malignancy, it seems that Fig. 3 shows better survival in patients with a hepatic metastasis. This finding may relate to the strict selection of patients with metastatic cancer who are candidates for RF ablation in our center.

As either local recurrence or death can count as an event, the problem of dependent competing risks might arise. However, because all cases of local tumor progression occurred within 6 months and only one patient died before that time, in our study the assumption was made that dependency of competing risks did not play a role of significance, and that for the endpoint local tumor progression, the patient who died could be properly considered censored at time of death. Also, when a patient was treated with chemotherapy (or radiotherapy) in addition to an earlier ablation procedure, we considered the patient censored at the date of last imaging, to retain the ability to investigate the initial therapeutic efficacy of RF ablation. This possibly could have lead to an underestimation of the survival analysis.

Because studies that include patients with both a primary or secondary hepatic malignancy rarely publish survival rates (Table 5), we can only compare our results with an earlier study by Bowles et al.[25] that shows similar 1- and 2-year survival rates of 80% and 50%, respectively. Although our study population was relatively small and the data was retrospectively collected, our findings are consistent with the literature. Considering all studies in Tables 3, 4, and 5, 1- and 2-year survival rates ranged from 71%–97%* and 45%–94%,† respectively, for primary and secondary hepatic malignancies. Three- and 5-year survival rates ranged from 21%–94%[27,29,32–37,39–41,43,48] and 14%–54%, respectively.[27,32,34,41]

Cox regression showed no significant relationship between tumor diameter, tumor histology, and overall survival.

Although RF has many advantages in the treatment of liver tumors, it has disadvantages and complications as well, ranging from 0%–27%.‡ A large systematic review of 1931 patients treated with RF ablation from 1995 to 2002 by Mulier et al.[51] showed that major complications occurred in 7.1% of all patients. The most common complications were impairment of hepatic function, hemorrhage, infection, and biliary damage. The most severe complication is treatment-related death, occurring in 0%–2.2%.§ Mulier et al.[51] published a death rate of 0.7%.

We recorded any adverse event related to the procedure. Major complications were defined as events that might lead to substantial morbidity, disability or mortality, or result in hospital admission or substantially lengthened hospital stay.[52] All other complications were considered minor. Our study showed no major complications. Only six minor complications (three small intrahepatic hematomas, and some ascites in three patients) were observed, requiring no intervention.

Radiofrequency ablation techniques have continued to evolve since the current study was conducted. Our results relate to currently available techniques, and it is likely that, with the development of new tumor ablation techniques, real-time imaging, and new probes, even better results might be obtained. Current evidence suggests that in small tumors, RF can be performed with adequate local control and with few complications. Larger series and randomized clinical trials with other techniques and treatment algorithms are necessary to determine the exact role of RF ablation as a treatment modality for primary and secondary hepatic malignancies. Until then, proper selection of patients for RF treatment in high-volume hepatobiliary centers with a multidisciplinary team should be advocated.

* References 22, 25, 27, 29, 31–37, 39–41, 43, 44.
† References 25, 29, 32, 34–37, 39, 40, 43, 44, 47.
‡ References 17–29, 32, 34, 35, 37–39, 41–44, 48–50.
§ References 17–28, 31–33, 37–39, 41–44, 48–50.

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"Technological" Approach Versus Clamp Crushing Technique for Hepatic Parenchymal Transection: A Comparative Study

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We evaluated the feasibility and effectiveness of combining two different electronic devices, the ultrasonic dissector (UD) and the harmonic scalpel (HS), during hepatic resection. One hundred consecutive patients underwent liver resection using UD plus HS between January and December 2004 (UD + HS group). The ultrasonic dissector was used to fracture liver parenchyma and the uncovered vessel was sealed using the HS. Surgical outcomes were compared with 100 consecutive patients who underwent liver resection using the clamp crushing method. Operative variables, postoperative liver function, hospital stay, and type and number of complications were compared. The two groups were equivalent in term of demographic and pathologic variables. The UD + HS group had a decreased blood loss (500 ml versus 700 ml, $P = 0.005$), number of patients transfused (22 versus 39, $P = 0.009$), tumor exposure at the transection surface (4 versus 12, $P = 00.012$), and hospital stay (7 versus 8.5 days, $P = 0.020$). Postoperative major complications, in particular, fluid collection and biliary fistula, were significantly less frequent in the UD + HS group (2 versus 9, $P = 0.030$). A longer operative time was recorded in the UD + HS group (385 versus 330 minutes, $P = 0.001$). The combined use of UD with HS allows liver resection to be safely performed, with the advantage of reducing blood losses and surgery-related complications. The only major disadvantage may be a longer transection time. (J GASTROINTEST SURG 2006;10:974–979)

KEY WORDS: liver surgery, techniques of hepatic resection, ultrasonic dissector, harmonic scalpel

Major liver resections have recorded an impressive reduction in mortality and morbidity over the last decade.[1,2] The enhanced safety of liver surgery has been attributed to a number of factors, most notably, the development of anesthetic and surgical techniques. Parenchymal liver transection represents a fundamental phase of liver surgery. Serious intraoperative bleeding, together with injuries to vital structures of the liver remnant, may occur, leading to substantial morbidity and mortality.[3–7] The optimal method of transecting liver parenchyma has not been yet established. Transection of the liver was done in the previous decades by the finger fracture technique.[8] Although efficient in transecting the liver, it produced much bleeding and required prolonged and difficult hemostasis afterward. A more popular and efficient method involves the use of a surgical clamp to crush liver parenchyma, leaving bridging vessels intact to be secured before division.[9]

Availability of hi-tech surgical devices has further evolved the technique of parenchymal transection during hepatectomy from classic clamp crushing (CC) technique to a combination of different techniques.[10–13] The introduction of the ultrasonic dissector (UD) has allowed a more precise parenchyma division with preservation of small bridging vessels, reducing intraoperative blood loss.[14] A limit of this technique is that surgical ligation of bridging vessels is still required to achieve complete hemostasis during transection. The harmonic scalpel (HS), because of its simultaneous hemostatic and coagulating effect, has been introduced in both open and laparoscopic liver surgery.[15,16] The benefit of this

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device is to achieve local hemostasis of vessels of up to 3–4 mm, with minimal injury to surrounding tissue.[17,18] On the other hand, an increased rate of postoperative bile leaks has been described after hepatic resection performed using the harmonic scalpel alone.[19] In this study, we analyze multiple preoperative and perioperative variables related to 200 matched liver resections, comparing our experience of liver transection performed using the CC and combining the UD and HS (UD + HS).

MATERIAL AND METHODS

Beginning in January 2004, hepatic resections were performed at the Department of Surgery–Liver Unit at Scientific Institute H San Raffaele, Milan, Italy, using the UD plus HS for liver transection. In the previous years, liver transection was performed using the CC technique or the HS alone.

We searched from a prospectively collected hepatobiliary surgical database for patients who underwent elective liver resection for primary and metastatic malignancy or benign disease. After excluding cases with concomitant colonic resection or biliary-enteric anastomosis, we identified 100 consecutive liver resection performed from January 2004 to December 2004, in which the UD and HS were used for liver transection (UD + HS group). We then identified a group of 100 consecutive patients with similar demographic and pathologic features who underwent liver resection with the CC method (CC group). All patients in the CC group underwent liver resection between February 2002 and September 2003. Gender, presence of underlying liver diseases, cause of surgery, and size of disease were recorded. Intraoperative parameters were evaluated as well: operative procedure, operating time, Pringle time, blood loss, intraoperative amount of blood transfused, and histologic tumor exposure at the transection surface. Abnormal liver function tests including total bilirubin, alanine aminotransferase, aspartate aminotransferase, and prothrombin time through patient discharge were recorded. Morbidity and hospital stay were also compared between the two groups. Patients were monitored for the development of postoperative fluid collections and/or biliary fistulas. For the purpose of this study, we defined biliary fistula as bilious drainage lasting more than 7 postoperative days.[20] Bile leakage was suspected by evaluating drainage fluid color and confirmed assaying total bilirubin level into the drainage fluid. A total bilirubin level into the drainage fluid higher than 5 mg/dl in patients with normal serum bilirubin value or three times higher than the serum bilirubin value in jaundice patients was considered to be a sign of bile leakage.

Packed red blood cells were administered if the hemoglobin fell below 8 g/dl or with hemoglobin levels below 9 g/dl in patients with altered cardiopulmonary function or symptomatic anemia. Only transfusions of packed red blood cells have been considered as "transfusion" in the present report.

The terminology for liver anatomy and resection is based on the recommendations of the International Hepato-Pancreato-Biliary Association.[21] Removal of two segments or more was considered as major resection.[22]

Surgical Techniques

Laparatomy was performed through a right subcostal incision and a midline incision. Intraoperative ultrasound was performed before deciding on the type of resection. In cases of major resection, control of vascular inflow and outflow was obtained beforehand. During right or left hepatectomy, ligation of the main portal and arterial branches was performed before parenchymal resection. In contrast, section of the main biliary duct was performed during parenchymal transection. The extent of intended hepatic parenchymal division was marked on the surface with electrocautery.

In the UD + HS group, the superficial liver tissue was divided using the HS (Ultracision G220; Ethicon Endosurgery, Cincinnati, OH); with the absence of large vessels and bile ducts, nearly all of the peripheral liver parenchyma can easily be divided without causing bleeding or bile leakage. The UD (Soring; Sonoca, Quickborn, Germany) was used to fracture hepatocytes along the proposed line of division. The UD is moved in a "painting" motion on the liver surface or along the transection groove. This leaves intact arteries, veins, and bile ducts crossing the line of division and the uncovered bridging structures were sealed and divided using the HS. Blood vessels up to 2–3 mm in diameter are coagulated in 3–4 seconds, while coagulation of larger vessels requires a longer contact time of 5–6 seconds. The HS was set at power level 3, because at this setting the risk of injuring nearby structure is minimal.[18] Both the blunt edge and the flat side of the blade can be used for dissection; however, we believe that the flat side provide a better coagulation effect. Repeated, alternating use of the UD and the HS is continued until resection is complete. Vessels larger than 5 mm were clipped with titanium clip or sutured.

In the CC group, parenchymal transection was performed with Kelly clamps, progressive hemostasis

of the vessels with titanium clips or ligations, and coagulation by electrocauterization or argon beam.

No other hemostatic agents or devices were used, and a closed suction drain was routinely placed along the transection surface in all patients. The intermittent Pringle maneuver was applied during liver transection as needed. Three hepatobiliary surgeons (L.A., M.C., M.A.) were always present as the operator or the first assistant. Anesthetic technique, transfusion policy, and postoperative management were not modified during the study period.

Statistical Analysis

Demographic, pathologic, operative details, and surgical outcomes between the two groups were compared using the χ^2 test or Fisher's exact test for categorical data and the Mann-Whitney *U* test for ordinal data. Peak liver function test values were compared between the groups using the Student t test. All data were expressed as the median and range or the mean plus the standard deviation. Significance was defined as $P < 0.05$. All analyses were performed using the statistical package SPSS 12.0 (SPSS, Chicago, IL).

RESULTS

The two groups were well matched for all baseline characteristics. The distribution of tumor size, tumor number, and extent of resection were also similar between the two groups (Table 1).

Patients in the UD + HS group had a median blood loss of 500 ml versus 700 ml in the CC group ($P = 0.005$). Blood transfusions were necessary in 22 patients in the UD + HS group and in 36 patients in the CC group ($P = 0.029$). Operative time was significantly longer in the UD + HS group than in the CC group (380 minutes versus 330 minutes, $P = 0.001$). Postoperative peak values of alanine aminotransferase, aspartate aminotransferase, total bilirubin, and prothrombin time were comparable between the two groups. Final pathologic analysis identified 4 patients in the UD + HS group and 12 patients in the CC group with histologic tumor exposure at the transection surface ($P = 0.037$).

After surgery, 14 patients in the UD + HS group had 16 complications, and 23 patients in the CC group had 27 complications. A total of 20 events were minor: 4 were pleural effusion, 2 were wound dehiscence, 4 were wound infection, 2 were acute myocardium infarct, 2 were pneumonia, and 6 were transitory liver failure. Five major events occurred in the UD + HS: one case of intra-abdominal bleeding requiring relaparotomy, two self-limiting bile leaks, one patient who underwent a segmental resection for a hepatocellular carcinoma in a cirrhotic liver developed chronic liver failure, and a fatal liver failure occurred after a right hepatectomy in a cirrhotic liver. One death was recorded in the UD + HS group while three patients died in the CC group.

In the CC group, 14 major events occurred: 3 subphrenic abscess (requiring drainage), 6 bile leaks (in 2 cases an endoscopic retrograde cholangiopancreatography procedure was necessary), 2 cases of intra-abdominal bleeding requiring relaparotomy, 2 cases of fatal liver failure, and 1 case of massive pulmonary embolism. A significant difference in major postoperative complication was present between the two groups ($P = 0.030$). The incidence of fluid collection and/or biliary fistula was also significantly lower in the UD + HS group than in the CC group ($P = 0.030$).

According to the presence of cirrhosis, three and six minor complications occurred respectively in

Table 1. Patient characteristics and surgical procedures

Patient characteristics and surgical procedures	UD + HS group	CC group
Median age (range, y)	64 (19–83)	60 (32–81)
Male/female (n)	57/43	58/42
Background liver status		
Normal/cirrhosis/steatosis (n)	61/32/7	63/30/7
Presence of comorbidities	58%	44%
Disease		
HCC	34%	31%
Metastatic liver tumor	39%	37%
Bile duct cancer	8%	13%
Benign diseases	16%	15%
Other	3%	4%
Lesion size (mm)		
≤40	32%	35%
>40	68%	65%
Main surgical procedure (n)		
Left hepatectomy	17	9
Right hepatectomy	22	15
Extended left hepatectomy	2	2
Extended right hepatectomy	4	2
Left lateral sectionectomy	9	13
Right posterior sectionectomy	2	2
Bisegmentectomy	13	19
Segmentectomy	18	26
Wedge resection	12	10
Cystopericystectomy	1	2
Type of hepatic resection (major/minor) (n)	69/31	62/38
No. of resections		
Single	86	88
Multiple	14	12

the UD + HS group and CC group ($P = 0.235$). Three major complications including one case of bleeding and two cases of severe liver failure occurred in the UD + HS group, and five major complications including two bile leaks, one bleeding, and two severe liver failures occurred in the CC group ($P = 0.392$).

Median hospital stay was 7 days (range, 5–53) in the UD + HS group and 8.5 (range, 5–60) in the CC group ($P = 0.02$). Intraoperative and surgical outcome results are summarized in Table 2.

DISCUSSION

Parenchyma transection is the most important stage of liver resection. The majority of intraoperative complications that affect patients' outcome, including bleeding, bile leakage, abdominal collection, and infections, are related to this procedure.[3,6,7,23,24]

Various techniques for splitting the liver have been used, including CC,[9] UD,[14] hydrodissection,[25] stapling with vascular endostaplers,[15] laser system,[4] bipolar scissors,[6] saline-linked radiofrequency sealer,[26] and HS.[15,16] UD and the CC technique appear to be the most frequently used.[1,2,27] The UD, although more costly and time consuming than CC, has gained wide acceptance because it may reduce bleeding during liver resection.[14] Fan et al.[27] reported a 30% reduction in blood loss after changing their technique from CC to UD. A limit of this technique is that blood vessels and biliary tract branches need to be clipped or sutured to achieve complete hemostasis and biliostasis during dissection. Monopolar and bipolar electrocoagulation have been proposed as a simple and inexpensive method to achieve hemostasis during hepatic resection; however, the heat generated is considerable and may cause damage far from the plane of dissection.[28,29]

High-power ultrasonic activated scalpel systems that cut and coagulate tissues have been used in both open and laparoscopic surgery. The most widely used system (Ultracision) incorporates piezoelectric transducers that induce a frequency of 55,500 vibrations per second on the applied blade. At this frequency, the ultrasonic energy induces the denaturation of proteins by destroying hydrogen bonds and by the generation of heat in the vibrating tissue.[16,30] Because of its simultaneous haemostatic and coagulating effect, with minimal injury to surrounding tissue, it might theoretically offer a considerable advantage over electric coagulation.[29]

Our prospective series of hepatic resections demonstrates that the combined use of UD and HS is a safe and effective technique for transection of liver parenchyma. Surgery-related complications in the UD + HS group were significantly lower than in the CC group. Even though the use of HS alone has been associated with a significant increase in the incidence of postoperative bile leaks,[19] our study do not confirm this data. Bile leakage rate in the UD + HS group was 2%, a lower rate than in major series reported in literature.[1,2]

We suppose that the combined use of UD and HS offers several advantages that may explain the low

Table 2. Surgical outcomes

Surgical outcome variable	UD + HS group	CC group	*P*
Blood loss (ml), median (range)	500 (100–2000)	700 (100–6000)	0.005
Intraoperative blood transfusions (n)	22	39	0.009
Median units transfused (range)	2 (1–4)	2 (1–10)	
Operative time (min), median (range)	385 (150–660)	330 (120–660)	0.001
Pringle maneuver (n)	58	64	0.729
Median total occlusion time (range)	40 (10–83)	42 (10–78)	0.849
Liver function tests			
Mean peak ALT (±SD)	493 (398)	610 (430)	0.223
Mean peak AST (±SD)	422 (410)	510 (490)	0.343
Mean peak total bilirubin (±SD)	1.5 (0.9)	1.4 (0.8)	0.579
Mean peak prothrombin time INR (±SD)	1.19 (0.11)	1.23 (0.25)	0.539
Histologic tumor exposure (n)	4	12	0.037
Postoperative morbidity (%)			
None	86	75	0.69
Minor	9	11	0.637
Major	5	14	0.030
Fluid collection or biliary fistula (%)	2	9	0.030
Hospital stay (days)	7 (5–53)	8.5 (5–60)	0.025
Mortality (%)	1	3	0.312

morbidity rate reported in the UD + HS group. The UD allows clear visualization of liver parenchyma, especially when exposure of the major hepatic veins or portobiliary pedicle is required for delineation of the transection plane and helps to avoid inadvertent injury to vital structure to the remnant liver. With cautious application of portal clamping and the UD, the transection surface is usually clean, making easier the identification of key vascular and biliary structures, which can then be divided or preserved in a precise fashion. This prevents inadvertent damage to important structures and best ensures recognition of all intraparenchymal biliary branches, which may cause of bile leaks, abscesses, and sepsis in the postoperative period. The occlusion of arteries, veins, and bile ducts crossing the line of transection by the HS is therefore made easier and safer by the use of UD, which exposes the covered vessel. With the HS, the cut surface appears even and brownish, making identification of biliary leaks or persistent bleeding easy to detect and suture. Considering the known correlation of intraoperative tissue damage and postoperative infective complications, the absence of collateral damage to adjacent tissues represents an additional important advantage of HS.[30]

Our data showed that, in two well-matched patient groups undergoing liver resection, the combined use of UD with HS for parenchymal transection resulted in significantly less operative blood loss when compared with the CC technique. This translated into a significant difference in the number of patients transfused between the two groups. Most bleeding during transection with inflow occlusion results from tears in the hepatic venous tributaries or liver parenchyma. Different blood loss between the two groups suggests a different risk of such injury between the two methods, which agrees with the concept that CC is more blunt than ultrasonic dissection.[14,27] Furthermore, the optimal hemostatic and coagulating effect of HS might have played a significant role. The HS seems to be a good alternative compared with other vessel-sealing devices such as the bipolar scissor, which seems to be less efficient in achieving a satisfactory hemostasis during transection of cirrhotic parenchyma.[31] In our study, 32 patients in the UD + HS group had cirrhosis, and even in this category of patients, the combined use of Ultrasonic Dissector and Harmonic Scalpel was effective, with limited blood loss and no need for changes in the transection technique.

Surgical experience is of paramount importance in designing a transection plane that guarantees tumor-free margins. Nevertheless, the importance of the technique of resection cannot be underscored.[7,10] While the incidence of histologic tumor exposure at the transection surface in the CC group was similar to that reported in other series of patients undergoing liver surgery,[32,33] only four cases of histologic tumor exposure at the transection surface were recorded in the UD + HS group. From the oncologic point of view, UD seems to reduce the risk of tumor exposure at surgical margin, which is a well-established independent variable influencing tumor recurrence and long-term survival. The CC technique sacrifices at least 5 mm of normal liver parenchyma during each application of the clamp, as this is the space occupied by the blade of the clamp. By contrast UD can achieve adequate surgical margin with minimal sacrifice of normal liver parenchyma. This is particularly important for the excision of lesions adjacent vascular structures like the portal vein or vena cava.

Even though a recent randomized study has demonstrated any apparent benefits from the use of a single technology (UD, Hydrojet, dissecting sealer) compared with the CC technique,[34] the results of this study support the idea that the combined use of two modern devices—UD and HS—is associated with a reduced incidence of tumor exposure and bile leak, less blood loss, and a lower frequency of blood transfusion compared with CC.

CONCLUSIONS

The combined use of UD and HS allows liver resection to be safely performed, with the advantage of minimal surgical complication and reduced blood looses. The only major disadvantage may be a slower transection speed. A prospective randomized trial is needed to clarify the clinical benefits of liver resections performed using these novel techniques.

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Outcome Using Hemihepatic Vascular Occlusion Versus the Pringle Maneuver in Resections Limited to One Hepatic Section or Less

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Consensus is lacking concerning how to manage afferent vessels during hepatectomy, particularly as to the Pringle maneuver vs. selective hemihepatic clamping. Data for 81 hepatocellular carcinoma patients with chronic hepatitis or liver cirrhosis whose liver resection was limited to one section or less, including intraoperative data and postoperative liver function data, were analyzed retrospectively to compare two strategies. No significant differences of intraoperative data or postoperative clinical course were seen between the two groups, even in patients with chronic hepatitis or liver cirrhosis whose postoperative deterioration of liver function could be expected to be more than patients with a normal liver. The difference was evident only in serum alanine aminotransferase level on postoperative day 10 (mean ± SEM, 64.5 ± 5.1 IU in the Pringle group vs. 51.6 ± 4.4 IU in the selective clamping group; $P < 0.05$). During liver resection limited to one section or less, even with underlying chronic hepatitis or cirrhosis, intermittent use of the Pringle maneuver preserved liver function to the same extent as selective clamping. (J GASTROINTEST SURG 2006;10:980–986)

KEY WORDS: Hepatic resection, hemihepatic vascular occlusion, pringle maneuver

Although hepatic resection does not necessarily require any kind of liver vascular control, the possibility of life-threatening hemorrhage and ways to manage it successfully should always be considered in advance. Conventionally, the Pringle maneuver,[1] which involves compression of the hepatoduodenal ligament, is used during hepatectomy to control blood loss, but at the cost of profound hepatic ischemia and possible adverse effects upon the metabolic function of hepatocytes. In addition to this warm ischemic injury,[2] further deterioration of liver function would be expected from reperfusion injury[3] after the maneuver. Furthermore, the Pringle maneuver produces intestinal congestion unless it is frequently interrupted.

An alternative method of hemostasis is selective clamping of hepatic inflow vessels, such as hemihepatic vascular occlusion as described by Makuuchi et al.[4] and Takasaki et al.[5] This represents temporary, selective (mostly unilobar) occlusion of afferent vessels in the hepatic hilum supplying liver to be resected. With this method, visceral congestion is considered to be limited because considerable portal blood flow is preserved, and because only portions of the liver are rendered anoxic. Compared with similar resections without vascular control, this technique was reported to reduce intraoperative blood loss and postoperative hyperbilirubinemia in patients with or without cirrhosis.[4] However, at present, outcomes of hepatectomy patients who have undergone the Pringle maneuver and those who have had selective clamping to occlude hepatic afferent vessels have not been directly and unambiguously compared.

We retrospectively investigated the efficacy of these two vascular inflow occlusion techniques during liver resection limited to one section or less for patients with chronic hepatitis or liver cirrhosis whose postoperative deterioration of liver function could be expected to be more than in patients with a normal liver, with special attention to intraoperative data and postoperative liver function.

PATIENTS AND METHODS

Patients

From 1992 to 2004, 482 patients underwent initial hepatic resections without choledochojejunal anastomosis in the Department of Gastroenterological

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Surgery at the Yokohama City University Graduate School of Medicine (223 patients with colorectal liver metastases, 34 patients with liver metastases from other organs, 210 patients with hepatocellular carcinoma [HCC], and 15 patients with benign liver diseases, including 4 patients with intrahepatic cholelithiasis). Of 210 patients with HCC, 128 underwent limited liver resection involving one section or less. Of these patients, 22 underwent hepatectomy with no inflow vessel occlusion. After retrospective investigation of data for the other 106 patients, 81 patients were included in the analysis, whereas 12 patients were excluded because their liver parenchyma was pathologically free of hepatitis and cirrhosis, and 13 others were excluded because a local ablation technique such as microwave tissue coagulation or radiofrequency ablation was used together with hepatectomy. Of the 81 patients, 41 underwent resection with Pringle's maneuver, whereas 40 underwent resection with selective vascular control. Hemihepatic vascular occlusion as reported by Makuuchi et al.[4] was performed in three patients, whereas in 37 patients, the method of Takasaki et al.[5] of en masse occlusion of Glisson's sheath of the appropriate hemipedicle at the bifurcation was used.

Hepatectomy and Vascular Clamping Procedures

As a rule, the hepatectomy procedure—anatomic resection involving resection of the tumor together with the portal vein related to the tumor and the corresponding hepatic territory—was performed. Intraoperative ultrasonography was used to identify any occult tumors not detected preoperatively and also to confirm relationships between tumors and vasculobiliary structures. Parenchymal dissection was performed using ultrasonic dissectors: from 1992 to 1998, the SONOP SUS201D dissector (ALOKA, Tokyo, Japan) was used, and since 1999, the CUSA system (Valley Lab, Boulder, CO) and bipolar irrigation electrocautery (Codman & Shurtleff, Randolph, VA) were used.

The Brisbane 2000 terminology of the International Hepato-Pancreato-Biliary Association was used for operative procedures,[6] with segmentectomy representing resection of a Couinaud segment[7] and sectionectomy denoting resection of one of Healey's segments.[8]

One of the first three authors (K.T., H.S., and S.T.) of this report participated in every operation. Decisions regarding which vascular occlusion method was to be used during hepatectomy were made on a case-by-case basis. Occlusion for 15 minutes followed by 5 minutes of release was carried out in both Pringle's maneuver and selective clamping. When a tumor was located between the right and left hemiliver in a patient with selective clamping, hepatic resection and inflow control was performed as previously reported.[9] With the establishment of hemihepatic inflow control to the side containing most of the tumor, excision of the tumor commenced from the corresponding hemiliver. When this ipsilateral resection was completed, the vascular control was released and contralateral hemihepatic inflow was occluded immediately for completion of the resection.

As a rule, prostaglandin E1 (0.01 to 0.05μg/kg per minute) was administered intraoperatively to indirectly reduce ischemia-reperfusion injury by enhancing portal venous flow,[10] and also directly by stabilizing the hepatocellular membranes via a decrease in cytoplasmic Ca2+ and by controlling superoxide radical production.[11] Methylprednisolone (10 mg/kg) was also injected before occlusion of afferent vessels to protect the liver during warm ischemia.[12]

Postoperative Complications

Hyperbilirubinemia was noted as a postoperative complication when the bilirubin concentration on day 7 was 3 mg/dL or more. Biliary fistula was diagnosed when bile drainage was apparent from the abdominal wound and drain, with a total bilirubin concentration in the drainage fluid of more than 5 mg/ml or three times the serum concentration. Subphrenic or intra-abdominal abscess was confirmed by percutaneous drainage. Ascites or pleural effusion was noted as a complication when it required percutaneous drainage or control by administration of diuretic agents.

Perioperative Factors

The prognostic nutritional index,[13] initially reported as a measure of nutritional status in patients with gastrointestinal carcinoma, was calculated based on peripheral blood lymphocyte counts and serum albumin concentrations. Ischemic time was the cumulative total duration of the Pringle maneuver or of selective clamping.

Grade of hepatitis activity and stage of fibrosis of liver parenchyma in the resected specimen were assessed pathologically according to the New Inuyama Classification[14] as follows: Hepatitis-related necrosis and inflammatory activity was graded from A0 to A3, whereas degree of fibrosis was staged from F0 to F4. On the basis of these two types of pathologic findings in the liver parenchyma, patients with chronic liver disease included in this study were subdivided into two combined categories: 1 is equivalent to A1

to A3 and F1 to F3 (mild to severe hepatitis and fibrosis) and 2 is equivalent to F4 (cirrhosis).

The cut surface of the resected specimen was traced on paper, then the tracing was scanned digitally with a laser scanner. The corresponding area was calculated by computer to yield the transection area.

Data Analysis

Statistical comparisons of baseline data were performed byusing the Mann-Whitney *U* test or the Fisher exact test, as appropriate. A difference was considered significant at $P < 0.05$.

RESULTS

Preoperative Data for Patients Undergoing Hepatectomy

Comparable hepatectomy procedures were conducted in the Pringle group and the selective clamping group ($P = 0.54$). In the Pringle group, partial resection was performed in 11 patients, segmentectomy in 19 patients, sectionectomy in 10 patients, and sectionectomy with partial resection in one patient. In the selective clamping group, partial resection was performed in 11 patients, segmentectomy in 17 patients, segmentectomy with partial resection in two patients, and sectionectomy in 10 patients.

The Pringle group and the selective clamping group were comparable in terms of other prehepatectomy variables such as age, gender, platelet count (Plt), prothrombin time ratio, serum concentrations of activated partial thromboplastin time, hepaplastin test, total bilirubin (TB), liver enzymes (including serum aspartate aminotransferase [AST] and serum alanine aminotransferase [ALT]), prehepatectomy serum albumin concentration, indocyanine green retention rate at 15 minutes, and the prognostic nutritional index. Patient characteristics are shown in Table 1. The combined histologic designation concerning hepatitis grade and liver fibrosis was also similar in the two groups.

Table 1. Preoperative data for hepatectomy patients with chronic hepatitis or liver cirrhosis, by group

Variables	Pringle (n = 41)	Selective clampling (n = 40)
Age, yr	66 (43–75)	64 (41–80)
Gender, M/F, no.	33/8	35/5
PLT, $\times 10^4$/ml	12.2 (4.6–21.1)	13.9 (4.2–31.3)
PT ratio	1.09 (0.92–1.26)	1.09 (0.93–1.90)
APTT, sec	29.8 (22.6–36.1)	30.7 (23.2–38.0)
HPT, %	82.0 (56.0–176.0)	83.5 (47.0–177.0)
Serum		
TB, mg/dL	0.7 (0.4–1.6)	0.7 (0.3–1.8)
AST, IU	53.0 (21.0–136.0)	55.5 (17.0–133.0)
ALT, IU	52.0 (12.0–181.0)	59.5 (10.0–125.0)
Alb, g/dL	3.95 (2.6–4.9)	3.9 (3.2–4.94)
ICG R15, %	16.3 (5.4–32.0)	15.0 (8.1–39.0)
PNI	48.1 (28.5–57.6)	47.1 (36.4–58.0)
Hepatitis and fibrosis (combined)		
1	17 (41.5%)	24 (60.0%)
2	24 (58.5%)	16 (40.0%)

Values are given as median (range) except where indicated otherwise. The difference in gender distribution and hepatitis and fibrosis grade between groups was analyzed using the Fisher exact test. Other data were analyzed with the Mann-Whitney U test. No data show a significant difference between the groups.
PLT = palatelet count; PT = prothrombin; APTT = activated partial thromboplastin time, HPT = hepaplastin test; TB = total bilirubin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; Alb = albumin; ICG R15 = indocyanine green retention at 15 min; PNI = prognostic nutritional index.[13]

Intraoperative Data for Hepatectomy Patients

No significant difference was noted between the Pringle and selective clamping groups in intraoperative data such as hepatic ischemic time, operating time, resected liver volume, transection area, blood loss and blood transfusion, and central venous pressure. Median blood loss per square centimeter of transection area was 8.4 ml/cm2 for patients in the selective clamping group, slightly less than that for the Pringle maneuver group (10.4 ml/cm2). However, a slightly higher proportion of patients avoided transfusion in the Pringle maneuver group than in the selective clamping group (Table 2). Intraoperative similarities included the proportion of patients receiving prostaglandin E1 (Pringle vs. selective clamping, 97.6% vs. 87.5%) or methylprednisolone administration (82.9% vs. 67.5%).

Postoperative Clinical Course for Patients Undergoing Hepatectomy

Maximum TB, AST, and ALT, and minimum Plt after hepatectomy, did not differ between the Pringle maneuver group and the selective clamping group Table 2).

Peak AST and ALT occurred on the first postoperative day, and peak TB occurred on the third postoperative day and gradually returned to preoperative values after 1 week. A gradual decrease in Plt was observed until the third postoperative day, with recovery to the normal range within 7 days. The postoperative increases in serum AST and ALT were greater in the Pringle group than in the selective clamping group. Serum ALT (mean ± SEM) on postoperative day 10 was 64.5 ± 5.1 in the

Table 2. Intraoperative and postoperative data for hepatectomy patients with chronic hepatitis or liver cirrhosis, by group

Variables	Pringle (n = 41)	Selective clamping (n = 40)
Ischemic duration, min	90.0 (11–240)	80.0 (23–180)
Operative time, min	435.0 (247–787)	465.0 (276–720)
Resected liver volume, g	175.0 (55.0–453.0)	223.5 (40.0–690.0)
Transection area, cm^2	74.4 (15.0–139.2)	70.8 (35.0–182.0)
Total blood loss, L	1.19 (0.1–4.9)	1.25 (0.2–3.9)
Blood loss per transection area, ml/cm^2	10.4 (1.5–73.3)	8.4 (2.1–30.5)
Blood transfusions, ml	0.0 (0.0–3600)	250.0 (0.0–2000)
Nontransfused patients, no. (%)	24 (58.5)	15 (37.5)
CVP during Hx, mm Hg	6.0 (2.5–15.0)	6.0 (1.0–11.0)
Minimum Plt, $\times 10^4$ ml	7.5 (1.9–19.8)	7.7 (1.1–16.4)
Maximum TB, mg/dL	1.8 (0.9–5.0)	1.9 (0.8–6.1)
Maximum AST, IU	421 (72–1497)	356.5 (155–2078)
Maximum ALT, IU	300 (46–1576)	309 (122–751)
Postoperative complication, no. (%)	10 (24.4)	9 (22.5)
Hospital stay after Hx, days	18.0 (9–84)	21.0 (12–101)

Values are given as median (range) except where indicated otherwise. Differences in number of nontransfused patients and complication rates were analyzed by the Fisher exact test; other data were analyzed by the Mann-Whitney U test. No data show a significant difference between the groups.
CVP = central venous pressure; Hx = hepatectomy; Plt = platelet count; TB = total bilirubin; AST = asparate aminotransferase; ALT = alanine aminotransferase.

Pringle group vs. 51.6 ± 4.4 in the selective clamping group ($P < 0.05$). However, no significant differences of serial changes in TB, Plt, or AST were evident between groups (Fig. 1).

No operative deaths occurred in either group. In the Pringle group, complications were noted in 10 patients (24.4%), including biliary fistula in one patient, biliary fistula with intraabdominal abscess in one patient, subphrenic abscess in one patient, wound infection in one patient, ascites in two patients, hyperbilirubinemia in one patient, pleural effusion in two patients, and arrhythmia in one patient. In the selective clamping group, nine patients (22.5%) had postoperative complications, including one patient with biliary fistula, one patient with wound infection, two patients with ascites, one patient with hyperbilirubinemia, two patients with intestinal obstruction, and two patients with pleural effusion. Postoperative complication rate and hospital stay after hepatectomy were comparable between groups (Table 2).

DISCUSSION

With refinements in surgical technique, improvements in perioperative care, and better criteria for patient selection, hepatectomy can be performed today with a low mortality rate. Avoidance of excessive bleeding and blood transfusion is a central aim of most present-day liver surgeons. Accordingly, many surgical techniques for vascular control during hepatectomy have been advocated, including continuous or intermittent Pringle maneuvers,[15,16] liver clamping,[17] crush clamping methods,[18] hemihepatic vascular occlusion,[4,5] and total vascular exclusion.[19]

Conventionally, the Pringle maneuver has been used to reduce blood loss during hepatectomy, but its efficacy still is controversial. The Pringle maneuver resulted in less blood loss and better preservation of liver function in the early postoperative period than operating without special vascular control procedures, according to some reports,[20,21] whereas according to others, the Pringle maneuver was associated with a higher complication rate and higher postoperative serum concentrations of bilirubin and liver enzymes.[22,23] To avoid adverse effects of the Pringle maneuver such as hepatic warm ischemia and splanchnic congestion, hemihepatic inflow control techniques[4,5] have been advocated to control hemorrhage from the liver parenchyma without causing ischemia in the contralateral hepatic lobe or splanchnic congestion. However, efficacy and safety have not been clearly compared between the Pringle maneuver and selective clamping methods.

Aiming to clarify these debated issues, we retrospectively studied HCC patients with chronic hepatitis or liver cirrhosis whose liver resections were one hepatic section or less without choledochojejunal anastomosis. Cases with choledochojejunal

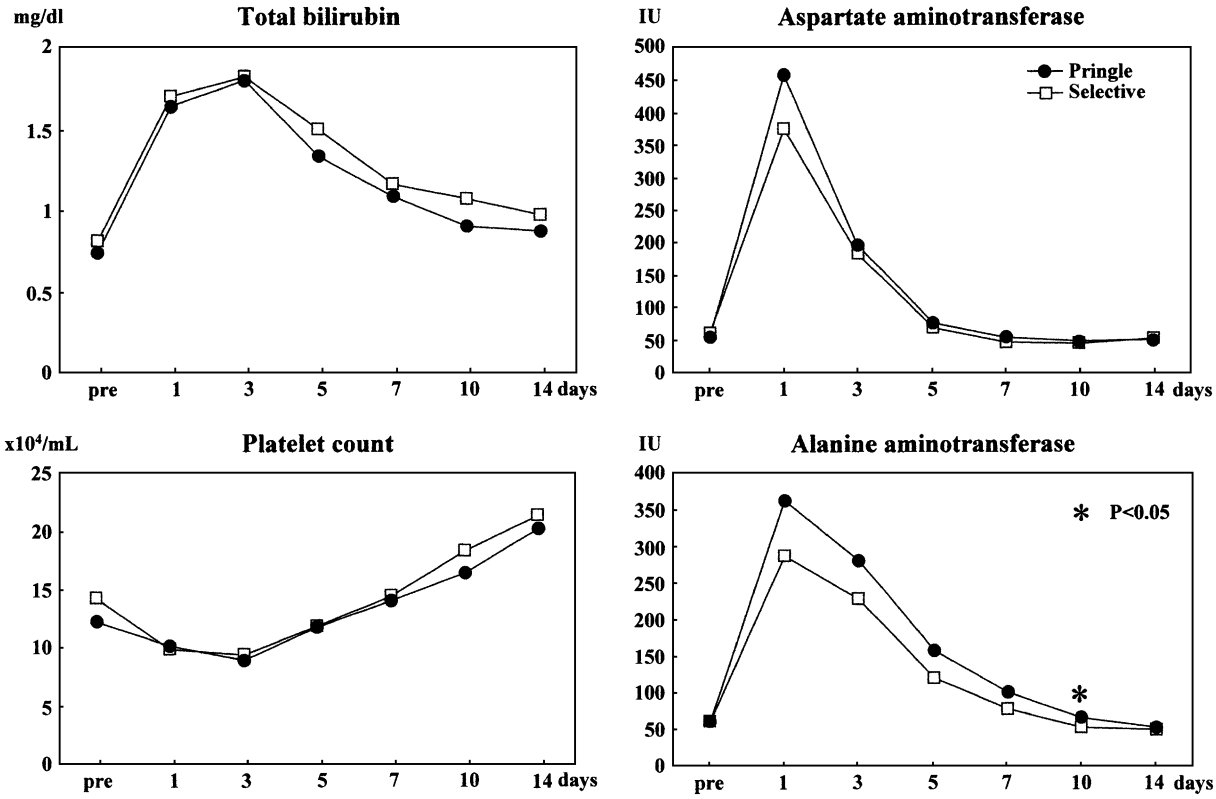

Fig. 1. Serial changes in results of postoperative liver function tests in patients whose anatomic resection specimen showed chronic hepatitis or cirrhosis involving the parenchyma. Each value is the mean. (●) Pringle maneuver, n = 41; (□) selective clamping, n = 40; *$P < 0.05$; pre = preoperative.

anastomosis were excluded to avoid confounding influences from biliary complications on serial changes of posthepatectomy data. To ensure that differences in liver function were influenced mainly by the extent of ischemic liver parenchyma, the hepatectomies studied were limited to one section or less. With selective clamping, only portions of the liver were anoxic, even when a tumor was located between the right and left hemiliver; hemihepatic inflow control was established and then released on each side in turn, beginning with the vessels supplying the side of the liver requiring the greatest tumor excision.[9] Postoperative deterioration of liver function would be expected in patients with chronic hepatitis or liver cirrhosis who had undergone vascular inflow occlusion techniques. Therefore, comparison was performed in patients with histologically evident chronic hepatitis or fibrosis.

No significant difference was seen between the groups in preoperative profiles. There were no significant differences by group of intraoperative data or postoperative clinical course. Neither postoperative complication rates nor hospital stay differed significantly between groups. There was also no significant difference in postoperative liver function between the groups. Postoperatively, when day-to-day changes in serum AST and ALT were considered, AST and ALT tended to be greater in the Pringle group than in the selective clamping group. However, maximum AST and ALT did not differ between the groups, and a difference became evident only in the serum ALT level on postoperative day 10.

Cirrhotic liver was less able to tolerate ischemia than normal liver in clinical observations[4,24] or animal experiments,[25] but our results suggested that the different vascular control procedures were equally applicable for patients with chronic hepatitis or cirrhosis. This may be because normothermic ischemic injury induced by the Pringle maneuver was only intermittent, or because the cirrhotic liver was perfused by collateral inflow pathways outside the portal triad.

The classic teaching that human liver cannot tolerate normothermic ischemia for longer than 15 to 20 minutes had led many surgeons to restrict use of portal triad clamping to critical hemorrhagic circumstances. In 1978, however, Huguet et al.[26] showed that the human liver could withstand much more prolonged normothermic ischemia, for up to

90 minutes. Elias et al.[15] reported that the human liver could tolerate the Pringle maneuver for more than 120 minutes during hepatic resection, provided that the occlusion was intermittent. Indeed, intermittent occlusion for more than 120 minutes occurred in 11 patients of the 41 that underwent the Pringle maneuver in the present series. An experimental study also concluded that intermittent clamping is preferable when prolonged periods of vascular inflow occlusion are required during liver resection.[27] In a controlled clinical study of continuous vs. intermittent portal triad clamping,[28] better parenchymal tolerance of intermittent clamping also was reported. Furthermore, safety of the intermittent Pringle maneuver recently was reported in a living donor during liver transplantation.[29]

In this study, the gastrohepatic ligament was not clamped to ensure occlusion of an aberrant left hepatic artery. In addition, not all surrounding ligaments were dissected, because liver resections were limited to one hepatic section or less. The liver is considered to be supplied by collateral vessels in surrounding ligaments more frequently in cirrhosis than in the normal state. These details might account for the lack of difference between the Pringle maneuver and selective clamping in patients with chronic hepatitis or cirrhosis. Furthermore, the liver was perfused by retrograde flow from the hepatic veins.[30] Tatsuma et al.[31] showed that such backflow could maintain liver adenosine triphosphate synthesis and therefore liver viability during the Pringle maneuver. Accordingly, the Pringle maneuver was reported to induce only partial hepatic ischemia.[30]

During limited liver resection, that is, of one hepatic section or less, the Pringle maneuver performed in an intermittent manner did not result in worse clinical outcomes than hemihepatic vascular occlusion. Performing the intermittent Pringle maneuver may induce less ischemic stress and allow better preservation of posthepatectomy liver function, even if chronic hepatitis or cirrhosis is present. Because the intermittent Pringle is often a simpler and less time-consuming technique to apply, it is superior at least in patients with chronic liver disease requiring this extent of resection. Further investigation of a greater number of patients is needed to verify these results.

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Clinical and Pathological Features of Allen's Type C Classification of Resected Combined Hepatocellular and Cholangiocarcinoma: A Comparative Study with Hepatocellular Carcinoma and Cholangiocellular Carcinoma

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The clinical features of Allen's type C of combined hepatocellular and cholangiocarcinoma (cHCC-CC) are not well known. In this study, we aim to define the clinicopathologic features of cHCC-CC and to evaluate the preoperative diagnosis and surgical treatment results in comparison with those of hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC). We retrospectively analyzed 13 patients with cHCC-CC, 509 patients with HCC, and 41 patients with CCC treated in our hospital within past two decades. Viral hepatitis B or C backgrounds were more prominent in HCC and cHCC-CC groups than in the CCC group. Elevated serum alpha-fetoprotein (AFP) levels were found in 60.3% of HCC patients and in 46.2% of cHCC-CC patients. Only one patient of cHCC-CC was correctly diagnosed before surgery. The postoperative survival rates between the cHCC-CC and HCC or the CCC group were not significantly different. Both intrahepatic and extrahepatic postoperative recurrences were frequent in cHCC-CC patients, and CCC component recurrences were more frequently seen. In conclusion, the preoperative diagnosis is difficult; liver masses similar to those of HCC, together with moderately elevated serum AFP and CA19-9 levels, are reliable indicators of cHCC-CC. Surgical resection of this tumor yields results intermediate between those of HCC and CCC in character. More cases are needed to further define the characteristics of this tumor. (J GASTROINTEST SURG 2006;10:987–998)

KEY WORDS: Primary liver cancer, combined hepatocellular and cholangiocarcinoma, diagnosis, prognosis, surgery, tumor recurrence

Primary liver cancer (PLC) is one of the most common cancers in Eastern Asia in regions such as Japan and China. There are three major PLC types in adults, classified according to the histopathologic components of the tumor. The most common PLC type is hepatocellular carcinoma (HCC) that originates from hepatocytes. The second most common type is cholangiocellular carcinoma (CCC), which originates from the intrahepatic bile duct epithelium. The least common type is combined hepatocellular and cholangiocarcinoma (cHCC-CC), representing 0.40% to 14.2% of PLC cancer cases.[1–6] To date, the origins of cHCC-CC remain unclear, although various possibilities have been suggested.[7,8] The World Health Organization defined cHCC-CC as a rare tumor comprised of two groups of malignant cells with the histological features of HCC or CCC. However, this general definition is not discriminating enough to define various histopathologic conditions when elements from these two tumor types occur together. To date, Allen's classification has been widely used in various reports.[2,3,9] This classification includes: type A, double cancer of HCC and CCC, with HCC and CCC present at different sites without contact; type B, HCC and CCC are present at adjacent sites and mingle with

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continued growth; and type C, HCC and CCC are mixed, growing within the same tumor. Type C is also named as mixed hepatocellular and cholangiocellular carcinoma (MHC) by the Liver Cancer Study Group of Japan.[10] Histologically, only type C displays the characteristic of genuine mixture of both HCC and CCC elements, and in many published studies of cHCC-CC, only Allen's type C was included and regarded as true cHCC-CC.[2,3,5,11,12] In the present study, we have focused solely on Allen's type C of cHCC-CC.

The clinical features, therapy modalities, and the prognosis of HCC and CCC have been extensively researched and reported on by various groups. However, cHCC-CC remains relatively uninvestigated, and there is little information to enable useful comparisons of this tumor type with HCC or CCC. Furthermore, results from the few cHCC-CC studies have shown great variability between research groups.[3]

In this study, we summarized the clinicopathological features, surgical management, and results of 13 histologically confirmed cases of resected cHCC-CC. In addition, we compared our data with 550 cases of resected HCC and CCC and defined the clinicopathologic features of cHCC-CC. We also evaluated the preoperative diagnosis and surgical treatment of this rare tumor.

MATERIAL AND METHODS

Patients

From 1982 to 2003, in the Department of Surgery, Osaka University Hospital, 13 patients with resected Allen's type C of cHCC-CC were included in this study. In our series, number of HCC patients is 509, number of CCC patients is 41 and number of cHCC-CC patients is 13. The percentage of cases showed among all 563 patients. HCC patients are 509 of all 563 patients (90.4%) and CCC patients are 41 of all 563 patients (7.3%). Typical hepatocellular differentiation of cHCC-CC was recognized by the following features: (1) trabecular pattern composed of tumor cells forming tissue strands of various thicknesses, separated by sinusoidlike blood spaces lined with a single layer of endothelial cells, (2) abundant eosinophilic cytoplasm and bile production, and (3) intracytoplasmic hyaline globules. The typical cholangiocellular differentiation was recognized by the following features: (1) a tubular and/or papillary structure covered by small cubical to low columnar cells resembling the biliary epithelium, (2) mucin production, and (3) accompanying abundant fibrous stromal. All 13 cases of cHCC-CC (Tables 1 and 2) were confirmed by pathological examination including immunohistochemistry of alpha fetoprotein (AFP) and cytokeratin (CK7) (Figs. 1 and 2). Preoperative diagnosis was based on preoperative laboratory investigations and imaging diagnosis.

Clinicopathologic Features

Preoperative investigation of patient data, including demographics, was collated from computer-based medical records. Hepatitis B or C infection status of each patient was determined by screening for hepatitis B virus surface antigen and hepatitis C antibody. Chronic hepatitis and liver cirrhosis status of each patient was confirmed by a liver biopsy of peripheral nontumor liver tissue. Levels of serum

Table 1. Clinical characteristics of patients with cHCC-CC

Patient's No.	Age (Yr)	Sex	HBsAg	HCVAb	AFP (ng/ml)	PIVKA-II (ng/ml)	CEA (ng/ml)	CA 19-9 (U/ml)
1	65	M	−	ND	8	ND	ND	ND
2	57	M	−	−	<5	4037	ND	ND
3	47	F	+	−	171	<62.5	1	14
4	36	M	+	−	5773	<62.5	ND	ND
5	61	M	−	−	117	359	27	50
6	52	M	+	−	<5	69	ND	1218
7	74	M	−	−	3297	9408	3	37
8	58	M	−	−	6	71	5	<5
9	60	M	−	−	<5	<40	<1	20
10	58	M	−	−	7	162	2	19
11	75	M	−	−	564	1698	2	892
12	47	M	+	−	<5	626	ND	ND
13	50	M	+	−	52	2733	ND	ND

M = male; F = female; HBsAg = hepatitis B surface antigen; HCVAb = hepatitis C virus antibody; AFP = alpha fetoprotein; PIVKA = protein in vitamin K absence; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; ND = note done.

Table 2. Preoperative diagnosis, macroscopic findings, surgical treatment, and outcome of cHCC-CC

Case No.	Tumor size (mm)	Preoperative diagnosis	Macroscopic differentiation	Segment resected[†]	Additional surgery	Recurrent time (mo)	Recurrence (organs)	Recurrent component	Survival (mo)
1	12.5	Liver abscess	Infiltrative	7, 8	Right pulmonary lobectomy	1	Skin	CCC?	13 (dead)
2	5.0	HCC	Simple nodular	6, 7	—	24	LN, bone, sacrum	ND	61 (dead)
3[‡]	5.6	HCC	Infiltrative	6, 7, 5*	Hilar LN dissection	15	Liver	CCC	135 (alive)
4	4.5	HCC	Infiltrative	6, 7, 8	—	2	Pleura	CCC?	2 (dead)
5	9.0	CCC	Infiltrative	2, 3, 4	Hilar LN dissection	2	bone, lung	CCC	7 (dead)
6	6.9	HCC	Infiltrative	5, 8, 4*	—	8	Liver	CCC	21 (dead)
7	6.8	HCC	Confluent multiple nodular	6, 6*, 8*	—	6	Liver, bone	HCC	28 (dead)
8	4.3	HCC	Simple nodular type with extra nodular growth	8	—	56	Liver	CCC	57 (alive)
9	11.5	HCC	Infiltrative	7, 8*	Portal vein thrombi extraction	—	—	—	36 (alive)
10	3.0	HCC	Confluent multiple nodular	6, 5	—	18	Lung, liver	NA	19 (alive)
11	9.4	cHCC-CC	Infiltrative	2, 3, 4, 5, 8	—	—	—	—	14 (dead)[§]
12	3.0	HCC	Simple nodular	6, 7	—	—	—	—	9 (alive)
13	4.2	HCC	Confluent multiple nodular multinodular	2, 3, 5, 8	—	—	—	—	10 (alive)

CCC? = clinically suspected CCC; NA = data not available.
*Partial resection of Couinaud's segment.
[†]Segments are based on Couinaud's classification.
[‡]Re-resection was performed after recurrence.
[§]Patient died of carcinoma of the prostate.

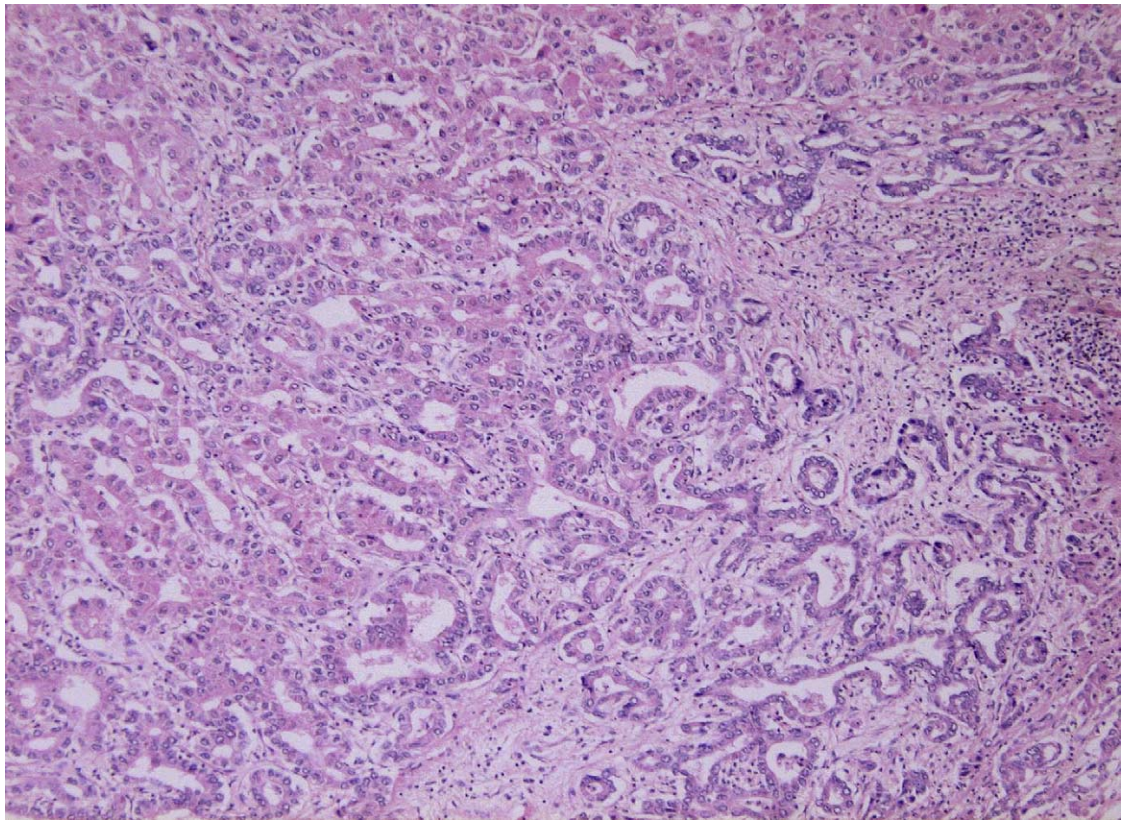

Fig. 1. A representative specimen of histological features of cHCC-CC, Allen's type C. HCC-like area (left), and (right) a CC-like area (magnification ×100).

tumor markers AFP, carcinoembryonic antigen (CEA), and CA19-9 were collected when available. Data were also collected about surgical procedures, including surgical time, bleeding, and additional lymph node dissection. Macroscopic and microscopic features such as lesion diameter, capsule formation, bile duct invasion, vascular involvement, and lymph node metastasis were also collected from the computer-based medical records. Macroscopic classification of cHCC-CC was carried out using criteria of HCC classification by the Liver Cancer Study Group of Japan,[13] including simple nodular type, infiltrative type, confluent multiple nodular type, and simple nodular type with extra nodular growth. Tumor staging was performed according to the Union International Contrele Cancer TNM staging system. Surgical procedures were grouped according to the classification by the Liver Cancer Study Group of Japan,[13] including the following categories: Hr0, resection of less than one Couinaud's segment; HrS, resection of Couinaud's segment; Hr1, anterior, posterior, or lateral segmentectomy; Hr2, right or left lobectomy or central bisegmentectomy; and Hr3, right or left trisegmentectomy. Survival time was calculated from the date of operation to the date of death due to tumor recurrence, or from the last follow-up date.

Immunohistochemical Staining

Formalin-fixed, paraffin-embedded specimens including tumors and peripheral tissue were selected for analysis. Sections measuring 4 μm in thickness were deparaffinized in xylene and rehydrated and stained with hematoxylin-eosin solution for histopathologic examination. After deparaffinization in xylene and rehydration in a graded series of ethanol, immunohistochemistry was performed using a Vectastain ABC peroxidase kit (Vector Labs, Burlingame, CA). Briefly, the sections were treated with an antigen retrieval procedure in 0.01 mmol/L sodium citrate buffer (pH 6.0) for 40 minutes at 95° C and were incubated in methanol containing 0.3% hydrogen peroxide at room temperature for 20 minutes to block endogenous peroxidase. The sections were incubated with normal protein block serum solution (room temperature, 20 minutes) to block nonspecific staining, and then incubated overnight at 4° C with anti-AFP (mouse monoclonal IgG, diluted 1:400; Sigma-Aldrich, Inc., St Louis,

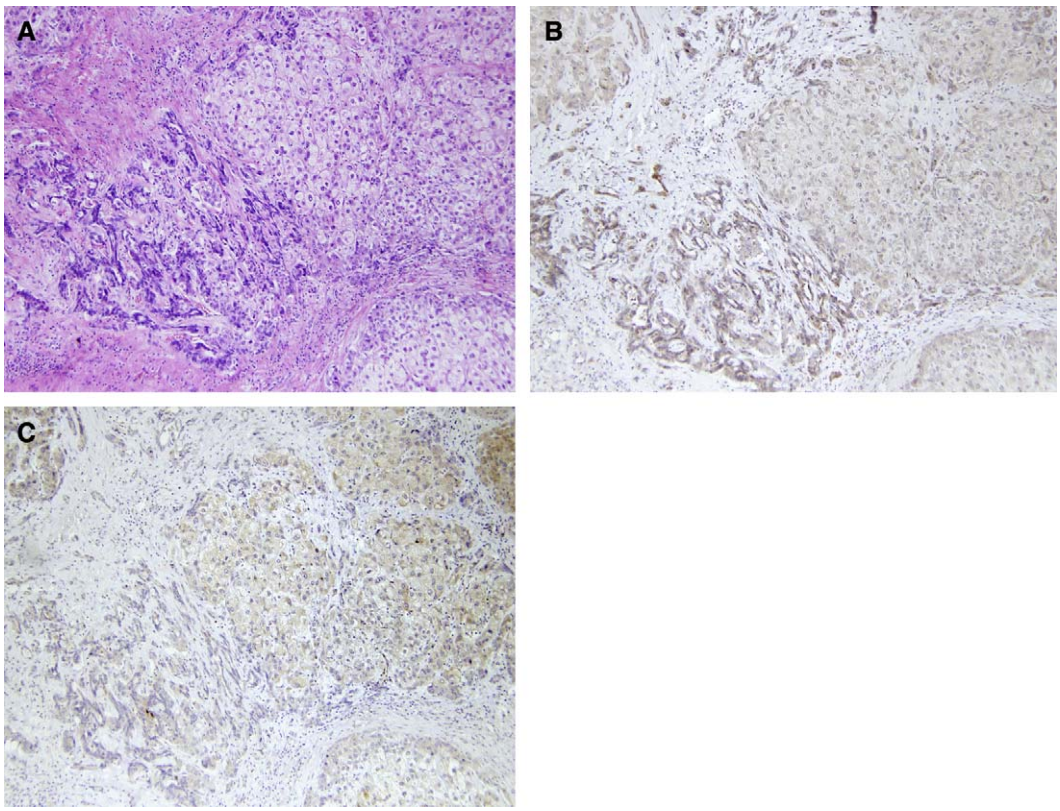

Fig. 2. (**A**) Hematoxylin-eosin stain of one specimen of cHCC-CC. (**B**) In CCC-like area (right), a strong reaction for CK7 is seen. However, in HCC-like area (left), negative reaction is seen. (**C**) In HCC-like area (right), a weak positive reaction for AFP is seen. However, in CCC-like area (left), a negative reaction is seen (magnification ×100).

MO), anti-CK7 (mouse monoclonal IgG, diluted 1:200; Sigma-Aldrich, Inc., St Louis, MO). Sections were washed three times for 5 minutes in phosphate-buffered saline, incubated with a biotin-conjugated secondary antibody (horse antimouse; room temperature, 20 minutes), and finally, incubated with peroxidase conjugated streptavidin (room temperature, 20 minutes). Peroxidase reaction was developed with 3, 3′-diaminobenzidine tetrachloride (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Sections were counterstained with Meyer's hematoxylin. For negative controls, sections were incubated with Tris-buffered saline instead of the primary antibody.

Statistical Analysis

Unless otherwise indicated, numerical data are presented as mean ± SD. Differences in proportions of categorical data were tested by chi-square test. Unless otherwise indicated, differences in mean values of numerical data were tested using a two-tailed Student's *t* test. Survival (mean, medium survival days, and 1-, 3-, and 5-year cumulative survival rates) was assessed using the Kaplan-Meier method, and comparisons were made using the log-rank test. Statistical software used for all assessments was SPSS 11.5 (SPSS, Inc., Chicago, IL).

RESULTS

Patient Demographics and Laboratory Investigations of HCC, CCC, and cHCC-CC

Of the 563 cases of PLC resected, 2.3%, 90.4%, and 7.3% were cHCC-CC, HCC, and CCC, respectively. On average, the age of the patients in each of these three groups was late 50s or early 60s. The sex ratio of cHCC-CC and HCC showed an apparent male predominance, whereas in the CCC group, the male predominance was far less prominent. A positive viral hepatitis B or C status was prominent in the HCC group and noticeable in cHCC-CC group, but not in the CCC group. Serum AFP levels were more frequently elevated and tended to be higher in the HCC group than in the cHCC-CC group. However, the difference was not statistically significant. Serum CEA and CA19-9 levels were more frequently elevated and tended to be higher in the CCC group than in the cHCC-CC group, but the difference was not statistically significant (Table 3).

Pathological Examinations and pTNM Stages of HCC, CCC, and cHCC-CC

The postoperative pathological examination of the peripheral nontumor liver tissue showed that the majority of cHCC-CC and HCC patients had liver cirrhosis or chronic hepatitis (Table 4). However, in the cHCC-CC group, the incidence of chronic hepatitis was more frequent than liver cirrhosis, whereas in the HCC group, the opposite scenario was noted. Tumor size was largest in the cHCC-CC group, followed by the CCC group, then the HCC group. However, differences in tumor size were not statistically significant. The incidence of microscopic vascular invasion (portal vein or hepatic vein) in cHCC-CC was almost the same as the other two groups. The cHCC-CC and HCC groups both had a low lymph node metastatic rate, whereas the CCC group had more than a 50% possibility of developing lymph node metastasis. This difference in the rate of lymph node metastasis was statistically significant. As for tumor's capsule, there were significantly more "no capsule formation" and "partial formation" in the cHCC-CC group than in the HCC group. Concerning the pTNM stages, we found that in the cHCC-CC and HCC groups, more than half the patients were in the early or middle stages (pTNM 1 or 2 stage), whereas significantly more patients in CCC group were in the advanced stages (pTNM 3 or 4 stage).

Surgical Procedure for HCC, CCC, and cHCC-CC

We combined Hr0 and HrS as one category because Hr0 was seldom performed in the cHCC-CC and CCC groups. Operation times and blood loss are summarized in Table 5 for the hepatic resections performed. We noticed that significantly more Hr2 or Hr3 were performed in the cHCC-CC group (33.4%) and the CCC group (39%) than in the HCC group (21%). There were no perioperative deaths in the cHCC-CC and CCC groups; six (1.2%) patients died in the perioperative stage in the HCC group. In addition to a hepatectomy, patient 1 had local invasion of the right diaphragm and the inferior lobe of the right lung by the tumor of Couinaud's segments 7 and 8. A right pulmonary lobotomy was also performed at the same time as the hepatectomy patient 1. Patient 9 was found to have cancerous thrombi in

Table 3. Demographic and laboratory findings in cHCC-CC, HCC, and CCC patients

Items	Groups of patients			*P* value*
	cHCC-CC (n = 13)	HCC (n = 509)	CCC (n = 41)	
Case number (%)	13 (2.3%)	509 (90.4%)	41 (7.3%)	
Age (yr), (range)	57 ± 10.9 (36–75)	60 ± 9 (29–84)	61.5 ± 9.7 (33–80)	
Sex ratio (M/F)	12/1	4.9/1	1.2/1	2–3 $P = 0.000$
				1–3 $P = 0.019$
Viral hepatitis marker				
HBsAg (+)	38.5% (5/13)	20.3% (101/498)	5% (2/40)	2–3 $P = 0.019$
				1–3 $P = 0.007$
HCVAb (+)	0% (0/12)	61.4% (227/370)	14.3% (5/35)	1–2 $P = 0.000$
				1–3 $P = 0.000$
				2–3 $P = 0.000$
Serum tumor markers				
AFP level (ng/ml) (range)	769 ± 1752 (0–5773)	899 ± 44380 (0–500500)	NA	
<20	53.8% (7/13)	39.7% (201/506)	NA	
20–10000	46.2% (6/13)	51.8% (262/506)	NA	
>10000	0% (0/13)	8.5% (43/506)	NA	
CEA (ng/ml) (range)	5.7 ± 9.5 (0–27)	3 ± 2 (0–12)	12.0 ± 39 (0–245)	2–3 $P = 0.005$
CEA ⩽10	85.7 (6/7)	98% (151/154)	90.2 (37/41)	2–3 $P = 0.037$
CEA >10	14.3 (1/7)	2.0% (3/154)	9.8% (4/41)	
			21126 ± 68594	2–3 $P = 0.000$
CA19-9 (ng/ml) (range)	254 ± 462 (5–1218)	30 ± 104 (0–1160)	(5–330000)	
CA19-9⩽10	88.9% (8/9)	66.7% (84/126)	89.2% (33/37)	2–3 $P = 0.007$
CA19-9>10	11.1% (1/9)	33.3% (42/126)	10.8% (4/37)	

M = male; F = female; HBsAg = hepatitis B surface antigen; HCVAb = hepatitis C virus anitbody; AFP = alpha-fetoprotein; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; NA = data not available.

*1–2 means comparison of cHCC-CC and HCC; 1–3 means comparison of cHCC-CC and CCC; 2–3 means comparison of cHCC-CC and CCC.

Table 4. The pathological features and pTNM stage in cHCC-CC, HCC, and CCC patients

Items	Groups of patients			*P* value*
	cHCC-CC (n = 13)	HCC (n = 509)	CCC (n = 41)	
Liver cirrhosis	23.1% (3/31)	59% (292/495)	NA	1–2 $P = 0.010$
Chronic hepatitis	61.5% (8/13)	34.9% (173/495)	NA	1–2 $P = 0.048$
Hepatitis of cirrhosis	84.6% (11/13)	94.0% (465/495)	NA	
Tumor size, cm (range)	6.5 ± 3.2 (3–12.5)	4.8 ± 3.9 (0.5–2.4)	5.3 ± 3.3 (0.5–16)	
Microscopic vascular invasion	23.1% (3/13)	31.6% (161/509)	36.6% (15/41)	
Bile duct invasion	0%	3.7% (19/509)	NA	
Lymph node metastasis	7.7% (1/13)	1.2% (6/509)	53.7% (22/41)	1–3 $P = 0.004$
				2–3 $P = 0.000$
Capsule formation				
No formation	46.2% (6/13)	20.3% (103/509)	NA	
Partial formation	38.5% (5/13)	9.6% (49/509)	NA	1–2 $P = 0.000$
Complete formation	15.4% (2/13)	70% (356/509)	NA	
pTNM stages				
1 or 2	61.5% (8/13)	58.5% (298/509)	24.4% (10/41)	1–3 $P = 0.013$
3 or 4	38.5% (5/13)	41.5% (211/509)	75.6% (31/41)	2–3 $P = 0.000$

NA = data not available.
*1–2 means comparison of cHCC-CC and HCC; 1–3 means comparison of cHCC-CC and CCC; 2–3 means comparison of HCC-CC and CCC.

both the right branch and trunk of the portal vein. Thrombi extraction was performed. During hepatectomy, patients 3 and 5 were suspected of having liver hilar lymph node metastasis. Additional hilar lymph node dissections were performed, but lymph node metastasis was found only in patient 5.

Survival of HCC, CCC, and cHCC-CC

The mean, median survival time and cumulative 1-, 3-, and 5-year survival of patients in the cHCC-CC, HCC, and CCC groups after hepatic resection are listed in Table 6 and illustrated in Fig. 3. Mean survival time and 1-, 3-, and 5-year cumulative survival rates were highest in the HCC group, followed by the cHCC-CC group, and were lowest in the CCC group. Patients in the cHCC-CC and HCC groups had similar median survival rates. Cumulative survival rates for 1, 3, and 5 years were significantly better in the HCC group than in the CCC group. There was no significant difference in postoperative survival rates between patients from the HCC group and the cHCC-CC group, or from the CCC group and the cHCC-CC group.

Recurrence of cHCC-CC

Up to the date of our investigations, 9 of the 13 cases experienced tumor recurrences (Table 2). This included four cases of extrahepatic recurrences,

Table 5. Surgical procedures in cHCC-CC, HCC, and CCC patients

Items	Group of patients			*P* value*
	cHCC-CC (n = 13)	HCC (n = 509)	CCC (n = 41)	
Hepatic resection				
Hr0 or Hrs	6.7% (1/13)	38.7% (197/509)	7.3% (3/41)	
Hr1	60% (8/13)	40.7% (204/509)	7.3% (3/41)	1–3 $P = 0.000^{\dagger}$
Hr2	26.7% (3/13)	17.5% (89/509)	53.7% (22/41)	2–3 $P = 0.000^{\dagger}$
Hr3	6.7% (1/13)	3.7% (19/509)	31.7% (13/41)	1–2 $P = 0.000^{\ddagger}$
Operation time (min)	397 ± 224	303 ± 147	548 ± 72	2–3 $P = 0.000^{\ddagger}$
				1–3 $P = 0.000^{\ddagger}$
Blood loss (ml)	2722 ± 3310	2196 ± 4085	2211 ± 1464	

*1–2 means comparison of HCC-CC and HCC; 1–3 means comparison of HCC-CC and CCC; 2–3 means comparison of HCC-CC and CCC.
†*P* value was calculated for the difference of the ratio of Hr0, Hr1, Hr2 to Hr3, Hr4 between groups.
‡One-way ANOVA test.

Table 6. Mean median survival time and cumulative survival rates in cHCC-CC, HCC, and CCC patients

Groups	Mean survival	Median survival (days)	1-year survival rate	3-year survival rate	5-year survival rate
cHCC-CC (n = 13)	1710.8	1801	84.6%	50.1%	50.1%
HCC (n = 504)	2453.9	1769	85.3%	66.3%	50.3%
CCC (n = 41)	1034.6	402	51.2	30.0%	25.8%

three cases of remnant liver recurrence, and two cases of both intrahepatic and extrahepatic recurrences. The recurrent sites included five cases of liver, two cases of lumbar bone, two cases of lung, one case of lymph node, one case of bone, one case of pleura, one case of skin, and one case of sacrum. The recurrent components included four cases of CCC, one case of HCC, two cases of clinically suspected CCC components, and two cases of unclear components.

DISCUSSION

cHCC-CC was first described by Wells in 1903.[14] However, the first comprehensive description and classification of this tumor type was reported in 1949.[6] Although various studies on the classification, origin, clinicopathological features, and therapy of cHCC-CC have been published (Table 7), several aspects of these data were inconsistent and often showed disparities. For example, in assessments of the clinicopathological features of cHCC-CC, (including sex ratio, background chronic liver disease, and hilar lymph node involvement), inconsistent and disparate results have been recorded.[2–5,11] Indeed, some studies from Asian regions have reported that, as with HCC, cHCC-CC was commonly seen in patients with backgrounds of liver cirrhosis or viral hepatitis.[2,11] However, this observation was not noted in other studies from Western regions.[3]

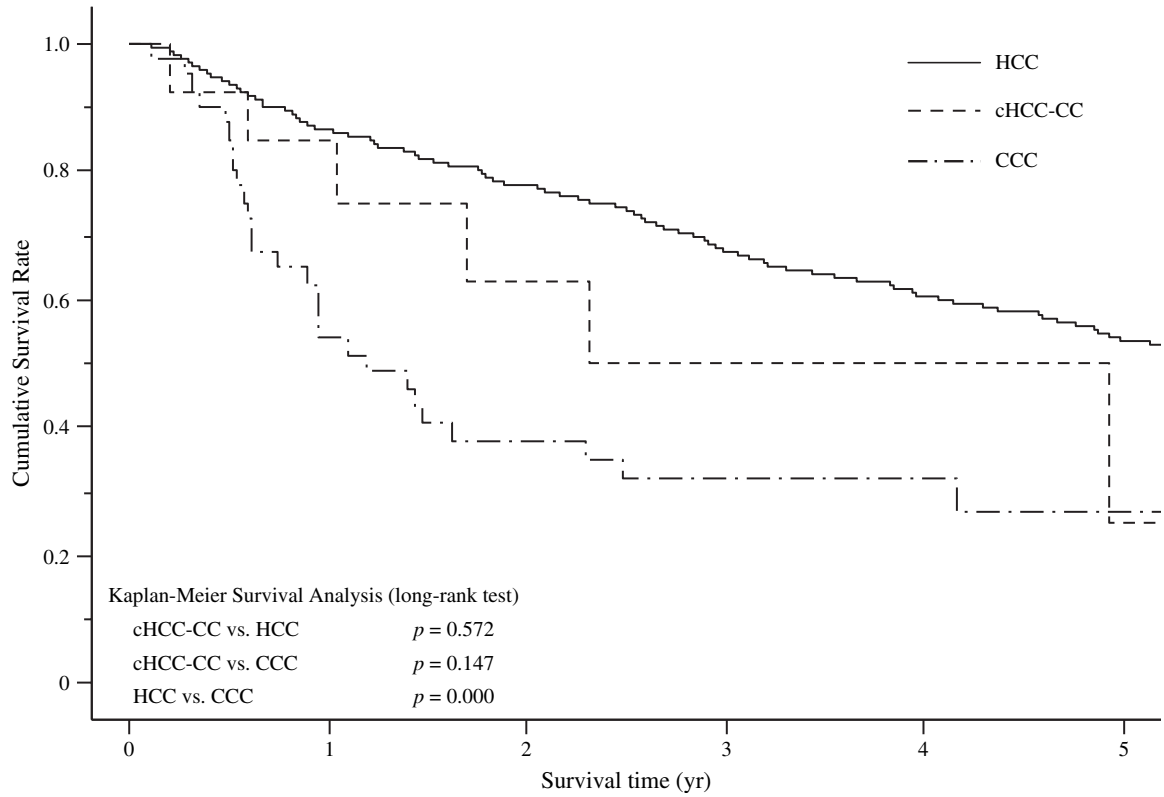

Fig. 3. Comparison of cumulative survival rates among cHCC-CC, HCC, and CCC patients. The HCC group had a significantly better postoperative survival rate than the CCC group. The postoperative survival rates between the cHCC-CC group and the HCC group or the cHCC-CC group and the CCC group were not significantly different.

Table 7. The clinico-pathologic features and postresection survival of a published series of cHCC-CC

Author	Time	Case No	Ratio	Type	M/F	Hepatitis		Mean AFP (ng/ml)	Mean CEA (U/ml)	Mean CA 19-9 (U/ml)	LN	Postoperative survival			
						B	C					Medium	1 year	3 years	5 years
Vano[2]	2003	26	2.4%	C	23/3	27%	38%	75.5†	3.6†	NA	0.76%	23	73%*	34.6	23.1
Liu[16]	2003	12	2%	NS	8/4	58%	0%	19.5	NA	NA	NA	17	58%*	35%	0%
Lee[1]	2002	17	0.4%	NS	15/2	45%	50%	16.8	19.8	172.3	23.5%	NA	NA	NA	NA
Jarnagin[3]	2002	27	3.6%	C	14/13	NA	NA	187	5	NA	NA	31*	78%*	38%*	24%
Sasaki[11]	2001	7	2.3%	C	6/1	57%	75%	32787	2.5	9.5	28.5%	13	NA	NA	NA
Ng[8]	1998	21	2.3%	ABC	18/3	75%	0%	4812.9	NA	NA	22%	7	NA	NA	NA
Nakamura[5]	1996	6	2.8%	C	5/1	50%	0%	350	3.7	58.4	0%	52.5	100%	60%	60%

LN = hepatic hilar lymph mode metastasis; NA = data not available.
*Estimated data.
†Medium value.

In some reports, cHCC-CC had an apparent male predominance,[2,5] whereas other reports did not show this.[3,4] Additionally, problems relating to the recurrence pattern, the necessity of additional hilar lymph node dissection, and the postoperative prognosis are yet to be fully clarified.

It would appear that the biggest obstacle to this research lies in the rarity of this tumor type. The largest published study included 36 cases of all cHCC-CC types, and was done by Maeda and colleagues in 1995.[9] Statistically, the results of small-sized study reports are more easily influenced by "randomized noise" and may not be able to show clearly the distinct features of this disease entity. Another problem is the unavailability of universally accepted diagnosis and classification criteria. This can result in inconsistent diagnosis criteria and selection of cases in these published studies. For example, some published reports regarding only type C of cHCC-CC showed a mixture of two lines of differentiations throughout and included what appeared to be true combined tumors[2,3]; others either included all three types of cHCC-CC[4,8,15] or did not state the criteria for diagnosis, classifications used, or cHCC-CC types included.[1,16] However, pathological diagnosis techniques for cHCC-CC are still being developed. Tickoo et al.[17] showed that in situ hybridization for albumin mRNA techniques could allow a more precise differentiation of hepatocellular and cholangiocellular elements in comparison to results of cytokeratin profile, polyclonal CEA, and AFP. Besides, differences in etiology and disease pattern between eastern regions and western regions might influence these aspects deeply. Fong et al.[18] claimed that, because HCC of Asian regions seems to be associated with a higher rate of concurrent viral hepatitis or chronic cirrhosis when compared with that of western cancer centers, cHCC-CC could also be influenced by such differences.[3] In the present study, we have focused just on the Allen's type C of cHCC-CC and used immunohistochemistry of AFP, CK-7, and periodic acid-Schiff stain (PAS) staining to examine the differentiation and diagnosis of CCC and HCC components.

According to published research, cHCC-CC accounts for 0.40% to 14.2% of all PLC cases (Table 7). Goodman et al.[19] reported frequency of 2.4%, Jarnagin et al.[3] reported frequency of 3.6%, and Allen[6] reported a frequency of 14.2%. The inconsistency of diagnosis criteria and selection of cases in published studies may have resulted in these variations. Besides, some ratios were calculated only from resected clinical samples[2,4] and some were calculated from both resected clinical samples and autopsied or biopsied samples.[9] Differences in

regional/temporal variances and etiology may also play a role. In addition to the cHCC-CC to HCC ratio, the HCC to CCC ratio is also interesting. In Japan, Sharp and colleagues[20] reported a ratio of 6.4 in a population-based follow-up of the extended life span study. Our data showed that the percentages of HCC, CCC, and cHCC-CC in PLC were 90.4%, 7.3%, and 2.3%, respectively.

The clinical pathological features differed from each other in many of the published studies. In our study (Table 1), a prominent male predominance (93.3%) and noticeable viral hepatitis rate (38.5%) were found in cHCC-CC group. These made cHCC-CC, Allen's type C, more similar to HCC than CCC. As for tumor marker, Nakamura reported serum AFP and CA19-9 levels were increased in three of five patients and four of five patients, respectively.[5] Our data showed that elevated AFP and CA19-9 levels were detectable in almost half of cHCC-CC patients. The tumor size in the present studies was generally larger in the cHCC-CC group in comparison to the HCC and CCC groups. This finding was in line with data from several other research groups.[2,3,16] We speculate that the relatively small portion of viral hepatitis rate in cHCC-CC group (30% in cHCC-CC vs. 81.4% in HCC) may result in the major part of patients not being aware of the possibility of liver cancer. This hypothesis is supported by the fact that in our present study, the patients with negative viral hepatitis status had larger tumors than patients positive for viral hepatitis (7.68 vs. 4.84, $P = 0.067$).

The preoperative diagnosis of cHCC-CC appeared to be challenging. Taguchi et al.[4] reported a study of 23 cases of cHCC-CC, among which none could be correctly diagnosed before hepatectomy procedures. In the present study, although preoperative angiography/CT scan were routinely carried out before surgery (data not shown), only one case of a patient with both elevated serum AFP and CA19-9 could be correctly diagnosed. The other 12 cases were misdiagnosed either as HCC (10 cases), CCC (one case), or liver abscess (one case). It seems that imaging diagnosis alone could not differentiate cHCC-CC from HCC. Strategies for improving the preoperative diagnosis of cHCC-CC have been developed. Nakamura et al.[5] hypothesized that a hypervascular tumor with high CEA and CA19-9 levels or a hypovascular tumor with a high level of AFP may indicate a preoperative diagnosis of MHC. In our studies, liver mass similar to that of HCC in CT scans plus moderately elevated serum AFP and CA19-9 were reliable indicators of cHCC-CC. Primary colon or rectum cancer with liver metastasis can also present both elevated serum AFP and CA19-9. However, these possibilities can be easily excluded if a primary lesion is not found.

Surgical procedures in the present study showed that more Hr2 or Hr3 were performed in the cHCC-CC group (33.4%) and the CCC group (85.4%) than in the HCC group (21%). The operation time was longest in the CCC group, followed by the cHCC-CC group, and then the HCC group. The increase in surgical time in the CCC group was largely due to the additional hilar lymph node dissections for CCC patients. Blood loss in the cHCC-CC group was larger than that of HCC and CCC. However, the difference between the groups was not significant due to the small size of the cHCC-CC group. These facts implied that patients in the cHCC-CC group, as a whole, probably underwent more invasive surgical procedures than the HCC group. The necessities of additional hilar lymph node dissection for cHCC-CC are still under debate. Sasaki et al.[11] reported that two in three cases of the multinodular type of cHCC-CC had hilar lymph node metastasis and indicated that additional hilar lymph node dissection should be necessary for this type of cHCC-CC. However, many series did not show a high lymph node metastasis rate or did not mention it.[2,3,16] Nakamura et al.[5] suggested that additional hilar lymph node dissection was unnecessary because of the negative finding of lymph node metastasis at the time of surgery. In the present study, only two cases were suspected to have metastatic carcinoma in hilar lymph node during surgery, so additional hilar lymph node dissection was performed. The results showed one positive and one negative. The low incidence of metastatic hilar lymph nodes in our study (7.7%) does not support the necessity of additional hilar lymph node dissection for cHCC-CC patients, especially in cases of accompanying liver cirrhosis, which inevitably leads to further surgical invasion.

In many studies, the survival of cHCC-CC after surgery was worse than that reported for HCC. Yano et al.[2] reported that cHCC-CC patients had a significantly poorer rate of postoperative survival than patients with either HCC or CCC. Jarnagin et al.[3] also reported a worse survival of cHCC-CC patients in comparison to HCC or CCC patients. However, these differences in survival were not significant. In the present study, the mean and the 1-, 3-, and 5-year survival rates were highest in the HCC group, followed by the cHCC-CC group, and then the CCC group. The HCC group had a significantly better postoperative survival rate than the CCC group. Although cHCC-CC had an intermediate survival rate between that of HCC and CCC in the graph of postoperative survival (Fig. 3 and Table 6),

the difference between the cHCC-CC and the HCC group or the cHCC-CC and the CCC group was not statistically significant due to the small number of patients in the cHCC-CC group.

The recurrence of cHCC-CC has been frequently reported. Yano et al.[2] reported a higher prevalence of intrahepatic recurrence than extrahepatic recurrence, whereas Sasaki et al.[11] reported more prevalence of extrahepatic recurrence than intrahepatic recurrence. Although both extrahepatic and intrahepatic recurrences were frequently seen in the present study, extrahepatic recurrences were more frequent than intrahepatic recurrence. It is well known that HCC can easily develop intrahepatic recurrence after hepatectomy, and local therapeutic approaches such as percutaneous radiofrequency ablation have been proved effective.[21] The extrahepatic recurrence of cHCC-CC might not be suitable for these local therapeutic modalities. Besides, the recurrence components were mainly CCC in this present study; the HCC component was pathologically proven in only one case. These phenomena indicate that the postoperative recurrence pattern of cHCC-CC was more similar to CCC than HCC. These results are in agreement with the report of Uenishi et al.[22] that the CCC component of MHC seems to determine the prognosis, because metastases are usually composed of the CCC elements. Recent trials using combination systemic chemotherapy and neoadjuvant chemoradiation have shown promise for recurrent CCC.[23] Furthermore, we have also reported one case of cHCC-CC of local recurrence, with long-term survival after a combination of reoperation and hepatic arterial infusion chemotherapy.[12] Therefore, systemic chemotherapy might also be helpful for extrahepatic recurrent cHCC-CC.

CONCLUSION

In conclusion, cHCC-CC Allen's type C is a rare type of PLC with clinicopathological features that are more similar to HCC and recurrence patterns that are more similar to those of CCC. The preoperative diagnosis is difficult; however, liver masses similar to those of HCC, together with moderately elevated serum AFP and CA19-9, are reliable cHCC-CC indicators. Surgical resection of this tumor can yield results that are intermediate, between HCC and CCC in characteristics. However, although differences were not significant due to the small number of patients in the cHCC-CC group, both extrahepatic and intrahepatic recurrences can easily occur. More cases are needed to further elucidate the characteristics of this type of tumor.

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Prognostic Significance of the Number of Positive Lymph Nodes in Gallbladder Cancer

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The aim of this study was to assess the prognostic impact of the number of lymph node metastases. The medical records of 33 patients with node-positive gallbladder cancer (GBC) treated at our institution from January 1985 through December 2002 were reviewed. There were 10 cases with a single node metastasis. The sites were as follows: the cystic duct node, the pericholedochal node, the retroportal node, the hilar node, the lymph node around the common hepatic artery, and the paraaortic node. According to the International Union Against Cancer (UICC) 5th edition, 5-year survival rates for the patients with pN1, pN2, and greater than pN2 were 19.2%, 10%, and 0%, respectively (not significant). Patients with a single node metastasis had a higher 5-year survival rate (33%) than patients with two or more lymph node metastases (0%; $P < 0.05$). There were no lymph node recurrences in patients with a single node metastasis. Number of positive nodes and liver metastasis were factors predictive of significantly worse survival. Rather than using the topographic classification, or even simply classifying whether nodal involvement is positive or negative, classification according to the number of positive nodes will contribute to establishing a more practically useful staging system. (J GASTROINTEST SURG 2006;10:999–1007)

KEY WORDS: Gallbladder carcinoma, lymph node metastasis, prognostic factor, adjuvant therapy, sentinel lymph node

Surgical treatment for advanced gallbladder cancer (GBC) has gradually improved. However, a considerable number of patients have suffered postoperative recurrence. Lymph node metastasis was formerly reported as one of the poor prognostic factors.[1–3] It occurs in many cases of advanced gallbladder cancer. Its spread in various directions is one of its peculiar characteristics.[1,4]

Generally, the grade of lymph node metastasis has been classified based on topographic location, which includes the distance between the primary lesion and regional lymph nodes. Regional lymph nodes of GBC were classified into the following two subgroups in the 5th edition of the TNM staging system of the International Union Against Cancer (UICC): N1, metastasis along the hepatoduodenal ligament, and N2, metastasis in the lymph nodes around the pancreas and/or celiac and superior mesenteric artery.[5] In the latest version of the UICC cancer staging manual, grade of lymph node metastasis was unified.[6] Although long-term survivors with positive nodes rarely exist, conditions that influence prognosis with surgical results have not been clarified. Accurate subgrouping systems are important to permit comparisons of treatment results and to predict outcomes.

Recently, the prognostic significance of the number of metastatic lymph nodes in breast, gastric, and colorectal cancer has been emphasized in several reports.[7–9] However, there was no report concerning the prognostic significance of the number of lymph node metastases in gallbladder cancer.

The aim of this study was to assess the survival impact of lymph node metastases based on the number of positive nodes.

MATERIAL AND METHODS

From January 1985 to December 2002, 108 patients with GBC were treated for curative intent at Yokohama City University Hospital. Histological

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examination revealed lymph node metastasis in 51 patients.

In this series, to observe the particular influence of lymph node metastasis on patient survival, patients with any of the following findings were excluded: histologically confirmed positive surgical margins (n = 13), and postoperative mortality (n = 5). The remaining 33 patients were subjects in this study.

We used the following techniques of partial hepatectomy: gallbladder bed resection in 15 patients, medial-inferior and anterior-inferior segmentectomy (S4a + S5) in 12 patients, S4a + S5 and posterior segmentectomy in one patient, extended right hepatectomy with caudate lobectomy in three patients, and right trisegmentectomy with caudate lobectomy in two patients. The extent of liver resection was determined by the extent of direct invasion by the gallbladder cancer. The standard procedure was extended cholecystectomy (cholecystectomy plus partial resection of liver segment 4 and 5 approximately 3 cm from the gallbladder bed) for stage II and III gallbladder cancer. Since 1995, anatomic resection of liver segment 5 and the lower part of segment 4 has been the standard procedure. Right hemihepatectomy was performed for the patients with massive direct liver invasion. When the hepatic hilum was involved, extended right hemihepatectomy with caudate lobectomy and bile duct resection was performed. All patients in this study underwent extended lymphadenectomy that included N1 and N2 of the TNM classification, UICC 5th edition, and paraaortic lymph nodes.[1]

All patients received standardized follow-up examinations that included laboratory tests at 1- to 2-month intervals, chest radiographs at 3-month intervals, abdominal computed tomography at 3-month intervals, and ultrasonography of the liver at 3-month intervals. The outcome of patients was investigated, and only those who clearly died from recurrent gallbladder carcinoma were regarded as having tumor-related death.

Resected specimens and lymph nodes were immediately fixed in 10% buffered formaldehyde solution. Five-millimeter multiple step sections of the resected specimens were embedded in paraffin and stained with hematoxylin-eosin. Maps of distribution of the metastatic lymph nodes were made for each case. Microscopic liver metastasis was investigated as described previously.[10]

The extent of disease was described mainly by using the TNM classification system of the UICC, except in the case of extraluminal, parenchymal, cancerous infiltration of the hepatoduodenal ligament, in which case the *General Rules for Surgical and Pathological Studies on Cancer of the Biliary Tract*[11,12] were used.

Regarding the classification of lymph node metastases, the lymph nodes around the abdominal aorta were treated as distant metastasis in TNM staging. On the other hand, the *General Rules for Surgical and Pathological Studies on Cancer of the Biliary Tract* defined these lymph nodes as regional lymph nodes. In this study, these nodes were treated as regional lymph nodes.

Statistical Analysis

Chi-square (Fisher exact probability) analysis was used for comparison of the two groups. The cumulative survival rate was evaluated by the Kaplan-Meier method, and significance was assessed by the log-rank test. Multivariate analysis using Cox's proportional hazard model was used to estimate the significance of the prognostic factors. Program SPSS 6.1J (SPSS Inc., Chicago, IL) software for Windows was used for statistical analysis. Only the valuables having a P value of less than 0.1 in the univariate analyses were entered into the Cox regression model in a backward stepwise regression. A P value < 0.05 was considered statistically significant.

RESULTS

Nodal Status

The nodal status in patients was as follows: 14 patients with pT2, 9 with pT3, and 10 with pT4. The average number of regional lymph nodes examined per patient was 29.5 ± 5.2, range, 13–55. The rates of nodal metastasis were 52%, 88.9%, and 61.5% in pT2, pT3, and pT4, respectively. The number of metastatic lymph nodes varied from 1–43. The mean numbers of metastatic lymph nodes were 4.2, 9.0, and 5.9 in pT2, pT3, and pT4 patients, respectively. The incidence of cancer metastasis for each lymphatic station is shown in Table 1. The most commonly involved lymph nodes were the lymph nodes around the common bile duct (36.4%), the posterior pancreaticoduodenal lymph nodes (21.8%), the cystic lymph nodes (21.8%), the retroportal lymph nodes (16.4%), and the paraaortic lymph nodes (16.4%). The frequencies of lymph node metastasis among pN1 or pN2 were not uniform. There were some nodes that belonged to the pN1 and pN2 stations, but these were involved less than the paraaortic lymph nodes.

There were 10 cases with a single node metastasis. The sites were as follows: the cystic duct node, the pericholedochal node, the retroportal node, the hilar node, the lymph node around the common hepatic artery, and the paraaortic node (Fig. 1). These were broadly distributed from N1 to greater than

Table 1. Frequency of lymph node metastasis in patients with gallbladder carcinoma

Station	N stage (UICC 5th ed.)	Incidence of nodal metastasis (%)
Cystic duct node	1	21.8
Pericholedochal node	1	36.4
LN around the PHA	1	9.1
Retroportal node	1	16.4
Hilar node	1	5.5
LN around the CHA	2	14.5
Posterior pancreaticoduodenal node	2	21.8
LN around the coeliac artery	2	3.6
LN around the SMA	2	0.0
Paraaortic node	>2	16.4

LN = lymph node; CHA = the common hepatic artery; SMA = the superior mesenteric artery; PHA = proper hepatic artery.

N2 according to the UICC 5th edition classification. All cases with multiple nodal metastases had at least one of these six lymph nodes.

Morbidity and Mortality

During this period, 77 patients with GBC that invaded the subserosal layer or deeper underwent resection with a curative intent. Thirty-two of 77 (41.6%) patients suffered from postoperative complications, including five deaths (Table 2). All deaths occurred in patients who had the hepatoduodenal ligament invasion with multiple lymph node metastases. Four of five patients had lymph node metastases in the paraaortic region. Regarding the operative procedures, two patients underwent extended right hemihepatectomy with caudate lobectomy. Another three patients underwent S4-S5 resection. Three patients underwent portal vein reconstruction.

Four of five patients had bleeding from the right hepatic artery or the arteries around the head of the pancreas. Transcatheter arterial embolization (TAE) was performed to rescue the patients from hemorrhagic shock. However, patients fell into fatal liver failure along with multiple organ failure.

Survival Rate

The 5-year survival rates for patients staged pN1, pN2, and more distant lymph node metastases were 19.2%, 10.0%, and 0%, respectively (Fig. 2). There were no significant differences between pN1 and pN2.

The relationship between lymph node metastasis and survival is given in Table 3. The 5- and 10-year survival rates were excellent in node-negative patients. The survival rate decreased in node-positive patients, especially when the number of metastatic nodes exceeds one.

The 5-year survival rates and the median survival for patients with a single positive node and two or

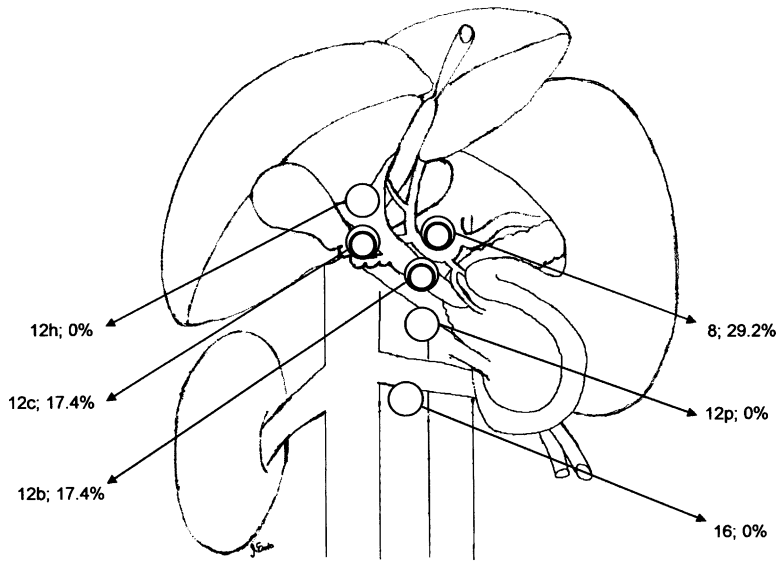

Fig. 1. The sites of a single node metastasis were as follows: 12c, the cystic duct node; 12b, the pericholedochal node; 12p, the retroportal node; 12h, the hilar node; 8, the lymph node around the common hepatic artery; and 16, the paraaortic node. The cumulative survival of patients with particular nodes was not equivalent. Patients who had a nodal metastasis in 12b, 12c, or 8 survived over 5 years.

Table 2. Relation between lymph node metastasis and cumulative survival fo patients with gallbladder cancer

Lymph node metastasis	No. of patients	Survival rate %			MST (mo)
		3 yr	5 yr	10 yr	
Negative	22	76.9	76.9	69.2	
Positive					
1 node	10	55.6	33.3	33.3	48
2–3 nodes	7	19.1	0	0	22
4–5 nodes	7	28.6	0	0	18
⩾6 nodes	9	13.3	0	0	15

MST = median survival time.

more positive lymph nodes were 33.3% and 48 months, and 0% and 18 months, respectively (Fig. 3). There was a statistical significance between these two groups ($P = 0.0301$). To observe the particular influence of lymph node metastasis on patients' survival, patients with microscopic liver metastasis were excluded. Then, the 5-year survival rates of patients with a single nodal metastasis and 2 or more nodal metastasis were 60.0% and 0% (the median survival time was 22 months), respectively. There was a statistical significance between these two groups ($P = 0.0051$).

Only the patients who have a positive node in the cystic nodes, the pericholedochal nodes, or the lymph node around the common hepatic artery survived over 5 years among the patients with a single node metastasis (Fig. 1).

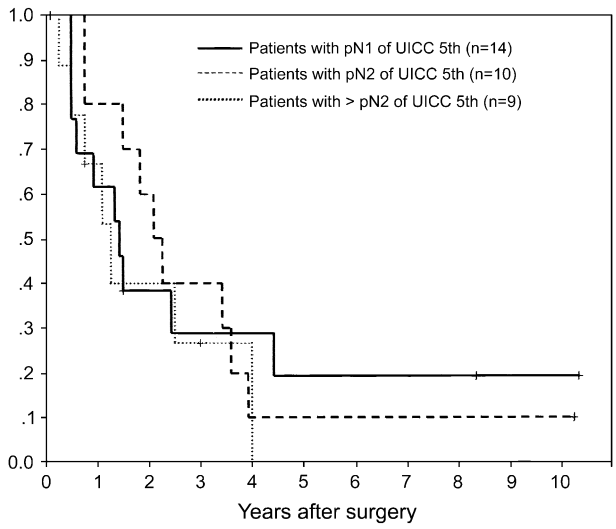

Fig. 2. Cumulative survival according to pN grade of UICC 5th edition classification. There were no statistically significant differences between the pN1 group and the pN2 group.

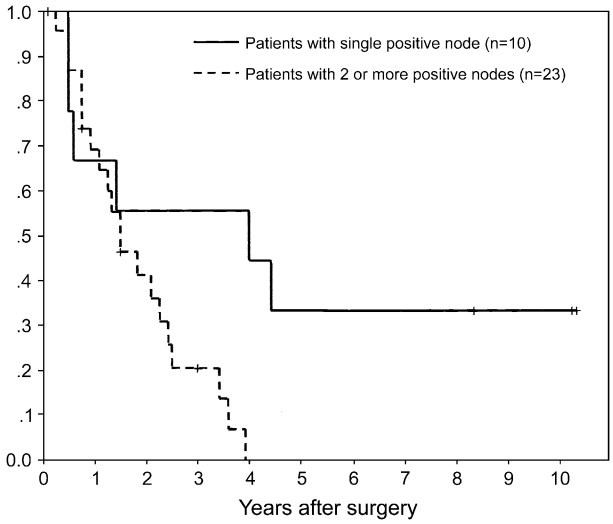

Fig. 3. Cumulative survival of GBC patients with lymph node metastasis after surgery, stratified by the number of positive nodes. There was a statistically significant difference between the patients with a single positive node and the patients with two or more positive lymph nodes ($P < 0.05$).

Mode of Recurrence

Recurrence was observed in 30 patients, with the main sites being systemic lymph node metastasis in 16, the remnant liver in 12, and peritoneum in 7. There was no lymph node recurrence in the patients with a single node metastasis (Table 4). On the other hand, 13 of 21 patients with multiple nodal metastases had lymph node recurrence beyond the scope of lymph node dissection, such as the retrocaval area, left side of the celiac artery, and the supraclavicular lymph nodes. On the other hand, regarding the UICC 5th edition classification, 2 of 14 patients with pN1 had lymph node recurrence. Eleven out of 19 patients with pN2 and more distant nodal metastasis had lymph node recurrence. There was no statistically significant difference between these two groups in frequency of lymph node recurrence.

Univariate Analysis

The log-rank test for Kaplan-Meyer analysis was performed to investigate the clinicopathologic factors that influenced patient survival. Three of the following 15 factors showed a statistically significant difference (Table 5): age, gender, size of the tumor, direct liver invasion, serosal invasion, hepatoduodenal ligament invasion, liver metastasis including microscopic metastasis, vascular invasion, pN of the UICC 5th edition classification, number of positive nodes, tumor differentiation, lymphatic invasion, small vessel invasion, perineural invasion, and

Table 3. Cause of surgery-related death in patients with lymph node metastasis

Patient	Age/sex	Stage	No. of LNM	Surgical procedure	Cause of operative death
1	63/M	T4N1M0	6	Ext. right + caud + PV	Bleeding, liver failure
2	60/M	T4N1M0	5	Ext. right + caud	Bleeding, liver failure
3	75/M	T4N1M0	6	S4aS5S6 + PD + PV + Distal gastrectomy	Bleeding, liver failure
4	69/M	T4N1M0	19	S4aS5 + PD + PV	Leakage of C/J, pneumonia, MOF
5	67/F	T4N1M0	24	S4aS5 + PD	Bleeding, MOF

LNM = lymph node metastasis; caud = caudate lobectomy; PV = portal vein resection; PD = pancreatoduodenectomy; S4aS5 = medial-inferior and anterior-inferior segmentectomy; C/J = choledochojejunostomy; MOF = multiple organ failure; S4aS5S6 = medical inferior, anterior-inferior and posterior-inferior segmentectomy.

adjuvant chemotherapy. Concerning lymph node metastases, classification by TNM staging of the UICC, 5th edition, does not reveal statistically significant differences.

Multivariate Analysis

Cox proportional hazard regression analysis was performed to determine the factors that affected outcome. Three valuables having a *P* value of less than 0.1 in the univariate analyses were entered into the Cox regression model in a backward stepwise regression. Liver metastasis, including microscopic metastasis and number of positive nodes, was retained as an independent prognostic factor (Table 6).

DISCUSSION

Lymph node metastasis frequently occurs in advanced gallbladder cancer. The dissection of regional lymph nodes is therefore recommended for patients with pT2 or greater advanced T-grade.[13–15] However, the prognosis of patients with lymph node metastasis is still poor. Lymph node metastasis was formerly reported as one of the prognostic factors.[16–18] Some surgeons were doubtful whether the prophylactic lymph node dissection is beneficial, because there are many cases that recurred after radical operations for advanced GBC with lymph node metastasis.[16,17] On the contrary, lymph node dissection is thought to have a beneficial effect for selected patients, because there is a small number of long-term survivors with lymph node metastasis.[19–21] We also reported that extended lymph node dissection improved outcomes in advanced gallbladder cancer.[14] However, it is unclear which subgroup will provide potential candidates for extended lymph node dissection by the UICC staging methods. In the UICC 5th edition classification, regional lymph nodes were classified into two subgroups according to their topographic locations. However, the incidence of nodal involvement in some of the N2 subgroups was higher than those of the N1 subgroup. Furthermore, there was no significant difference in survival rates between the two subgroups. Thus, in the UICC 6th edition, lymph node metastasis is simply classified as whether nodal involvement is positive or negative. Because the overall survival rate of patients with lymph node metastasis is surely low, this staging system seems reasonable. However, this staging system cannot provide the information that would predict which patients are expected to be long-term survivors. It would be useful to be able to clarify which patients would benefit from extended lymph node dissection to avoid futile procedures. It may also provide an indication for adjuvant therapy.

Table 4. Mode of recurrence according to lymph node status

	pN of UICC, 5th ed.				Number of positive nodes	
Mode of recurrence	0 (n = 22)	1 (n = 14)	2 (n = 10)	>2 (n = 9)	1 (n = 10)	>2 (n = 23)
Lymphatic	1	2	5	7	0	14
Local	0	1	1	0	0	2
Liver	3	8	2	2	5	7
Lung	0	1	2	1	2	2
Brain	0	0	1	1	1	1
Bone	0	1	0	0	1	0
Peritoneum	1	3	4	0	2	5

Table 5. Univariate analysis of various dinicopathological factors in patients with node-positive gallbladder cancer

Variables	No. of patients	Median survival (mo)	Survival rate (%)			*P* value
			1 yr	3 yr	5 yr	
Gender						
Male	6	16	66.7	16.7	0	0.2731
Female	27	25	72.3	36.9	15.8	
Age (yr)						
<70	22	25	77.3	33.8	5.6	0.7626
>71	11	13	55.6	33.3	33.3	
Size of tumor (mm)						
<30	8	17	75.0	50.0	33.3	0.2453
>31	25	22	69.7	27.9	5.6	
Direct liver invasion						
(−)	17	18	75.0	42.9	25.7	0.1103
(+)	16	22	67.3	22.4	0	
Serosal invasion						
(−)	28	27	77.6	37.6	14.1	0.0213
(+)	5	9	26.7	0	0	
Hepatoduodenal ligament invasion						
(−)	28	22	70.2	38.0	14.3	0.2601
(+)	5	25	80.0	0	0	
Liver metastasis (induding microscopic)						
(−)	25	25	82.9	39.9	17.1	0.0146
(+)	8	9	37.5	12.5	0	
Vascular invasion						
(−)	29	22	74.8	36.3	13.6	0.1566
(+)	4	9	37.5	0	0	
pN of UICC (5th ed.)						
1	14	17	61.5	28.9	19.2	0.7919
2	10	25	80.0	40.0	10.0	
>2	9	15	66.7	26.7	0	
Number of positive nodes						
1	10	48	66.7	55.6	33.3	0.0301
≥2	23	18	69.3	20.5	0	
Tumor differentiation						
Pap + tub 1	16	16	62.5	18.8	6.3	0.1186
Mod + por	17	30	79.8	49.7	19.9	
Lymphatic invasion						
0	14	27	77.4	30.1	10.0	0.8158
1	19	16	66.7	33.3	13.3	
Small vessel invasion						
0	18	27	88.9	32.9	19.8	0.1686
1	15	11	47.1	31.4	0	
Perineural invasion						
0	21	27	74.7	41.5	17.8	0.1948
1	12	15	64.8	18.5	0	
Adjuvant chemotherapy						
No	25	18	65.6	28.9	9.6	0.3830
Yes	8	27	87.5	43.8	21.9	

Pap = papillary differentiated; tab1 = well differentiated; Mod = moderately; Por = poorly differentiated.

In this study, we focused our attention on the number of positive nodes, because it has been introduced into several staging systems for gastrointestinal malignancies. Our results revealed that the prognosis of patients with a single node metastasis was fairly favorable, even if the patient had a pN2 lymph node metastasis. It has been shown that the modes of recurrence in patients with one positive

Table 6. Multivariate analysis of relative survival using the number of positive nodes

	Hazard ratio	*P* value
Liver metastasis	2.843 (1.178–6.864)	0.020
Number of positive nodes	3.786 (1.167–12.284)	0.027

node did not differ from patients without nodal involvement. Prognosis of these patients tends to be influenced by liver metastasis. On the other hand, out of 23 patients with multiple nodal metastases, 14 patients had lymph node recurrence, 7 patients had liver metastases, and 5 patients had peritoneal recurrence. It suggests that long-term survival would hardly be expected in patients showing two or more positive nodes. From the viewpoint of prognosis, patients with two or more positive lymph nodes were indicated to have generalized disease. It is obvious that these patients cannot survive by extended resection alone.

Regarding the high mortality rate of these patients who underwent extended hepatectomy with portal vein resection and/or pancreatoduodenectomy, indications for curative resection should be reconsidered. For the curative intent, arteries around the hepatoduodenal ligament and the head of the pancreas were skeletonized due to complete clearance of the lymphatic tissues. In such instances, arterial bleeding from ruptured pseudoaneurysm may occur. Not only our present data but also that of Kondo et al.[22] reported high mortality rates in extensive surgery for stage III or IV disease. They also reported that an extended right hepatic lobectomy in a cholestatic liver and portal vein resection strongly correlated with in-hospital mortality.[23] Thus, such extensive resection for node-positive disease might be considered only in patients with single lymph node metastasis. When two or more lymph node metastases were detected before or during the operation, a life-threatening operation should be avoided for curative intent. If postoperative histological findings indicate that there are two or more lymph nodes involved by the tumor, patients should be informed and consideration given to systemic chemotherapy. Thus, the total number of positive nodes provided worthwhile information about patients' prognoses.

Should extended lymph node dissection be applied routinely for all patients, though the few patients who have a single node metastasis received benefit from this procedure? Presently, sentinel node navigation surgery has been adopted in surgery for breast cancer, malignant melanoma, and other tumors.[24,25] However, the sentinel lymph node concept for GBC is rarely reported. Our study indicates that the cystic node, pericholedochal node, retroportal node, hilar node, lymph nodes around the common hepatic artery, and the paraaortic node are considered the first stations of lymphatic metastases. There was no evidence of lymph node recurrence in patients with a single node metastasis. Furthermore, patients with nodal metastasis in some of the 11 stations always had nodal metastasis in at least one of the six nodes considered the first stations. We consider these six nodes as possible sentinel lymph nodes of gallbladder cancer, whereas the other nodes may be the secondary or subsequent stations. These six nodes varied widely from the hepatoduodenal ligament to the paraaortic region. If reliable sentinel lymph node (SLN) detection methods are established, sentinel node navigation surgery could be applied to patients who were diagnosed without lymph node metastasis. When these sentinel nodes show no metastasis, lymph node dissection can be omitted for patients without lymph node metastases. On the other hand, lymph node dissection should be radically performed for patients with a single node metastasis, because these patients are expected to be cured by surgical resection. However, it is technically and temporally difficult to find and examine six nodes during the operation to prove no nodal involvement. Thus, en bloc resection of regional lymph nodes N1 and N2 in the UICC 5th edition classification should be performed as the second best procedure to resect all possible sentinel lymph nodes. Future detecting methods for sentinel lymph nodes by using dye or radioisotopes are expected to be developed.

Concerning the paraaortic lymph nodes, we have only one patient who had a single node metastasis in this region. She died of multiple lung and brain metastases 4 years after the operation. Median survival of patients with paraaortic node metastasis was 15 months in this study. Kondo et al.[19] also reported paraaortic lymph node dissection provides no survival benefit to the patients with paraaortic disease. Thus, paraaortic node dissection should be omitted.

Would extensive surgery be the best treatment for patients with two or more positive nodes? Some authors suggested that pancreatoduodenectomy is beneficial for the patients with lymph node metastasis around the head of the pancreas[26–28] due to the complete dissection of the soft tissue around the pancreas. However, actual survivors over 5 years are still rare. Araida et al.[28] reported that the 5-year survival rate after hepatopancreatoduodenectomy (HPD) in patients with positive lymph node metastases without hepatoduodenal ligament invasion was significantly better than that after non-HPD resections (87% vs 17%). The number of lymph node

metastases in the long-term survivors was not described in their reports. There is still room for argument about indication of HPD for GBC based on the number of lymph node metastases.

For the patients with multiple nodal metastases, adjuvant chemotherapy might be considered, even though positive nodes are only detected in the hepatoduodenal ligament region. Although there is no established regimen for lymph node metastases, we have observed a few cases that showed a remarkable effect by combined chemotherapy of cisplatin, mitomycin C, etoposide, and UFT.[29] Recently, newer regimens using gemcitabine were reported as promising regimens for unresected gallbladder cancer.[30,31] Some investigators reported that gemcitabine therapy was effective adjuvant treatment in pancreatic cancer.[32,33] This agent might have a favorable effect even on adjuvant setting for gallbladder cancer. Thus, we expect that long-term outcomes after resection of GBC will gradually improve in the future with the combination of an effective adjuvant therapy and a highly selected indication with extended surgery.

CONCLUSION

Patients with a single node metastasis are expected to be cured by resection alone, whereas extended lymph node dissection does not provide the same anticipation for patients with multiple nodal metastases. Rather than using the topographic classification, or even simply classifying whether nodal involvement is positive or negative, our subgrouping of positive nodes will contribute to the establishment of a more practically useful staging system.

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Early Warning Scores Predict Outcome in Acute Pancreatitis

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The Early Warning Score (EWS) is a widely used general scoring system to monitor patient progress with a varying score of 0–20 in critically unwell patients. This study evaluated the EWS system compared with other established scoring systems in patients with acute pancreatitis. EWS scores were compared with APACHE scores, Imrie scores, computed tomography grading scores, and Ranson criteria for 110 admissions with acute pancreatitis. A favorable outcome was considered to be survival without intensive therapy unit admission or surgery. Nonsurvivors, necrosectomy, and critical care admission were considered adverse outcomes. EWS was the best predictor of adverse outcome in the first 24 hours of admission (receiver operating curve, 0.768). The most accurate predictor of mortality overall was EWS on day 3 of admission (receiver operating curve, 0.920). EWS correlated with duration of intensive therapy unit stay and number of ventilated days ($P < 0.05$) and selected those who went on to develop pancreas-specific complications such as pseudocyst or ascites. EWS of 3 or above is an indicator of adverse outcome in patients with acute pancreatitis. EWS can accurately and reliably select both patients with severe acute pancreatitis and those at risk of local complications. (J GASTROINTEST SURG 2006;10:1008–1015)

KEY WORDS: Acute pancreatitis, early warning scores, prognosis

Acute pancreatitis affects up to 38 per 100,000 population per year.[1] For most patients, pancreatitis is a mild and self-limiting illness; however, in around 20–30% of cases, severe life-threatening complications may ensue with the development of associated organ dysfunction.[2,3] The reliable identification of these patients would be useful in selecting individuals requiring critical care support and could be used to compare patient outcome in the context of clinical trials or audit.[4–6]

An ideal prognostic index should be able to identify severe cases of acute pancreatitis within 2–3 days of the onset of symptoms. Such a test should be sensitive, with a high negative predictive value, to avoid "missing" cases of severe pancreatitis. To this end, several clinical scoring systems have been adopted including Ranson, Imrie, and APACHE II. Isolated serum markers have been evaluated such as C-reactive protein (CRP), serum glucose, and interleukin (IL) 6.[5] Finally, attempts have been made to correlate radiological findings, usually on computed tomography (CT), with outcome, such as the Balthazar index.[7] While most prognostic evaluating systems have their relative merits, they all have disadvantages, ranging from being cumbersome in their application and calculation, to difficulty in regularly repeating them to monitor an individual patients' progress.

In the United Kingdom, the Early Warning Score (EWS) are commonly used in the assessment of unwell hospital patients. The EWS is a simple physiological scoring system that can be measured at the patient's bedside.[8] The EWS is calculated from six simple physiological parameters: blood pressure, urine output, respiratory rate, pulse rate, and conscious level. Derangement in these scores is assigned a number, and the sum of these numbers is used to calculate an overall EWS (Table 1). These scores can be calculated at an hourly rate for close monitoring of patients. Within Leicester, EWS is routinely used to monitor patients with acute pancreatitis. There are still comparatively few data validating the use of EWS, but its use has been encouraged by several professional bodies.[9–11] Although EWS does not measure pancreas-specific variables, we hypothesized that it would be an accurate measure of the SIRS response in acute pancreatitis. This study attempted to determine if EWS could used to identify patients with

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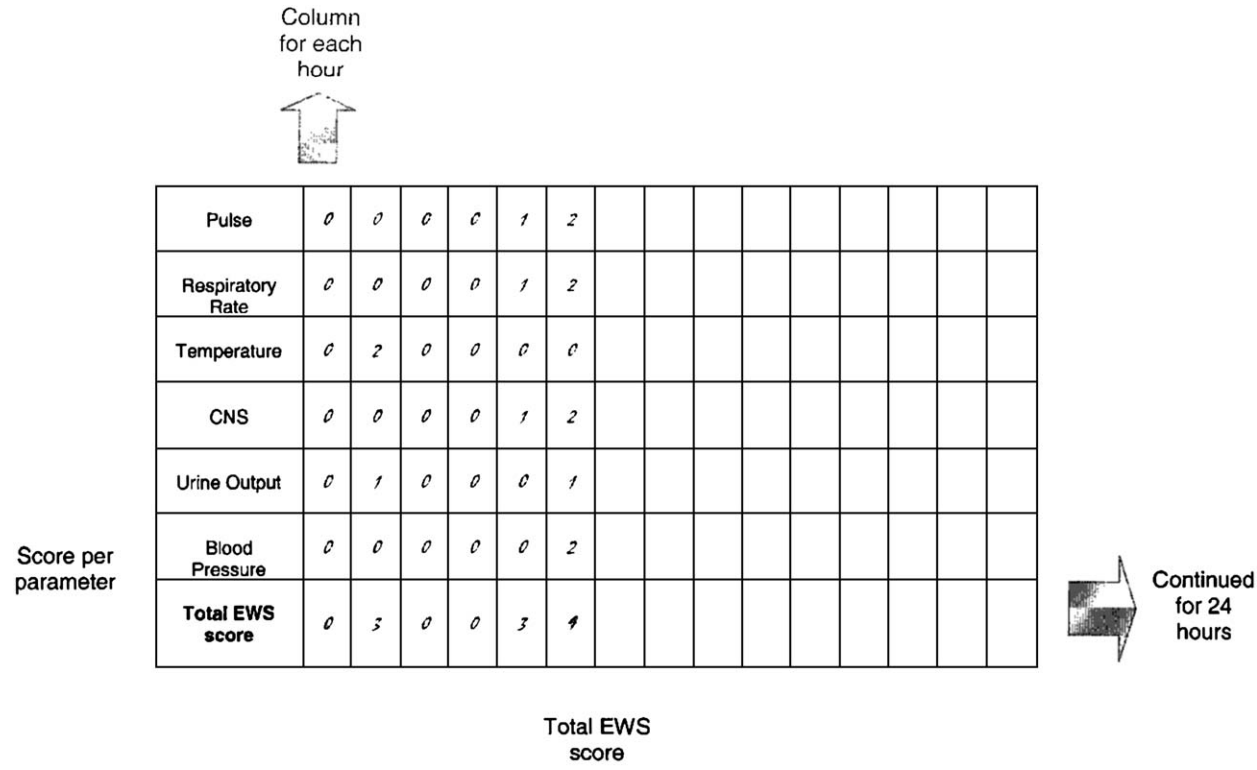
Table 1. Calculation of final EWS



severe pancreatitis and if any correlation of EWS could be made with outcome or mortality.

METHODS

The case-notes of 110 patients admitted with a coding diagnosis of acute pancreatitis were identified from computerized records from 2002 to the present. Diagnostic criteria for acute pancreatitis were serum amylase three times the limit of normal, in patients with upper abdominal pain. All patients presenting with these criteria were included in the study. This cohort of patients was subsequently divided into those with a favorable or adverse outcome. A favorable outcome was defined as patients surviving their episode of pancreatitis without ITU/HDU (intensive therapy unit/high dependency unit) admission or necrosectomy. An adverse outcome was defined as nonsurvivors, admission to HDU or ITU, or requiring necrosectomy. Patients were also classified into severe and nonsevere acute pancreatitis according to the Atlanta classification, for further analyses.[12]

Patient age, etiology of pancreatitis, APACHE scores, EWS, ASA grade, Ranson score, Imrie score, CT grades (Balthazar grading index),[13] and CRP levels from the time of admission were all collected and compared. The worst values within a 24-hour period for each physiologic scoring system were compared on days 1, 2, and 3 of admission. In addition, the final scores recorded in the case-notes prior to death, surgical intervention, or cessation of monitoring due to patient improvement were also compared.

Long-term complications secondary to pancreatitis were also recorded. Patients requiring admission to a critical care bed were identified, and the number of ventilated days, number of days requiring inotrope support, and number of days requiring renal support were recorded.

Statistical analysis was undertaken using the two-tailed Student's *t*-test, Mann-Whitney test, and Spearman rank correlation where appropriate. Receiver operator characteristics (ROC) analysis was applied as a measure of the overall accuracy of individual markers. The software used was GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA; www.graphpad.com).

RESULTS

Eighty-nine patients were classified as having had a favorable outcome (survivors with no admission to critical care and no surgery). Twenty-one patients were designated as having had an adverse outcome; these included survivors who required necrosectomy

(n = 5), survivors requiring critical care admission (n = 9), nonsurvivors without surgery (n = 5), and nonsurvivors following surgery (n = 2). The median age in the poor outcome group was significantly higher than that in the favorable outcome group (66 years and 55 years, respectively); however, other demographic parameters such as ASA grade and underlying etiology were equal.

Fifteen of the patients in the adverse outcome (71.4%) group underwent CT scanning, compared with only 4 (4.5%) in the favorable outcome group, within the first 3 days of admission. Hence, no meaningful comparison of CT grading could be undertaken, and the data are not shown. Likewise, there was no difference in CRP levels between groups, and these data are also omitted. APACHE II scores were significantly higher in the adverse outcome group within the first 24 hours of admission, but this difference was not maintained. Ranson scores were also higher in the adverse outcome group (3 versus 2 respectively) (Table 2). In contrast, EWS was consistently higher throughout the hospital stay in patients with an adverse outcome, and this difference was highly statistically significant.

Comparison of the scoring systems between survivors and nonsurvivors again showed that median EWS was consistently significantly higher in the nonsurvivors (2 versus 5, respectively).

EWS within the first 24 hours of admission was significantly higher among survivors who required admission to critical care compared with survivors who did not require admission to high dependency beds (4 versus 2, respectively). In addition, EWS recorded prior to transfer to critical care correlated with both the duration of ventilation ($P = 0.0013$, Spearman rank correlation) and total duration of critical care stay ($P = 0.0384$, Spearman rank correlation). Among all the survivors as one group, EWS was also found to strongly correlate with the total duration of hospital stay ($P = 0.0012$, Spearman rank correlation).

EWS scores were also compared among the survivors who had not undergone surgery to see if there was any relationship between EWS and the development of long-term pancreatic complications (Table 3). The data demonstrate that EWS of 4 or above within the first 24 hours of admission was significantly associated with an increased risk of developing a pancreas-related complication.

Table 4 provides a breakdown of the scoring systems among patients with severe or mild acute pancreatitis, as defined by the Atlanta congress in 1992.[12] Within this classification, all the scoring systems, apart from CRP, were higher in patients with severe acute pancreatitis. Finally, receiver operator characteristics analysis was applied to the APACHE II, EWS, Imrie, and Ranson scores as a measure of their overall accuracy in predicting the severity of pancreatitis, mortality, and outcome (Table 5). Receiver operator characteristics was not applied to

Table 2. Median and range of values for scoring systems for severity of acute pancreatitis between favorable and poor outcome groups

	Favorable outcome		Adverse outcome		
	Median value	Range	Median value	Range	Significance (*P*)
Day 1					
EWS	2	0–9	4	1–9	0.0001
Imrie	2	0–4	2	1–4	NS
APACHE II	6	0–17	9	6–23	0.0005
Day 2					
EWS	2	0–8	4	2–9	<0.0001
Ranson	2	1–7	3	1–6	<0.0001
Imrie	2.5	0–5	2.5	1–4	NS
APACHE II	8	0–18	10.5	4–30	NS
Day 3					
EWS	1	0–7	5	1–9	<0.0001
Imrie	2	1–2	3	1–3	NS
APACHE II	11	0–16	11	5–12	NS
Day final					
EWS	1	0–5	3	2–5	0.0042
Imrie	2	1–3	2	1–4	NS
CRP	139	64–372	166	10–282	NS
APACHE II	10	0–15	13	4–21	NS

Values recorded for each individual was the single worst score within a 24-hour period.

Table 3. EWS within the first 72 hours of admission among patients with local complications of pancreatitis

		Day 1		Day 2		Day 3	
	No.	Median	Range	Median	Range	Median	Range
Pancreatic complications (n = 13)							
Pseudocyst	7	4	0–7	5	0–8	5	0–9
Necrosis	5						
Infected Pancreas	3						
Pancreatic Ascites	3						
Pancreatic Fistula	1						
ARDs	6						
No complications (n = 82)		2	0–9	2	0–7	1	0–6
Significance		$P = 0.0037$		$P < 0.001$		$P = 0.0005$	

CRP levels, due to the consistent lack of measurable differences. The results show that EWS was the best predictor of adverse outcome on days 1, 2, and 3 from admission (receiver operator characteristics, 0.768, 0.811, and 0.825, respectively). EWS compared well to both the Imrie and Ranson scores in predicting the severity of pancreatitis within the first 24 hours of admission. The most accurate predictor of mortality overall was EWS on day 3 of admission with an receiver operator characteristics of 0.920.

In summary, an EWS of 3 or above within the first 24 hours can predict an adverse outcome with a sensitivity and specificity of 70.0% and 79.1%, respectively (negative predictive value of 92.5%) (Table 6). EWS can predict mortality with a sensitivity and specificity of 85.7% and 28.3%, respectively (negative predictive value of 94.3%), and can predict the severity of pancreatitis with a sensitivity and specificity of 81.0 and 81.6%, respectively (negative predictive value of 92.0%).

DISCUSSION

Experienced clinical judgment at the time of admission may detect patients with severe acute pancreatitis, however, many patients with acute pancreatitis may appear relatively well at the time of admission. Hence, there is a need for a more

Table 4. Scoring systems according to the Atlanta classification of severe pancreatitis

	Mild pancreatitis (n = 89)		Severe pancreatitis (n = 21)		
	Median value	Range	Median value	Range	Significance (*P*)
Day 1					
EWS	2	0–9	4	0–9	<0.0001
Imrie	2	0–4	4	1–9	<0.0001
CRP	12.5	5–217	87	5–178	NS
APACHE II	6	0–15	9	3–23	<0.0001
Day 2					
EWS	2	0–7	4	0–8	<0.0001
Ranson	1	0–5	3	1–7	<0.0001
Imrie	1	0–4	4	2–8	0.0015
CRP	104	11–183	136	16–153	NS
APACHE II	6	0–15	10	4–30	0.0373
Day 3					
EWS	1	0–6	3.5	0–9	<0.0001
Imrie	2	1–3	3	1–9	NS
CRP	193	49–332	238	10–257	NS
APACHE II	10	4–12	11	5–21	NS
Day final					
EWS	1	0–4	3	0–7	<0.0001
Imrie	2	0–3	3	2–7	<0.0001
CRP	139	64–372	142	10–166	NS
APACHE II	9	2–13	14	4–21	0.0434

Table 5. Receiver operator characteristics analysis for APACHE II, EWS, Imrie, and Ranson scores for predicting adverse outcome, mortality, and the severity of acute pancreatitis

	Area under the receiver operator characteristics	Standard error	95% Confidence interval	Significance (*P*)
Adverse outcome				
Day 1				
EWS	0.768	0.064	0.676–0.845	<0.001
Imrie	0.667	0.076	0.561–0.762	0.0272
APACHE II	0.754	0.067	0.655–0.836	0.0002
Day 2				
EWS	0.811	0.060	0.723–0.881	<0.0001
Imrie	0.625	0.093	0.451–0.778	NS
Ranson	0.773	0.063	0.678–0.851	<0.0001
APACHE II	0.672	0.113	0.461–0.861	NS
Day 3				
EWS	0.825	0.060	0.736–0.894	<0.0001
Imrie	0.773	0.063	0.678–0.851	<0.0001
APACHE II	0.500	0.137	0.278–0.722	NS
Mortality				
Day 1				
EWS	0.604	0.117	0.504–0.698	NS
Imrie	0.818	0.100	0.722–0.892	0.0014
APACHE II	0.714	0.113	0.613–0.802	0.0580
Day 2				
EWS	0.784	0.105	0.693–0.859	0.0068
Imrie	0.561	0.147	0.362–0.746	NS
Ranson	0.789	0.104	0.696–0.864	0.0057
APACHE II	0.722	0.138	0.536–0.865	NS
Day 3				
EWS	0.920	0.077	0.848–0.965	<0.0001
Imrie	0.904	0.151	0.641–0.988	0.0073
APACHE II	0.750	0.174	0.516–0.910	NS
Severity of pancreatitis (Atlanta Classification)				
Day 1				
EWS	0.763	0.055	0.671–0.840	<0.0001
Imrie	0.842	0.0050	0.753–0.909	<0.0001
APACHE II	0.746	0.060	0.646–0.826	<0.0001
Day 2				
EWS	0.824	0.049	0.737–0.892	<0.0001
Imrie	0.866	0.063	0.702–0.958	<0.0001
Ranson	0.713	0.055	0.619–0.715	0.0001
APACHE II	0.725	0.090	0.539–0.867	0.0123
Day 3				
EWS	0.771	0.055	0.675–0.849	<0.0001
Imrie	0.825	0.106	0.617–0.947	0.0021
APACHE II	0.662	0.134	0.426–0.851	NS

objective assessment system as an adjunct to clinical acumen. This has lead to the development of laboratory-based scoring systems, such as the Imrie and Ranson scores, which are the mostly widely used and known scoring systems in the United Kingdom. For the most part, these systems provide optimum accuracy only 48 hours after the onset of symptoms.[5] The APACHE score has been shown to be accurate in predicting severity within the first 24 hours of admission,[14] an observation reflected also in our results. However, APACHE II is dependent on up to 14 clinical and biochemical variables, and its complexity precludes its use as a regular surveillance tool, outside of the field of clinical studies. More recently, the Multiple Organ System Score (MOSS) was developed, which combines a number of physiological

Table 6. Sensitivity and specificity values with negative predictive values for EWS, APACHE II, Ranson, and Imrie scores

	Sensitivity (%)	Confidence interval (%)	Specificity (%)	Confidence interval (%)	Negative predictive value (%)
Adverse outcome					
Day 1					
EWS ⩾3	70.0	45.7–88.0	79.1	69.0–87.1	92.5
Imrie ⩾3	11.1	1.7–34.8	94.6	86.7–98.5	80.9
APACHE II ⩾7	75.0	50.9–91.2	67.1	55.4–77.5	93.5
Day 2					
EWS ⩾3	71.4	47.8–88.6	73.8	63.1–82.8	96.6
Imrie ⩾3	0.0	0.0–26.6	75.0	42.8–94.2	46.2
Ranson ⩾3	36.4	17.2–59.3	91.0	82.4–96.3	90.0
APACHE II ⩾7	81.2	54.3–85.7	50.0	24.7–75.3	70.0
Day 3					
EWS ⩾3	52.4	39.8–74.3	79.5	68.7–87.8	91.8
Imrie ⩾3	9.1	1.5–14.3	100	40.2–100	37.5
APACHE II ⩾16	21.4	4.9–50.8	100.0	58.9–100.0	35.3
Mortality					
Day 1					
EWS ⩾3	85.7	42.2–97.6	28.3	19.7–38.2	94.3
Imrie ⩾3	28.6	4.5–70.7	95.1	88.0–98.6	96.9
APACHE II ⩾7	71.4	29.3–95.5	60.7	49.7–70.9	97.8
Day 2					
EWS ⩾3	71.4	28.3–90.5	67.4	57.1–76.5	98.3
Imrie ⩾3	0.0	0.0–52.0	90.5	69.6–98.5	85.7
Ranson ⩾3	42.9	10.4–81.2	87.1	78.5–93.1	98.6
APACHE II ⩾7	80.0	28.8–96.7	37.0	19.4–57.6	90.0
Day 3					
EWS ⩾3	100.0	54.1–100.0	77.4	67.6–85.4	100.0
Imrie ⩾3	50.0	8.2–91.8	100.0	75.1–100.0	92.6
APACHE II ⩾7	66.7	11.6–94.5	33.3	13.4–59.0	85.7
Severity of pancreatitis (Atlanta classification)					
Day 1					
EWS ⩾3	81.0	43.0–85.4	81.6	71.0–89.5	92.0
Imrie ⩾3	38.1	18.2–61.5	95.5	87.5–99.0	88.1
APACHE II ⩾7	75.0	50.9–91.2	70.6	58.3–81.0	93.0
Day 2					
EWS ⩾3	71.4	47.8–88.6	78.4	67.3–87.1	96.4
Imrie ⩾3	38.1	18.2–61.5	95.5	87.5–99.0	88.1
Ranson ⩾3	28.6	14.7–46.3	93.2	84.9–97.7	78.2
APACHE II ⩾7	81.2	54.3–95.7	66.7	34.9–89.9	70.0
Day 3					
EWS ⩾3	55.0	31.6–76.9	82.6	71.6–90.7	92.7
Imrie ⩾3	52.4	29.8–74.3	100.0	19.3–100.0	16.7
APACHE II ⩾12	31.2	11.1–58.6	100.0	48.0–100.0	30.8

A cut off value of 3 for both Ranson and Imrie scores has been chosen following previously published data.

and biochemical parameters to predict outcome, with a sensitivity of 86% but a negative predictive value of only 81%.[4]

In addition to these multiparameter scoring systems, single-marker assays have been studied. These include serum glucose,[15] serum calcium,[16] and CRP. CRP has been reported as having prognostic significance, 48 hours after the onset of symptoms.[17,18] Our study did not find CRP to be significantly different between any groups. CRP production is initiated by proinflammatory cytokines such as IL-1 and IL-6. Hence, one may surmise that any prognostic value attached to CRP elevation may occur secondary to establishment of SIRS and may

be of little practical clinical value. To circumvent this problem, studies have focused on the interleukins themselves as prognostic indicators, such as IL-10[16] and IL-6.[19] Despite encouraging early results, the disadvantage of these assays and others, such as urine concentrations of trypsinogen activation peptide,[20] is that they rely on laboratory techniques and equipment that may not be available in most centers or that may not be robust enough for widespread clinical application.

Our study had insufficient data to reliably compare CT findings with outcome, mortality, or severity of pancreatitis. The usefulness of CT as a prognostic indicator is a matter of some debate.[21–24] It has been suggested that CT is most accurate 48–72 hours after the onset of acute pancreatitis,[25] and indeed constraints in obtaining CT scans, may make very early on-admission scans difficult to procure. This would seem to put CT at the same temporal disadvantage as CRP, Ranson, and Imrie scoring in giving an early prediction of pancreatitis severity and outcome. The advantage to CT is that it relies wholly on pancreatic-specific changes.

A major confounding factor in comparing results from studies is the lack of standardization for outcome and pancreatic severity. Ideally, the number of hours after onset of symptoms should be used to assess marker levels, rather than admission to hospital. In our retrospective study, this was clearly not achievable, but it should certainly be the objective of prospective studies. In addition, standardizing the definition of severe pancreatitis is required, such as using the Atlanta classification. Another potential flaw in our study is the small number of patients who had severe and an adverse outcome compared with patients who recovered well and quickly. One possible reason for this is the stringent criterion on which patients were included into the study. Patients with a mildly raised amylase due to severe disease, or delayed admission to hospital, may have been missed by the entry criterion. Finally, our morality in patients following necrosectomy is higher than would be expected from most modern series. Once again, the small numbers of patients in the adverse outcome group does not allow for any reasoned conclusions as why this might be.

Buter et al.[26] reported in 2002 that resolving organ dysfunction within the first week of admission from pancreatitis was associated with a benign course of the disease and that deteriorating organ function was the main determinant of mortality. Our study did not evaluate change in EWS scores with outcome. However, the data on EWS and morality alone also suggest this to be the case. An EWS of 3 or above by day 3 of admission was a much better predictor of mortality than EWS on day 1 of admission. Hence, patients whose EWS had failed to improve or had deteriorated to 3 within 72 hours of admission were the most likely to die of their disease.

We believe that EWS offers a new perspective on scoring for outcome in pancreatitis. Our results show that it is suitably sensitive with a high negative predictive value to select patients with severe pancreatitis within 24 hours of admission. EWS also serves as a prognostic indicator of outcome (i.e., whether a patient may need critical care or surgery or die of their disease). EWS is simple to use and measures only six physiological variables. It is routinely calculated by nursing staff and can be repeated hourly. Apart from the prognostic information provided by EWS on admission, it can also be used to monitor a patient's progress through the course of their hospital stay. For some outcomes, such as mortality, EWS has shown itself to be an extremely accurate predictor.

EWS offers a reliable and easily reproducible scoring system to monitor patients with acute pancreatitis. It is much less cumbersome than other scoring systems, and we advocate its use with patients.

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Overexpression of Vascular Endothelial Growth Factor-C Correlates With Lymph Node Micrometastasis in Submucosal Esophageal Cancer

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Lymph node metastasis, including lymph node micrometastasis (LMM), is one of the most important prognostic factors in esophageal squamous cell carcinoma (ESCC). Vascular endothelial growth factor C (VEGF-C) plays a key role in the process of lymphangiogenesis. We examined VEGF-C expression and tumor microvessel density of the primary tumors in ESCC and analyzed relationships between VEGF-C expression and clinicopathologic findings including LMM in submucosal ESCC. The subjects were 87 patients with submucosal ESCC. Immunohistochemical staining of VEGF-C and CD34 was performed with primary tumors, and staining of cytokeratin was performed with dissected lymph nodes. Microvessel density was calculated from CD34 expression, and LMM was detected by cytokeratin staining. VEGF-C overexpression significantly correlated with depth of tumor invasion, lymphatic invasion, and lymph node metastasis ($P < 0.05$, $P < 0.0001$, and $P < 0.0001$, respectively). High microvessel density also correlated with lymphatic invasion and lymph node metastasis ($P < 0.005$ and $P < 0.05$, respectively). LMM was detected in 8 cases and 14 lymph nodes by cytokeratin staining. VEGF-C overexpression and high microvessel density were found in tumors with lymph node metastasis and/or LMM, compared with tumors without nodal metastasis or LMM ($P < 0.0001$ and $P < 0.01$, respectively). The present findings indicate that in ESCC with submucosal invasion, VEGF-C overexpression of the primary tumor is a strong high risk factor for lymph node metastasis, including LMM. (J GASTROINTEST SURG 2006;10:1016–1022)

KEY WORDS: VEGF-C, microvessel density, lymph node micrometastasis, esophageal cancer

Esophageal cancer is one of the most common malignancies and grows relatively fast. The prognosis of patients with esophageal squamous cell carcinoma (ESCC) is worse than that of patients with other gastrointestinal cancers.[1] One of the most important prognostic factors is the presence of the lymph node metastasis. However, the molecular mechanisms underlying lymph node metastasis in ESCC are unclear.

Tumor progression and metastasis require angiogenesis, which is induced by various factors including the vascular endothelial growth factor (VEGF) family of polypeptide growth factors.[2,3] It has recently been reported that vascular endothelial growth factor C (VEGF-C) induces not only angiogenesis but also lymphangiogenesis via VEGF receptor-2 and VEGF receptor-3.[4–6] Expression of VEGF receptor-3 is highly restricted to lymphatic endothelial cells and is only stimulated by VEGF-C and VEGF-D.[7–9] Several reports have described significant correlation between VEGF-C expression, tumor lymphangiogenesis, and lymph node metastasis in some cancers.[10–17]

Furthermore, even in patients who undergo complete resection and have no histological evidence of lymph node metastasis, tumors sometimes recur after resection. This suggests that tumor spread can be more advanced than indicated by the results of histological diagnosis. In recent years, the development of

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sensitive immunohistochemical techniques has allowed detection of lymph node micrometastasis (LMM). Cytokeratin (CK), which is a component of some intermediate filaments, is an epithelial marker. Assays of CK expression have been used to detect individual tumor cells in lymph nodes of patients with various types of carcinomas.[18–21] We have investigated the relationship between LMM and clinicopathologic findings in patients with esophageal carcinoma.[22–24]

Little is known about LMM or expression of VEGF-C in patients with ESCC. In ESCCs with submucosal invasion, the risk of lymph node metastases ranges from 20% to 60%. The deepest portion of a tumor has a higher malignant potential than other areas and is the part of the tumor that ultimately will invade, spread locally, and metastasize.[25] In the present study, we examined expression of VEGF-C immunohistochemically in ESCCs with submucosal invasion and assessed whether VEGF-C correlates with clinicopathologic findings, especially lymph node metastasis including LMM.

MATERIAL AND METHODS

Patients

Eighty-seven consecutive patients with ESCC with submucosal invasion (80 males and 7 females) underwent esophagectomy at Kagoshima University Hospital from 1985 to 2002. The ages of the patients ranged from 39 to 81 years (mean, 65.7 years). None of the patients received radiation therapy or chemotherapy before surgery.

Clinicopathologic findings were based on the criteria of the TNM classification for esophageal carcinoma of the International Union Against Cancer.[26] We classified 22 of the ESCCs as well differentiated, 40 as moderately differentiated, and 25 as poorly differentiated. Twenty of the tumors were located in the upper third of the esophagus, 45 in the middle third, and 22 in the lower third. Depth of cancer invasion was histologically classified as follows: sm1, invasion limited to the upper one third of the submucosa (15 patients); sm2, invasion to between one third and two thirds of the submucosal depth (26 patients); and sm3, invasion into the deepest third of the submucosa (46 patients). Lymph node metastasis was found in 41 of the 87 patients (47.1%). Lymphatic and venous invasion were found in 55.2% (48/87) and 29.9% (26/87) of the patients, respectively (Table 1).

Table 1. Clinicopathologic characteristics of 87 patients with pT1b esophageal squamous cell carcinoma

Gender	
Male	80
Female	7
Age (yr)	65.7 ± 8.3
Location of tumor	
Upper	20
Middle	45
Lower	22
Histopathologic grading	
Well	22
Moderate	40
Poor	25
Depth of tumor invasion	
sm1	15
sm2	26
sm3	46
Lymphatic invasion	
Negative	39
Positive	48
Venous invasion	
Negative	61
Positive	26
Lymph node metastasis	
Negative	46
Positive	41

Immunohistochemistry

After primary lesions were fixed in 10% formaldehyde and routinely embedded in paraffin, 3 μm-thick sections were prepared for immunohistochemistry. Sections were deparaffinized in xylene, rehydrated in graded ethanol, and incubated in 0.3% H_2O_2 solution in methanol for 30 minutes to block endogenous peroxidases. All sections were autoclaved in 10 mmol/L sodium citrate (pH 6.0) for 10 minutes and allowed to cool at room temperature. After incubation in 1% bovine serum albumin for 30 minutes at room temperature, the sections were incubated overnight at 4° C with mouse anti-VEGF-C monoclonal antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) and mouse anti-CD34 monoclonal antibody (1:100; Transduction Laboratories, Lexington, KY). These reactions were developed with an avidin-biotin immunoperoxidase technique (ABC method).[27] The reaction was visualized using the Vectastain Elite ABC kit and a 3,3′-diaminobenzidine solution (Vector Laboratories, Inc., Burlingame, CA). Sections were then slightly counterstained with hematoxylin.

Expression of VEGF-C in over 30% of the cells examined was considered positive, as described elsewhere.[17] The negative controls were sections not incubated with primary antibodies and sections incubated with serum from mice not immunized with VEGF-C or CD34. All immunostained slides were evaluated by two independent observers (M.M. and S.N.). In six sections, the results of

immunohistochemical evaluation differed between these two observers; these sections were evaluated by a third observer (H.O.).

For the 46 patients found to be node-negative by conventional HE staining, one additional section from each lymph node was stained immunohistochemically by using the AE1/AE3 monoclonal antibody cocktail (20:1 mixture of AE1 to AE3; Boehringer Mannheim, Germany), which reacts with a broad spectrum of human cytokeratins.[28,29] All sections were incubated at 60° C overnight. The tissue sections were then deparaffinized in xylene and rehydrated with a series of graded ethanols. After cooking the slides in citrate buffer solution (pH 6.0) for 6 minutes in a pressure cooker, the sections were incubated with CK monoclonal antibody at a 1:100 dilution. The CK reactions were developed using an alkaline phosphatase technique.[30] The immunohistochemical examination was limited to one slide per lymph node to expedite routine histological procedures. LMM was confirmed by clear cytoplasmic immunoreactivity and nuclear shape and size, compared with the morphology of macrophages, plasma cells, and reticular cells. In almost all the specimens, detection of a single metastatic tumor cell was followed by detection of several more scattered single metastatic tumor cells. The negative controls were sections treated with the same protocol but with the primary antibody omitted, and sections exposed to normal mouse IgG instead of the primary antibodies. Normal esophageal epithelium and the primary tumors of specimens were used as positive controls and were consistently positive.

Evaluation of Vessel Count

Vessel count was assessed by light microscopy in areas of the tumor containing the highest numbers of capillaries and small venules at the invasive edge. The highly vascular areas were identified by scanning tumor sections at low power (×40 and ×100). After six areas of highest neovascularization were identified, vessel count was performed in a ×200 field (×20 objective and ×10 ocular), and the average counts of the six fields were determined. As described by Weidner et al.,[31] identification of a vessel lumen was not necessary for a structure to be defined as a vessel.

Statistical Analyses

Statistical analysis was performed using Student's *t* test, the chi-square test, the Kaplan-Meier method, and the log-rank test. A *P* value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Expression of VEGF-C and CD34 in Esophageal Carcinoma Tissue

The expression of VEGF-C was distributed throughout the cytoplasm of cancer cells (Fig. 1, *A*). The positive rate of VEGF-C expression was 50.6% (44/87). CD34 expression was detected in both blood and lymphatic endothelial cells (Fig. 1, *B*). The median microvessel density (MVD) was 39.0 ± 22.6 (range, 8–105/field).

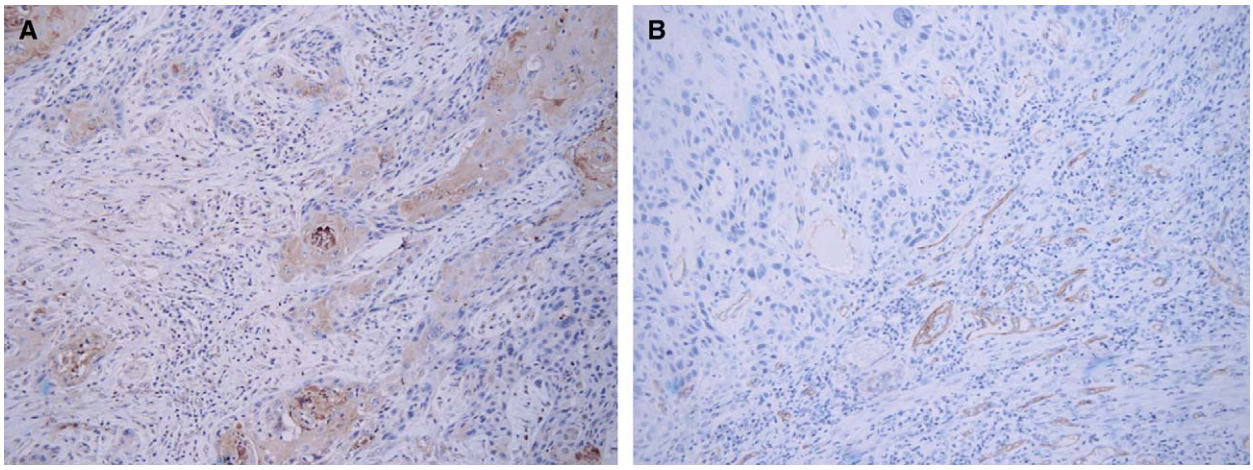

Fig. 1. Examples of immunostaining for VEGF-C and CD34 in ESCC with submucosal invasion. (**A**) Immunohistochemical expression of VEGF-C was detected in cytoplasm of carcinoma cells (original magnification, ×200). (**B**) Immunohistochemical expression of CD34 was detected in the blood or lymphatic microvessels (original magnification, ×200). Microvessels are visible as brown capillaries or small clusters, which stand out sharply from other tissue.

Correlation between Clinicopathologic Factors and Expression of VEGF-C and CD34

Table 2 shows the relationship between VEGF-C expression and pathological findings. VEGF-C expression significantly correlated with depth of tumor invasion, lymphatic invasion, and lymph node metastasis ($P < 0.05$, $P < 0.0001$, $P < 0.0001$, respectively). High MVD significantly correlated with lymphatic invasion and lymph node metastasis ($P < 0.005$, $P = 0.016$, respectively). Venous invasion tended to occur with greater frequency in patients with high MVD, but the association was not significant ($P = 0.08$).

LMM and Correlation With Expression of VEGF-C and CD34

Among the 46 patients shown to be node-negative by conventional HE staining, 14 LMM were detected by CK immunostaining (Fig. 2, *A*, *B*). VEGF-C overexpression and high MVD significantly correlated with LMM ($P < 0.0001$ and $P < 0.01$, respectively; Table 3). When dividing the patients without nodal metastasis by histological examination into two groups, the presence or absence of LMM and positive rates of VEGF-C and MVD were higher for patients with LMM than for those without LMM.

DISCUSSION

VEGF-C is a lymphangiogenic factor that stimulates mitogenesis in lymphatic endothelial cells via activation of the VEGF receptor-3.[4,6] Recent studies suggest that VEGF-C plays a clinicopathologic role in various malignancies. Immunohistochemical expression of VEGF-C in primary tumors has been shown to correlate well with lymph node metastasis and lymphatic invasion in patients with carcinoma of the thyroid gland, head, neck, esophagus, stomach, lung, pancreas, gallbladder, colorectum, uterus and ovary.[12,16,17,32–37] However, there have been few studies of the relationship between VEGF-C expression and LMM. We previously found that among patients with ESCC or gastric adenocarcinoma, those with LMM had significantly poorer prognosis and greater incidence of postoperative recurrence than those without LMM.[22–24] Thus, detection of LMM seems to be important for selection of treatment and prediction of clinical outcome.

In the present study, we examined patients with ESCC with submucosal invasion to investigate correlation between VEGF-C expression and clinicopathologic factors, especially lymph node metastasis and LMM. Although lymph node metastasis is rarely detected in cancers limited to the epithelial or mucosal layer, the rate of nodal metastasis of ESCC increases when the tumor invades into the submucosal layer.[38] Kishimoto et al.[39] described that the blood microvascular network is well developed in advanced cancers because of extensive tumor angiogenesis induced by VEGF, facilitating movement of cancer cells into blood vessels. Cancer cells that enter blood microvessels can move into lymphatic vessels via junctions between blood vessels and lymphatic vessels, and can

Table 2. Correlation between expression of vascular endothelial growth factor C and microvessel density in pT1b esophageal squamous cell carcinomas

Factor	VEGF-C expression (%)	P value	MVD	P value
Histopathological grading		NS		NS
Well	63		44.5 ± 27.6	
Moderate	55		37.7 ± 19.9	
Poor	32		36.2 ± 21.9	
Depth of tumor invasion		<0.05		NS
sm1	35		33.4 ± 26.8	
sm2	40		36.3 ± 18.2	
sm3	63		42.3 ± 23.3	
Lymphatic invasion		<0.0001		<0.005
Negative	26		31.5 ± 21.2	
Positive	71		45.1 ± 22.1	
Venous invasion		NS		NS
Negative	46		36.3 ± 20.1	
Positive	62		45.4 ± 26.9	
Lymph node metastasis		<0.0001		<0.05
Negative	28		33.5 ± 22.1	
Positive	76		45.1 ± 21.8	

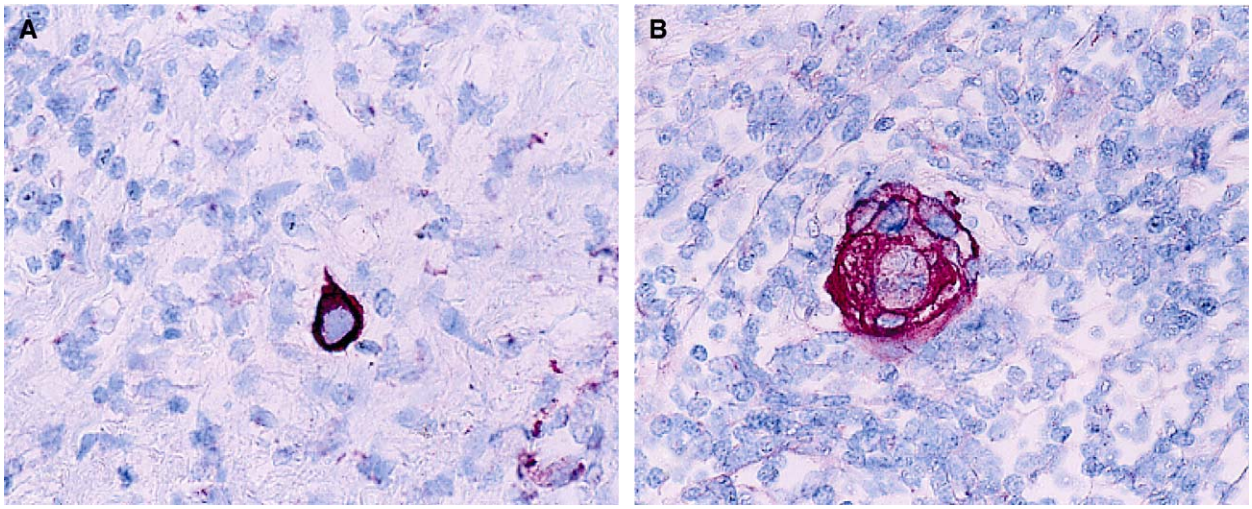

Fig. 2. Lymph node micrometastasis in lymph nodes found to be negative for metastasis by conventional hematoxylin-eosin staining. (**A**) Single cell micrometastasis. (**B**) Cluster-type micrometastasis.

eventually reach regional lymph nodes. In the early stage of cancer, numerous cancer cells can directly enter lymphatic microvessels rather than blood microvessels as a result of lymphovascular neogenesis and dilatation of lymphatic vessels induced by VEGF-C in the vicinity of the primary tumor and underdevelopment of tumor blood vessels. Thus, lymphatic microvessels may play a greater role in lymph node metastasis than blood microvessels. In the present study, we focused on lymph node metastasis in ESCCs with submucosal invasion, excluding advanced cases.

The present results indicate close correlation between VEGF-C expression and lymph node metastasis, especially LMM, which occurs in an early stage before overt lymph node metastasis. Although the positive rate of VEGF-C expression was higher in well differentiated tumors than those of poor differentiation, the difference was not significant. Li Q et al.[40] showed immunohistochemically that VEGF-C expression in nonsmall cell lung cancer negatively correlated with differentiation.[41] Various cells such as tumor cells and stromal cells secrete VEGF-C in tumors with high expression of VEGF-C. Some tumor cells may require the differentiation to secrete sufficient VEGF-C. Furthermore, VEGF-C overexpression significantly correlated with high MVD within the primary tumor. Despite the difficulty of discriminating between lymphatic vessels and blood vessels based on CD34 expression, several studies have shown positive correlation between VEGF-C expression and MVD.[17] These findings suggest extensive formation of microvessels in primary cancers with overexpression of VEGF-C. In the present study, VEGF-C expression and MVD were highest in cases with overt lymph node metastasis, and lowest in cases with no nodal involvement. The present results indicate that VEGF-C is a lymphangiogenic cytokine secreted by cancer cells, host cells, or stroma, and that after cancer cells entered into the lymphatic lumen, they moved in the mode of single cell or cluster cells to lymph node.[40]

In conclusion, in the present study, we characterized association between expression of VEGF-C and

Table 3. Correlation between expression of vascular endothelia growth factor C and microvessel density with or without lymph node micrometastasis and metastasis

Factor	pN(−)/LMM(−)	pN(−)/LMM(+)	pN(+)	P value
VEGF-C				<0.0001
Negative	30	3	10	
Positive	8	5	31	
MVD (mean ± SD)	31.5 ± 20.8	43.3 ± 26.6	45.1 ± 21.8	<0.01

pN(−) node-negative by conventional hematoxylin-eosin staining; pN(+) node-positive by conventional hematoxylin-eosin staining; LMM(−) node-negative by AE1/AE3 immunostaining; LMM(+) node-positive by AE1/AE3 immuniostaining; SD standard deviation.

both tumor MVD and clinicopathologic findings in ESCC. We found that overexpression of VEGF-C correlated with both LMM and overt metastasis. The present results suggest a close relationship between secretion of VEGF-C and the early stage of lymph node metastasis, particularly LMM. Due to the evidence indicating that submucosal ESCC with overexpression of VEGF-C is associated with a high risk of nodal metastasis, adjuvant therapy for treatment of lymph node metastasis should be considered in such cases.

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Clinicopathological Features of Gastric Carcinoma in Younger and Middle-Aged Patients: A Comparative Study

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Gastric carcinoma is relatively rare in patients under the age of 40. This study was undertaken to clarify the clinicopathological characteristics and surgical outcomes of gastric carcinoma in younger patients compared with those of middle-aged patients. The surgical results from 131 younger patients (aged ≤40 years) and 918 middle-aged patients (aged 55−65 years) were compared retrospectively. Female gender, undifferentiated tumor type and lymphatic invasion were significantly more common in the younger patients. Survival time did not differ between the two groups. The depth of tumor invasion was the only prognostic factor in younger patients, whereas macroscopic appearance, tumor diameter, depth of invasion, lymph node metastasis, and venous invasion were all significant prognostic factors in middle-aged patients. Peritoneal recurrence was significantly more common in younger patients. A family history of gastric adenocarcinoma was observed in 25.9% of younger patients, but this did not affect survival outcomes. As depth of invasion affects prognosis independently, and peritoneal metastasis is the predominant pattern of recurrence, it is essential to establish an optimal prophylactic treatment for peritoneal metastasis to improve surgical outcomes in younger patients with advanced gastric cancer. (J GASTROINTEST SURG 2006;10:1023–1032)

KEY WORDS: gastric cancer, younger patients, middle-aged patients, peritoneal metastasis, undifferentiated type

Gastric adenocarcinoma is the second most common malignant disease worldwide,[1] although its incidence has been decreasing.[2] The proportion of younger patients affected by gastric carcinoma and the corresponding survival rates for these individuals show considerable variability between studies.[3–6] Furthermore, there is controversy regarding the definition of clinicopathological features that are characteristic of gastric carcinoma in young patients.[7–10] The variability in the reported characteristics of gastric carcinoma in young patients might be due to differences in the criteria used to define young patients with gastric carcinoma or the number of patients analyzed. There have been reports of gastric carcinoma in patients younger than 30,[7] 35,[8] or 40 years.[9,10] A female predominance among younger patients with gastric cancer, compared with elderly gastric cancer patients, has been suggested[11]; however, another study reported a male predominance among younger patients.[12] There have also been reports suggesting a predominance of histologically undifferentiated tumor type,[13] advanced stage, or worse surgical outcome among young patients with gastric cancer.[14] Most reports have compared the clinicopathological characteristics and surgical outcomes between young patients and the elderly.[15,16] However, gastric cancer in elderly patients appears to have different characteristics.[17] Therefore, the clinicopathological characteristics and surgical outcomes in younger patients with gastric cancer should

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be compared with those of middle-aged patients, who represent the group most frequently affected by gastric cancer and might therefore display the most common features of gastric cancer. The present study was a retrospective comparative analysis of the clinicopathological characteristics, surgical outcomes and prognostic factors of younger (≤40 years old) and middle-aged (≥55 but <65 years old) patients with gastric cancer, in order to determine the best therapeutic strategy for these populations.

PATIENTS AND METHODS

A total of 2956 patients with histologically confirmed primary gastric carcinoma underwent curative gastrectomy between April 1985 and December 1999 at the Department of Gastroenterological Surgery, Yokohama City University, Graduate School of Medicine, Japan, and its satellite institutions. A total of 1049 patients (722 men and 327 women) were enrolled in this study. For the purposes of the analysis, we classified the patients into two groups: those aged 40 years or under ("younger"; 131 patients) and those aged 55−65 years ("middle-aged"; 918 patients). We defined the younger patients as those aged 40 or under in accordance with the criteria used in previously published literature[9,10] and in order to obtain a sufficient sample size to attain statistical significance. Among the 131 younger patients, 23 were aged 30 years or under, 25 were aged from 31 to 35 years, and 83 were older than 36 years of age.

A total of 948 patients underwent curative resection (121 younger patients and 827 middle-aged patients), and the remaining 101 patients were treated with palliative resection (10 younger patients and 91 middle-aged patients).

Preoperative imaging studies to determine the location of the tumor, its macroscopic appearance and diameter, its depth of invasion, and lymph node and distant metastases were routinely performed following an upper gastrointestinal barium-meal, an endoscopic examination, abdominal ultrasonography (US), and a computed tomography (CT) scan.

The Japanese Gastric Cancer Association has recommended a standardized lymph-node dissection procedure (standard D2 gastrectomy) for gastric cancer. In the present study, D2 gastrectomy was performed in accordance with the Japanese Classification of Gastric Carcinoma[18] as follows: distal gastrectomy principally for tumors in the lower third of the stomach; either distal or total gastrectomy for those in the middle third, depending on the direction of tumor invasion; and total gastrectomy for those in the upper third of the stomach or occupying the entire stomach. In addition, pancreaticosplenectomy was performed in patients with tumors extending into the upper third of the stomach with the aim of eradicating the lymph nodes at the splenic hilum and along the distal pancreatic artery. In every case, 15 or more lymph nodes were dissected according to the International Union Against Cancer (UICC) tumor, node, and metastasis (TNM) classification.[19]

All patients were followed up at least every 12 weeks for a minimum of 5 years according to our standard protocol, which includes tumor marker studies, endoscopic examinations, US, CT scans, and chest radiography. Patients in whom peritoneal metastasis was suspected from the results of a physical examination or the use of imaging modalities underwent an aspiration biopsy for confirmation. The median follow-up time was 56.3 ± 40.5 months for all registered patients and 60.3 ± 34.0 months for those receiving curative resection.

Of the 1049 registered patients, 384 had tumors located in the lower third of the stomach, 454 had tumors in the middle third, 183 had tumors in the upper third, and 28 had tumors occupying the entire stomach. Superficial tumors were macroscopically observed in 571 patients, well-defined tumors in 182 patients, and ill-defined tumors were seen in the remaining 296 patients. The tumor diameter was classified into three categories: tumors measuring <50 mm were found in 611 patients; tumors measuring ≥50 but <100 mm were present in 336 patients; and tumors measuring ≥100 mm were present in the remaining 102 patients. Differentiated tumors were histologically observed in 509 patients, and undifferentiated tumors were seen in 540 patients. Lymph-node metastasis, lymphatic invasion, and vascular invasion were observed in 445, 527, and 366 patients, respectively. The distribution of pathological stages among the patients was as follows: IA and IB (589 patients), II (139 patients), IIIA and IIIB (198 patients), and IV (123 patients).

Distal gastrectomy was performed in 740 patients, and total gastrectomy in 309 patients. D1 gastrectomy (complete dissection of tier 1 lymph nodes) plus the removal of the lymph node along the left gastric artery and the common hepatic artery was performed in 304 patients. D2 gastrectomy (complete dissection of tier 1 and 2 nodes) was performed in 655 patients, and D3 gastrectomy (complete dissection of tier 1 and 2 and para-aortic nodes) was performed in 90 patients. The anatomical location of all nodes was defined in accordance with the Japanese Classification of Gastric Cancer.[18] There were no cases with surgical complications, such as bleeding from the tumor or perforation of the tumor requiring emergency surgery. Surgery was performed

Table 1. Comparison of clinicopathological characteristics observed in younger and middle-aged patients with gastric cancer

Variables	Young (≤40 yr) (n = 131)	Middle aged (55–65 yr) (n = 918)	*P*-value
Age (y)	85.2 ± 5.0	60.2 ± 3.2	<0.0001
Gender			
Male/female	64/67	658/260	<0.0001
Location of tumor			0.0862
Lower third	44	340	
Middle third	69	386	
Upper third	15	168	
Entire	3	26	
Macroscopic appearance			0.7329
Superficial	75	496	
Well-defined	20	132	
Ill-defined	36	260	
Tumor diameter (mm)			0.0800
<50	76	536	
≥100	43	293	
≥10C	12	90	
Histologic type			<0.001
Differentiated	90	479	
Undifferentiated	101	439	
Depth of invasion			0.1954
pT1 (mucosa, submucoss)	72	430	
pT2 (proper muscle, subserasa)	22	228	
pT3 (serosa penetrated)	31	219	
pT4 (adjacent organs)	6	41	
Lymph node metastasis			
Absence/presence	83/48	521/397	0.1525
Lymphatic invasion			
Absence/presence	79/52	443/475	0.0099
Vascular invasion			
Absence/presence	97/34	586/332	0.0218
Peritonoal metastasis			
Absence/presence	125/6	874/44	0.9148
Liver metastasis			
Absence/presence	129/2	896/22	0.5334
Stage			
I/II/III/IV	79/16/24/12	510/123/174/111	0.6947
Lymph-node dissection			
D1 + α/D2/D3	24/92/15	280/563/75	0.0125
Operation method			
Partial/total	93/25	644/274	0.4622
Curability			
Curative/noncurative	121/10	827/91	0.4081

D1 + α = D1 plus lymph node along the left gastric artery and the common hepatic artery.

only after the procedure had been explained to the patient and their informed consent had been obtained.

Statistical Analysis

The SPSS program version 10.0 for Windows (SPSS Inc, Chicago, IL) was used for all statistical analyses. Patient characteristics were compared using the two-tailed Fisher exact test or the χ^2 test with Yates' correction, as appropriate. Quantitative variables were compared using the Student's *t* test and expressed as mean ± SD. Overall and disease-specific survival rates were calculated using the Kaplan-Meier estimation and examined by the log-rank test. The Cox proportional hazards regression model was used to identify prognostic factors. Step-forward regression was used to build a valid statistical model of the association of prognostic factors with

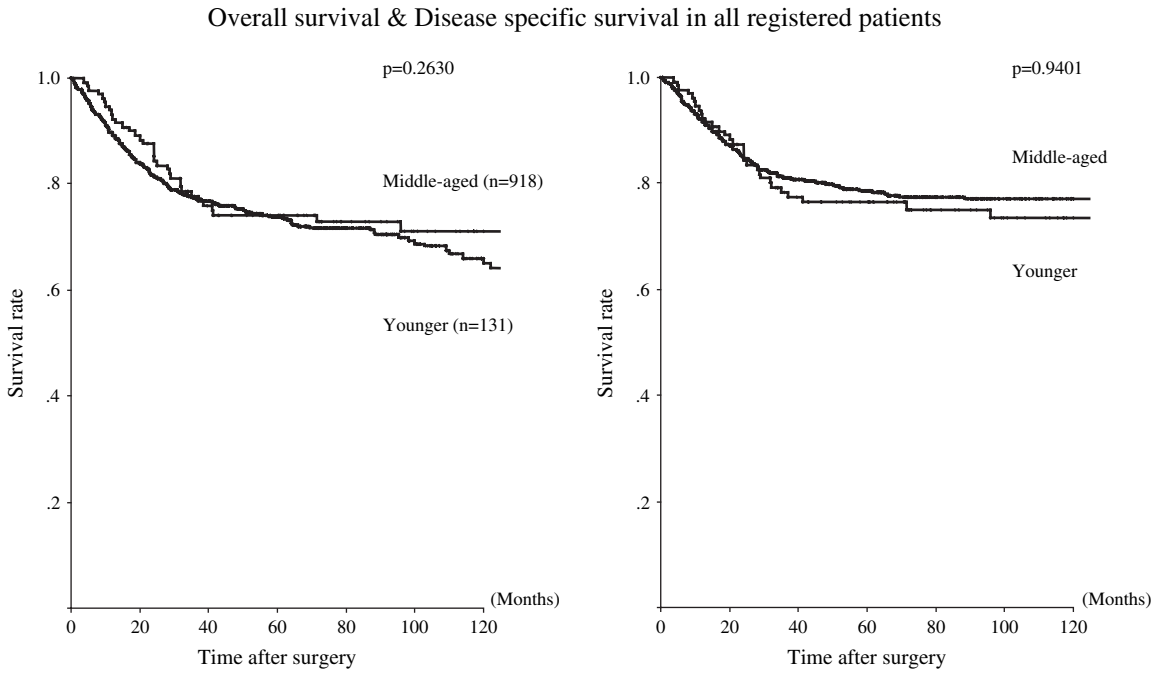

Fig. 1. Overall and disease-specific survival in younger and middle-aged patients.

disease-specific survival in those patients for whom we had complete data. The Cox proportional hazard-regression model was performed using the following variables: gender (male versus female), tumor location (upper versus middle versus lower third versus entire stomach), macroscopic appearance (superficial versus well-defined versus ill-defined), tumor diameter (<50 mm versus $\geqslant 50$ mm but <100 mm versus $\geqslant 100$ mm), histological type (differentiated versus undifferentiated), depth of invasion (pT1

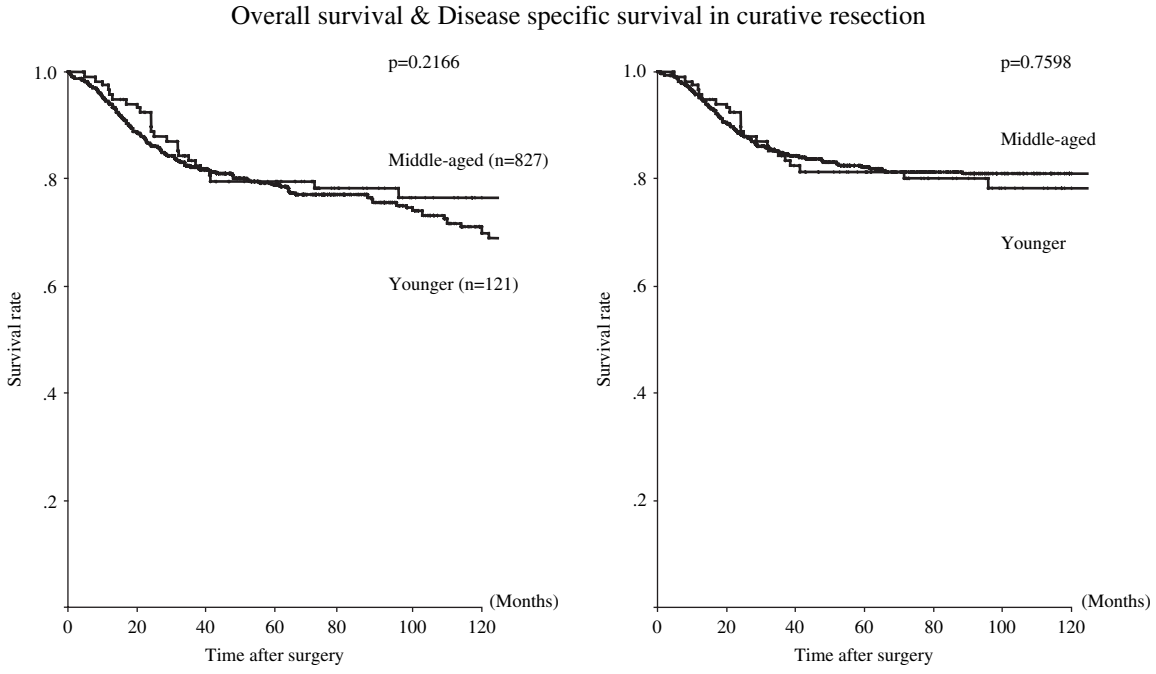

Fig. 2. Overall and disease-specific survival in younger and middle-aged patients receiving curative surgery.

Table 2. The 5-year survival rate according to clinicopathological factors in younger and middle-aged patients

	Young (n = 121)		Middle-aged (n = 327)		
	n	5-yr survival (%)	n	5-yr survival (%)	*P* value
Gender					
Male	58	85.6	595	82.6	0.6274
Female	63	79.3	232	82.9	0.3418
Location of tumor					
Lower third	41	79.0	305	82.1	0.5445
Middle third	67	89.0	361	90.0	0.6860
Upper third	12	55.6	146	70.6	0.3315
Entire	1	100	15	23.0	0.2609
Macroscopic appearance					
Superficial	75	100	489	96.7	0.1091
Well-defined	17	59.5	141	72.4	0.4312
Ill-defined	29	50.2	197	55.5	0.4048
Tumor diameter (mm)					
<50	75	94.4	526	91.5	0.5456
⩾50–100	40	66.7	248	70.8	0.6489
⩾100	6	33.3	53	47.0	0.2710
Histological type					
Differentiated	28	92.4	444	86.8	0.6602
Undifferentiated	93	79.4	383	77.9	0.7541
Depth of invasion					
pT1	72	98.5	425	97.1	0.4612
pT2	22	85.9	214	80.9	0.8020
pT3	23	37.0	163	54.3	0.1165
pT4	4	50.0	25	35.0	0.5879
Lymph node metastasis					
Absence	82	93.5	512	95.6	0.3167
Presence	39	58.6	315	62.3	0.6434
Lymphatic invasion					
Absence	79	94.6	437	94.6	0.7500
Presence	42	59.8	390	69.5	0.2205
Venous invasion					
Absence	93	88.5	563	92.3	0.1830
Presence	28	62.2	264	62.0	0.8857

versus pT2 versus pT3 versus pT4), lymph-node metastasis (pN0 versus pN1 versus pN2 versus pN3), lymphatic invasion (absence versus presence), and venous invasion (absence versus presence) according to the Japanese Classification of Gastric Carcinoma (18). According to this classification, macroscopically superficial type consisted of flat tumors with or without minimal elevation or depression. Histologically differentiated type included papillary adenocarcinoma, well-differentiated tubular adenocarcinoma, and moderately differentiated tubular adenocarcinoma. Undifferentiated type included poorly differentiated adenocarcinoma, signet ring cell carcinoma, and mucinous adenocarcinoma. Lymphatic invasion or venous invasion means the invasion of cancer cells into the lymphatics or microvessels. A probability value of $p < 0.05$ was considered statistically significant.

Table 3. The 5-year survival rate according to pathological stage in patients receiving curative resection

	Young (⩽40 yr) (n = 121)		Middle-aged (⩾55–65 yr) (n = 827)		
Stage	n	5-yr Survival rate (%)	n	5-yr Survival rate (%)	*P* value
IA	65	100	369	98.8	0.3854
IB	14	92.3	136	93.4	0.5869
II	16	59.7	123	80.2	0.0660
IIIA	15	64.7	102	59.2	0.9948
IIIB	8	25 mo (MST)	61	43.3	0.2400
IV	3	33.3	36	21.1	0.6860

MST = median survival time.

RESULTS

Comparison of Clinicopathological Characteristics of Younger and Middle-aged Patients With Gastric Cancer

There were significant differences in gender, histological type, lymphatic invasion, and venous invasion between the younger and middle-aged patients with gastric cancer. Among younger patients with gastric cancer, female gender, histologically undifferentiated tumor type, and lymphatic invasion were present significantly more often. More extensive lymph-node dissection was also necessary in younger patients. However, there were no significant differences in the other clinicopathological characteristics studied between the two groups (Table 1).

Survival

Among the 1049 registered patients, including those receiving palliative surgery, there was no significant difference in overall survival between the younger and middle-aged groups ($p = 0.2630$). The 5-year survival rates were 74.0% in younger patients and 73.7% in middle-aged patients. Furthermore, there was no significant difference in disease-specific survival at 5 years postoperation between the two groups (75.7% versus 77.1%, $p = 0.9401$) (Fig. 1). In patients receiving curative surgery, there was no significant difference in overall 5-year survival between the two groups (79.6% versus 79%, $p = 0.2166$). Similarly, there was no significant difference in disease-specific 5-year survival

Table 4. Prognostic factors in younger patients

Variable	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Gender		0.3072	—	
Male/female	0.6463 (0.2796–1.4938)			
Location of tumor		0.1158	—	
Middle/lower	0.6397 (0.2141–1.3607)			
Upper/lower	2.0983 (0.6958–6.3007)			
Macroscopic appearance		0.0003	—	
Well-defined/superficial	19.7485 (3.9737–98.1460)			
Ill-defined/superficial	19.6856 (4.4960–86.1933)			
Tumor diameter (mm)		<0.0001	—	
⩾50 < 100/<50	5.1055 (1.9269–13.5275)			
⩾100/<50	17.5874 (4.8766–63.4294)			
Histological type		0.1803	—	
Undifferentiated/differentiated	2.2939 (0.6810–7.7272)			
Depth of invasion		<0.0001		<0.0001
PT2/pT1	6.7865 (1.2403–37.1340)		6.7865 (1.2403–37.1340)	
PT3/pT1	36.2188 (8.2356–159.285)		36.2188 (8.2356–159.285)	
PT4/pT1	40.7174 (5.5460–298.938)		40.7174 (5.5460–298.938)	
Lymph node metastasis		0.0002	—	
pN1/pNo	4.5292 (1.6413–12.4984)			
pN2/pNo	10.0562 (3.4780–29.0763)			
pN3/pNo	7.6837 (0.9380–62.9441)			
Lymphatic invasion		0.0001	—	
Presence/absence	6.4299 (2.5302–16.3406)			
Venous invasion		0.0012	—	
Presence/absence	3.9127 (1.7188–8.9067)			

between the two groups (82.2% versus 81.4%, $p = 0.7598$) (Fig. 2).

The 5-year survival rates according to each clinicopathological factor between younger and middle-aged patients receiving curative resection are shown in Table 2. There were no significant differences between younger and middle-aged patients in any of the clinicopathological factors studied (Table 2). In addition, there was no significant effect of pathological stage on the disease-specific 5-year survival with curative resection between the two groups (Table 3).

Prognostic Factors

In younger patients treated with curative resection, univariate analysis revealed that macroscopic appearance, tumor diameter, depth of invasion, lymph-node metastasis, lymphatic invasion, and venous invasion significantly affected prognosis. Multivariate analysis in the same patients showed that depth of invasion independently predicted prognosis (Table 4). In middle-aged patients, univariate analysis showed that tumor location, macroscopic appearance, tumor diameter, depth of invasion, lymph-node metastasis, lymphatic invasion, and venous invasion significantly influenced prognosis. Multivariate analysis also showed that, with the exception of tumor location, these factors independently affected prognosis in these populations (Table 5).

Patterns of Recurrence

There was a significant difference in the pattern of recurrence between the younger and middle-aged patients; in the former, the incidence of peritoneal metastasis was significantly increased (Table 6).

Family History of Younger Patients With Gastric Cancer

Among the 131 younger patients with gastric cancer, 34 (25.9%) had a family history of gastric cancer among first- or second-degree relatives. The observed clinicopathological factors were compared between patients with and without a family history of gastric cancer. There was a significant difference in histological type between both groups. A histologically undifferentiated tumor type was observed significantly more frequently in younger patients with a family history of gastric cancer (Table 7). There

Table 5. Prognostic factors in middle-aged patients

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Gender		0.5354	—	—
Male/female	1.1309 (0.7664–1.6690)			
Location of tumor		<0.0001	—	—
Middle/lower	0.5139 (0.3345–0.7893)			
Upper/lower	1.6069 (1.0607–2.4343)			
Entire/lower	5.0335 (2.3872–10.6132)			
Macroscopic appearance		<0.0001		0.0326
Well-defined/superficial	9.5874 (5.3572–17.1578)		2.7203 (1.2094–6.1188)	
Ill-defined/superficial	15.6661 (9.1479–26.8287)		2.9519 (1.2999–6.7035)	
Tumor diameter (mm)		<0.0001		<0.0001
⩾50 < 100/<50	3.6342 (2.4835–5.3179)		1.0469 (0.7232–1.5156)	
⩾100/<50	7.6065 (4.5825–12.6258)		2.5993 (1.5733–4.2946)	
Histological type		0.0017		—
Undifferentiated/differentiated	1.7313 (1.2281–2.4406)			
Depth of invasion		<0.0001		
PT2/pT1	6.8548 (3.5892–13.0917)		2.0663 (0.8370–5.1009)	
PT3/pT1	19.3870 (10.4777–35.8719)		3.7651 (1.4705–9.6402)	
PT4/pT1	40.2652 (18.9829–85.4076)		5.8199 (2.0200–16.7678)	
Lymph node metastasis		<0.0001		
pN1/pNo	5.5453 (3.2800–9.3750)			
pN2/pNo	13.7443 (8.3504–22.6224)		2.0582 (1.1624–3.6445)	
pN3/pNo	36.6817 (19.7692–68.0627)		4.0150 (2.2764–7.0815)	
Lymphatic invasion		<0.0001		—
Presence/absence	6.6345 (4.1999–10.4805)			
Venous invasion		<0.0001		0.0116
Presence/absence	6.1395 (4.23889–8.8924)		1.5849 (1.1083–2.2666)	

was no significant difference in disease-specific survival between patients with and without a family history of gastric cancer. Among the 131 younger patients, the disease-specific 5-year survival rate was 75.6% in those with a family history of gastric cancer and 75.8% in those without a family history ($p = 0.8990$). Among the 121 younger patients treated with curative surgery, the disease-specific 5-year survival rates of patients with and without a family history were 77.9% and 82.8%, respectively ($p = 0.6562$) (Fig. 3).

Table 6. Patterns of recurrence in gastric cancer patients

	Younger (n = 23) (%)	Middle-aged (n = 123) (%)
Peritoneal metastasis	18 (78.3)	61 (49.6)
Lymph node metastasis	2 (8.7)	27 (22.0)
Hematogenous metastasis	3 (13.0)	35 (28.4)

$P = 0.0402$.

DISCUSSION

In the present study, female gender and a histologically undifferentiated type of tumor were frequently observed among younger patients with gastric cancer, although there was no significant difference in measures of survival between younger and middle-aged patients. The depth of invasion was the only independent prognostic factor present in younger patients. Peritoneal metastasis was present significantly more often in younger patients than in middle-aged patients. Among younger patients, 25.9% had a family history of gastric cancer, although there were no significant differences in survival and clinicopathological factors, with the exception of histological type, between those with and without a family history of gastric cancer. The incidence of a histologically undifferentiated tumor type was significantly increased in younger patients with a family history of gastric cancer.

The proportion of gastric carcinoma cases present in younger patients has been reported to be between 2% and 8% in the literature.[20,21] In the current study, the incidence of gastric carcinoma in younger patients

Table 7. Comparison of clinicopathological characteristic in younger gastric cancer patients with and without a family history of fastric cancer

	Family history (+) (n = 34)	Family history (−) (n = 97)	*P* value
Gender			0.5207
Male	15	49	
Female	19	48	0.7611
Location of tumor			
Lower third	11	38	
Middle third	19	60	
Upper third	4	11	
Entire	0	3	
Macroscopic appearance			0.1144
Superficial	16	59	
Well-defined	4	16	
Ill-defined	14	22	
Tumor diameter (mm)			0.5898
<50	22	54	
>50 <100	10	33	
⩾100	2	10	
Histological type			0.0312
Differentiated	3	27	
Undifferentiated	31	70	
Depth of invasion			0.9607
*P*T1	19	53	
*P*T2	6	16	
*P*T3	8	23	
*P*T4	1	5	
Lymph node metastasis			0.5464
Absence	23	60	
Presence	11	37	
Lymphatic invasion			0.5422
Absence	22	57	
Presence	12	40	
Venous invasion			0.0821
Absence	29	68	
Presence	5	29	
Peritoneal metastasis			0.5952
Absence	33	92	
Presence	1	5	
Hematogenous metastasis			0.3988
Absence	34	95	
Presence	0	2	
Stage			0.4123
I/II/III/IV	20/5/8/1	59/11/16/11	
Curability			0.2311
Curative	33	88	
Noncurative	1	9	

was 4.4%, reflecting findings from other reports. Moreover, most of the younger patients (83 patients; 63.4%) in the present study were between 36 and 40 years old. There have been conflicting reports regarding the gender distribution of younger patients with gastric cancer. One study suggested that, among younger patients, gastric cancer is present with equal frequency in both males and females,[8] whereas other studies have reported a male[12] or female[11] predominance, the latter also being observed in the present study. Evaluation of a larger number of patients with gastric carcinoma would be required in order to confirm a significant gender difference.

In this study, more than half of the tumors (52.7%) present in younger patients were located in the middle third of the stomach, although there were no significant differences in the distribution of tumors between the two groups ($p = 0.0862$). Predominance of tumors located in the middle third of the stomach has also been reported in some previous studies of younger patients,[7,11] although Choi et al.[20] reported more diversity of tumor location in this patient group. Another study reports a high frequency of tumors occupying the entire stomach in younger patients,[5] although this might be due to the relatively high frequency in the study population of hereditary diffuse gastric cancers, such as linitis plastica, which is prevalent in Western countries[22,23] but has seldom been reported in Japan.

Histologically, the undifferentiated tumor type was more common in younger patients with gastric cancer, a finding also reported by other investigators.[13] This unfavorable histological profile might be a property of gastric cancer in younger patients. Furthermore, lymphatic and venous invasion were more frequent in younger patients, whereas there was no difference in characteristics such as depth of invasion, lymph node metastasis, and tumor diameter, which are thought to be independent prognostic factors of gastric cancer in patients of all ages. Therefore, the survival in younger patients did not differ from that of middle-aged patients.

In this study, there was no significant difference in overall or disease-specific survival between younger and middle-aged patients. There was also no difference in disease-specific survival according to clinicopathological factors and pathological stage. These results suggest that the malignant potential of a tumor does not differ between younger and middle-aged patients. It is therefore essential to perform curative resection (R0) in younger patients, as well as middle-aged patients, in order to obtain satisfactory surgical outcomes. By contrast, other reports indicate that younger patients have a poorer prognosis.[14] This difference of prognosis might depend on the number of patients analyzed or differences in the control group used (such as middle-aged or elderly patients).

The prognostic factors observed in younger patients differed from those seen in middle-aged patients. In the former, only depth of invasion was selected as an independent prognostic factor,

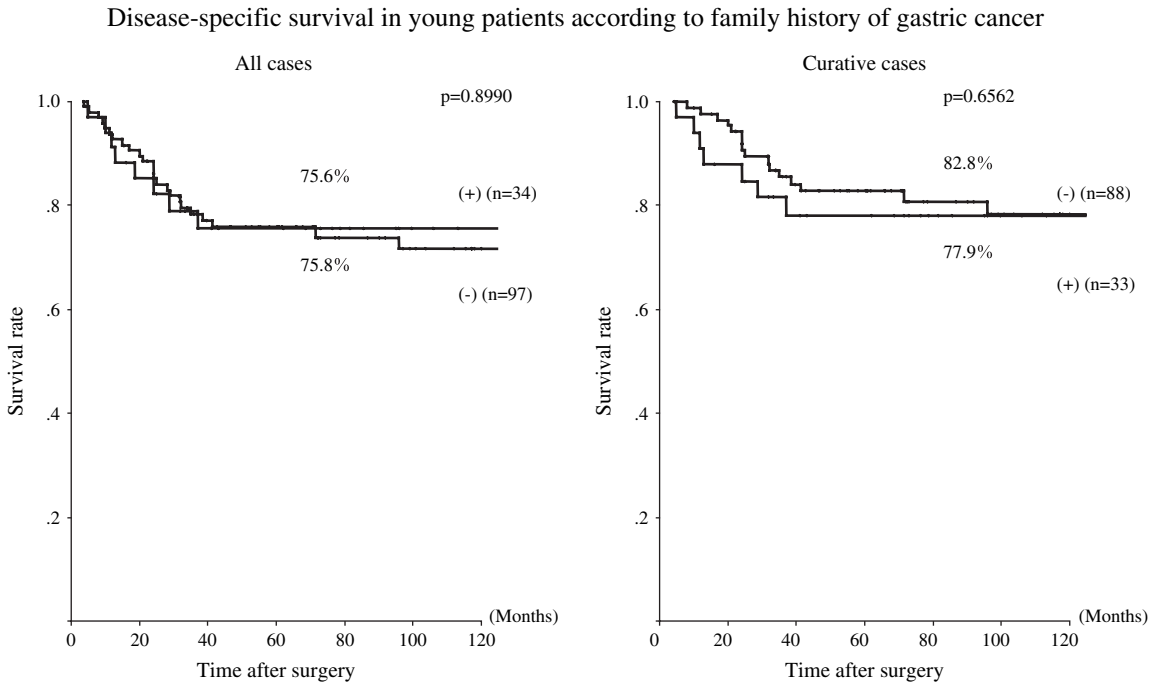

Fig. 3. Disease-specific survival in younger patients with and without a family history of gastric cancer.

whereas depth of invasion, lymph node metastasis, and tumor diameter were all independent prognostic factors in middle-aged patients. In general, depth of invasion, lymph-node metastasis, and tumor diameter are reported to be independent prognostic factors after curative resection in gastric cancer. In younger patients, lymph node metastasis and tumor diameter were not selected as independent prognostic factors. The results of multivariate analysis are relative; therefore, it appears that from the present data that the depth of invasion is the most significant factor compared with the other clinicopathological factors of gastric cancer present in younger patients.

In general, peritoneal metastasis is the most frequent pattern of recurrence in advanced gastric cancer, irrespective of age.[24,25] In this study, the incidence of peritoneal metastasis was more frequent in younger patients than in middle-aged patients. This result must reflect the outcome that depth of invasion was the only independent prognostic factor in younger patients. Therefore, it is necessary to establish a prophylactic treatment for peritoneal recurrence in younger patients with gastric cancer.

The current study revealed that 26% of younger patients have a family history of gastric cancer among their first- or second-degree relatives. This result indicates that genetic factors might play an important role in the development of gastric cancer in younger populations. However, there was no significant difference in clinicopathological factors, except histological type and survival, between patients with and without a family history of gastric cancer.

In Western countries, hereditary diffuse gastric cancer caused by germline E-cadherin/*CDH1* mutations has been reported.[26,27] In Japan, this type of tumor is uncommon and has not been observed in any of the patients that we have studied.

CONCLUSION

The surgical results in younger patients are similar to those in middle-aged patients, although younger patients have some different clinicopathological features. As depth of invasion is an independent prognostic factor and peritoneal metastasis is the predominant pattern of recurrence, it is essential to establish an optimal prophylactic treatment for peritoneal metastasis in younger patients with advanced gastric cancer in order to improve their surgical outcomes.

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Preoperative Nutritional Status of Patients Undergoing Roux-en-Y Gastric Bypass for Morbid Obesity

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Few data exist concerning preoperative nutritional status in patients undergoing bariatric surgery. We retrospectively analyzed the preoperative values of serum albumin, calcium, 25-OH vitamin D, iron, ferritin, hemoglobin, vitamin B_{12}, and thiamine in 379 consecutive patients (320 women and 59 men; mean body mass index 51.8 ± 10.6 kg/m^2; 25.8% white, 28.4% African American, 45.8% Hispanic) undergoing bariatric surgery between 2002 and 2004. Preoperative deficiencies were noted for iron (43.9%), ferritin (8.4%), hemoglobin (22%; women 19.1%, men 40.7%), thiamine (29%), and 25-OH vitamin D (68.1%). Low ferritin levels were more prevalent in females (9.9% vs. 0%; $P = 0.01$); however, anemia was more prevalent in males (19.1% vs. 40.7%; $P < 0.005$). Patients younger than 25 years were more likely to be anemic than patients over 60 years (46% vs. 15%; $P < 0.005$). This correlated with iron deficiency, which was more prevalent in younger patients (79.2% vs. 41.7%; $P < 0.005$). Whites (78.8%) and African Americans (70.4%) had a higher prevalence of vitamin D deficiency than Hispanics (56.4%), $P = 0.01$. Whites were the least likely group to be thiamine deficient (6.8% vs 31.0% African Americans and 47.2% Hispanics; $P < 0.005$). Nutritional deficiencies are common in patients undergoing Roux-en-Y gastric bypass, and these deficiencies should be detected and corrected early to avoid postoperative complications. (J GASTROINTEST SURG 2006;10:1033–1037)

KEY WORDS: Roux-en-Y gastric bypass, morbid obesity, obesity, nutrition, vitamin deficiency

Patients undergoing Roux-en-Y gastric bypass (RYGB) as treatment for morbid obesity are at risk for numerous vitamin and mineral deficiencies postoperatively. Iron deficiency anemia and deficiencies of several vitamins, including folate, thiamine, and vitamin B_{12},[1–8] have been well documented. More recently, concerns have been raised about the long-term effects of RYGB on calcium and bone metabolism.[4,9–11]

With the tremendous rise in the number of bariatric surgical procedures being performed annually, there is concern that these nutritional complications may be underdiagnosed and undertreated, leading to profound adverse effects on patients.[12] Although the incidence of neurological complications after bariatric surgery is well known,[5,13–15] several reports have been published in recent years emphasizing neurological complications after RYGB and attributing them to nutritional deficiencies.[16–21]

Few studies have examined the preoperative nutritional status of patients presenting for bariatric surgery in a systematic manner. The aim of this study was to evaluate the status of several vitamins, nutrients, and nutritional markers preoperatively in patients undergoing RYGB to assess their preoperative risk for nutritional deficiency.

METHODS

A retrospective review of 379 consecutive patients undergoing RYGB between August 2002 and February 2004 was conducted from a prospectively maintained PC-based database. The population consisted of 320 women (84%) and 59 (16%) men; mean body mass index was 51.8 ± 10.6 kg/m^2 (range 24–115 kg/m^2). The racial/ethnic breakdown of the population was white (25.8%), African American, (28.4%), and Hispanic (45.8%).

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Preoperative values of serum albumin, serum calcium, 25-OH vitamin D, serum iron, ferritin, hemoglobin, vitamin B_{12}, and thiamine (vitamin B_1) were examined and the prevalence of deficiencies noted. Values for serum albumin, serum calcium, serum iron, ferritin, and hemoglobin were available for all patients. Thiamine and 25-OH vitamin D levels were available for patients undergoing surgery after July 2003 (n = 141 and n = 144, respectively). Data were also stratified based upon age, gender, and race.

SPSS version 11.0 (SPSS Inc., Chicago, IL) was used to perform statistical analyses. Chi-square tests were used to evaluate gender and age differences and one-way ANOVA with two degrees of freedom was used to evaluate significance between racial groups.

RESULTS

The results for the overall group are summarized in Table 1. Significant preoperative deficiencies were noted for iron (43.9%), ferritin (8.4%), hemoglobin (22%), thiamine (29%), and 25-OH vitamin D (68.1%).

Analysis categorized by gender is summarized in Table 2. Interestingly, anemia was more common in men (40.7%) than in women (19.1%); $P < 0.005$. However, women were found to have a significantly higher prevalence of low ferritin levels than men (9.9% females vs. 0% males; $P = 0.01$).

Extremes of age were evaluated and results are shown in Table 3. Patients less than 25 years old were more likely to be anemic than patients greater than 60 years old (46% vs. 15%; $P < 0.005$, respectively). This directly correlated with iron deficiency, which was also more prevalent in the younger age group (79.2% vs. 41.7%; $P < 0.005$).

Nutrient deficiencies were stratified by race and are presented in Table 4. Vitamin D deficiency was significantly less common among Hispanics (56.4%) than among whites (78.8%) and African Americans (70.4%); $P = 0.01$. On the other hand, whites were the least likely group to be thiamine deficient (6.8%) compared with African Americans (31%) and Hispanics (47.2%); $P < 0.005$). Additionally, African Americans demonstrate significantly lower preoperative hemoglobin 12.6 g/dL ($P < 0.01$). This finding may have a dietary origin as this group also shared the lowest levels of vitamin D, 9.7 pg/ml ($P = 0.01$); iron, 65.9 ng/ml ($P = 0.02$); and thiamine, 108.6 ng/ml ($P < 0.01$). Although there are metabolic confounders due to the many enzymatic reactions involved in vitamin and iron metabolism, these data suggest that African Americans might benefit from additional attention directed toward vitamin supplementation and dietary counseling.

Table 1. Incidence of abnormal preoperative values

Nutrient	No. Abnormal	%
Albumin	4/357	1.1
Calcium	12/374	3.2
25-OH Vitamin D	98/144	68.1
Iron	159/362	43.9
Ferritin	30/358	8.4
Hemoglobin	85/378	22
Vitamin B_{12}	0/355	0
Thiamine (Vitamin B_1)	41/141	29

Table 2. Incidence of abnormal preoperative values by gender

Nutrient	Female Abnormal (%)	Male Abnormal (%)	*P* value
Albumin	3/301 (1.0%)	1/56 (1.8%)	.60
Calcium	11/316 (3.5%)	1/58 (1.7%)	.9
25-OH Vitamin D	87/128 (68%)	11/16 (8.8%)	.95
Iron	128/305 (42%)	31/57 (54.4%)	.08
Ferritin	30/302 (9.9%)	0/56 (0%)	.01
Hemoglobin	61/319 (19.1%)	24/59 (40.7%)	<.005
Vitamin B_{12}	0/302 (0%)	0/53 (0%)	NA
Thiamine (Vitamin B_1)	36/125 (28.8%)	5/16 (31.3%)	.84

Table 3. Incidence of abnormal preoperative values by extremes of age

Nutrient	Age < 25	Age > 60	*P* value
Albumin	1/26 (1.1%)	0/63 (0%)	.11
Calcium	1/26 (3.8%)	0/36 (0%)	.24
25-OH Vitamin D	14/74 (15.9%)	1/3 (33%)	.54
Iron	19/24 (79.2%)	25/60 (41.7%)	<.005
Ferritin	3/24 (12.5%)	8/59 (13.6%)	.90
Hemoglobin	12/26 (46%)	10/65 (15%)	<.005
Vitamin B_{12}	0/22 (0%)	0/60 (0%)	NA
Thiamine (Vitamin B_1)	5/15 (33.3%)	1/3 (33.3%)	1

DISCUSSION

The explosive rise in the number of bariatric surgical procedures performed annually has led to increasing concerns about its long-term risks and benefits in both the medical community and the public sector.

Table 4. Incidence of abnormal preoperative values by race

Nutrient	White Abnormal (%)	African American Abnormal (%)	Hispanic Abnormal (%)
Albumin	1/86 (1.2%)	3/167 (1.8%)	0/102 (0%)
Calcium	1/92 (1.1%)	6/173 (1.6%)	5/107 (4.7%)
25-OH Vitamin D	26/33* (78.8%)	50/71† (70.4%)	22/39* (56.4%)
Iron	33/90 (36.7%)	74/166 (44.6%)	50/104 (48.1%)
Ferritin	3/89 (3.4%)	19/164 (11.6%)	8/103 (7.8%)
Hemoglobin	16/96 (16.7%)	33/173 (19.1%)	36/107 (13.8%)
Vitamin B_{12}	0/91 (0%)	0/162 (0%)	0/101 (0%)
Thiamine (Vitamin B_1)	2/33* (6.1%)	22/71† (31.0%)	17/36† (47.2%)

*significant differences between groups by ANOVA.
†different superscripts denote significant differences between groups by ANOVA.

Iron, Ferritin, Hemoglobin, and Vitamin B_{12}

The effects of RYGB on iron and vitamin B_{12} metabolism have been extensively studied, described,[1–8,22–26] and reviewed.[27] Iron is normally absorbed in the duodenum, which is excluded from the food stream after RYGB, thus inevitably leading to iron deficiency. This propensity toward iron deficiency is often compounded by ongoing menstrual blood loss in women of childbearing age, since the vast majority of patients undergoing RYGB are women. Because of the high prevalence of anemia and iron deficiency after RYGB, virtually all centers routinely prescribe iron postoperatively to all menstruating women and many routinely prescribe iron to all patients after RYGB.[25,26]

Vitamin B_{12} is normally absorbed in the terminal ileum. However, in order for absorption to occur, vitamin B_{12} must be linked to intrinsic factor, a glycoprotein produced by parietal cells in the stomach and cleaved in the presence of hydrochloric acid and pepsin. This usually occurs in the distal stomach and duodenum, both of which are bypassed after a RYGB. Studies have documented that some centers also routinely prescribe vitamin B_{12} supplementation for all patients, whereas others monitor serial levels and prescribe supplementation when indicated.[25] Brolin et al.[8] have documented that although these deficiencies are relatively commonplace, they are rarely the cause of clinically significant adverse effects. Adachi and colleagues[27] have shown that oral vitamin B_{12} can reverse this problem. In addition, it is important to note that vitamin B_{12} deficiency was not present in any of our patients preoperatively. Our practice is to prescribe iron routinely to all patients after RYGB and to follow vitamin B_{12} levels every 6 months, prescribing supplemental vitamin B_{12} only when clinically indicated.

Our data regarding anemia, iron, ferritin, and vitamin B_{12} are mostly consistent with others in the literature. We did note a higher incidence of abnormally low hemoglobin levels in men than in women. The presence of inflammation, liver disease, and other medical comorbidities might explain the discrepancies in iron levels that were documented across obese patient populations and the two sexes.

Serum iron represents only 0.1% of the total body stores. Serum iron shows diurnal and positional variation. It is an acute phase reactant whose concentration at any given time is variable.[28] Although the concentration of ferritin often correlates well with the amount of stored iron in tissue, this storage system is dynamic, interacting with many factors that do not directly represent nutritional status. Inflammatory cytokines, including IL-1 and IL-6, promote ferritin translation to increase tissue storage at the expense of decreasing serum iron values.[29] Liver cell damage, defective liver metabolism, and insulin resistance have also been suggested to affect ferritin levels and iron metabolism.[30,31]

Thiamine

Thiamine deficiency can occur acutely after any bariatric surgical procedure in a patient that experiences prolonged vomiting and can be associated with severe neurological symptoms, which may be irreversible.[13–21] To our knowledge, no prior studies have evaluated the incidence of thiamine deficiency preoperatively. Our data demonstrated an alarming prevalence for thiamine deficiency preoperatively, especially among African Americans (31%) and Hispanics (47%). Therefore, prevention, early detection, and treatment of thiamine deficiency are critical to avoid a potentially devastating complication.

We currently recommend preoperative thiamine screening and perioperative replacement with 100 mg thiamine intramuscularly in the immediate postoperative period. Furthermore, any patient complaining of or presenting with prolonged vomiting should

be suspected of having a possible thiamine deficiency and treated as such empirically because the assay is not generally available immediately, and there are no adverse effects from thiamine replacement therapy.

Calcium and Vitamin D

Although calcium deficiency has not been described after RYGB, studies have recently been published describing the long-term effects of RYGB on bone metabolism.[10–11] Both of these studies demonstrated increased bone turnover in patients after RYGB, but the mechanism was uncertain. Both suggested that calcium and vitamin D supplements should be given to patients postoperatively, although the dose was unclear. We currently prescribe 1200 mg of calcium citrate with vitamin D to all our patients postoperatively, but this dose may indeed prove too low as new data become available.

Vitamin D deficiency is a major public health problem in the United States, It has been estimated that 21 to 58% American adults and adolescents are vitamin D deficient, depending on race and gender.[32] In addition, Buffington and colleagues,[12] in 1993, documented a 62% prevalence of vitamin D deficiency in morbidly obese patients and DiGiorgi and colleagues[33] found a 16% prevalence incidence of preoperative 25-OH vitamin D deficiency and advocated preoperative correction. Our data show an even higher prevalence of 25-OH vitamin D deficiency preoperatively (68%), which occurs in all racial/ethnic groups. Therefore, our current practice is to screen all candidates for 25-OH vitamin D deficiency, and if present, correct it either preoperatively or immediately postoperatively with ergocalciferol (50,000 IU) given orally once per week for 8 weeks.

CONCLUSION

In conclusion, patients undergoing RYGB rarely suffer from protein-calorie malnutrition. However, preoperative iron and ferritin deficiencies are common and correlate with the presence of anemia when evaluated for gender and age. Preoperative vitamin B_{12} levels are almost always normal. Of greater significance is the high prevalence of thiamine (29%) and 25-OH vitamin D (68%) deficiency, which may predispose these patients to serious neurological and metabolic complications postoperatively if not identified early and corrected.

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Staged Laparoscopic Infusion of Hyperthermic Intraperitoneal Chemotherapy After Cytoreductive Surgery

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Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy (HIPC) is a promising treatment for patients with peritoneal malignancies. Traditionally, HIPC is performed concurrently with cytoreductive surgery. However, this strategy is associated with significant morbidity and mortality. In this report, we describe our initial experience with staged laparoscopic infusion of HIPC. Five patients underwent complete open cytoreductive surgery followed by staged laparoscopic HIPC several weeks later. Primary malignancies included adenocarcinoma of the ileum (one patient), adenocarcinoma of the appendix (three patients), and adenocarcinoma of the gallbladder (one patient). At a subsequent operation, we performed laparoscopic HIPC. Quality of life was measured with the Functional Assessment of Cancer Therapy-Colon Subscale (FACT-C). Mean inflow and outflow cannula temperatures were 42.1° C and 40.5° C, respectively. Mean peritoneal perfusion flow rates were 689.8 ml/minute. The hospital stay for all patients was 1 to 2 days. One patient developed postoperative cellulitis, one patient died of progressive tumor, and four patients are alive without tumor progression. Quality-of-life measurements had returned to baseline 4 months after treatment. Staged laparoscopic HIPC after open cytoreductive surgery is safe, feasible, and can achieve uniform temperatures and perfusion flow rates. Although the results of this pilot study are encouraging, additional studies are required to determine long-term survival and quality of life. (J GASTROINTEST SURG 2006;10:1038–1043)

KEY WORDS: Carcinomatosis, laparoscopic, cytoreduction, hyperthermia

The survival and quality of life of patients with peritoneal metastases remains poor. The results of systemic chemotherapy for patients with peritoneal metastases from gastrointestinal malignancies have been disappointing. Cytoreductive surgery is a systematic attempt to remove all or nearly all peritoneal tumor nodules. Hyperthermic intraperitoneal chemotherapy (HIPC) is administered after cytoreduction to treat residual microscopic disease. The results from nonrandomized single- and multi-institutional studies suggest that this aggressive regional treatment may improve the survival of selected patients with peritoneal metastases from gastrointestinal malignancies.[1–4] A recent prospective randomized trial from the Netherlands demonstrated that cytoreductive surgery plus HIPC with mitomycin-C improves the survival of patients with peritoneal metastases from appendiceal and colorectal cancer.[5]

Although cytoreductive surgery plus HIPC is a promising therapy for patients with peritoneal metastases, the treatment is associated with significant morbidity. Complications include enteric fistula, small bowel obstruction, pancreatitis, and neutropenia.[6] HIPC is generally administered immediately after cytoreduction during the same anesthesia. However, the concurrent administration of immunosuppressive chemotherapy with major abdominal surgery may contribute to the complications of this aggressive regional treatment. Staged laparoscopic delivery of HIPC several weeks after cytoreductive surgery may prevent or reduce the severity of these complications. Nevertheless, the effectiveness of

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laparoscopic HIPC may be compromised by postoperative adhesions that limit perfusion flow rates and uniform peritoneal temperatures. The purpose of this pilot study is to determine the feasibility, safety, and outcomes of staged laparoscopic HIPC.

METHODS

This prospective pilot study of five patients with peritoneal metastases was approved by the Institutional Review Board at the University of Minnesota. Each patient had evidence of peritoneal metastases based on computed tomography findings and tissue diagnosis. Patients had not undergone previous abdominal radiation or intraperitoneal chemotherapy. The patients were otherwise healthy and had no radiographic evidence of extra-abdominal metastatic disease. Patients with liver metastases were excluded. Malignant ascites was not an exclusion criterion. Patients with uncontrolled cardiovascular disease were excluded. Additional inclusion criteria were granulocyte count greater than 1500, platelet count greater than 100,000, serum bilirubin less than 1.5, serum creatinine less than 1.5, and a serum albumin greater than 3.0. All patients had initial open cytoreductive surgery with removal of all peritoneal nodules; resection included at least one of the following procedures: small bowel resection, large bowel resection, omentectomy, peritonectomy, hysterectomy, oophorectomy, appendectomy, radical cholecystectomy, and portal lymph node dissection. Successful cytoreductive surgery was considered complete if all tumor nodules greater than 2.5 mm were removed.[1]

Three to 5 weeks after open cytoreductive surgery, patients underwent laparoscopic HIPC. The interval between cytoreductive surgery and laparoscopic HIPC was more often chosen for practical as opposed to theoretical purposes. Most of the patients were initially treated by referring surgeons. Thus, the time interval was usually determined by clinic and operating room schedules, review of pathology, and individual patient preference. A long interval (>6 weeks) between cytoreductive surgery and HIPC is not desirable because of the potential for recurrent tumor nodules.

For laparoscopic HIPC, a blunt tip trocar was placed into the peritoneal cavity under direct vision, pneumoperitoneum was established, and the laparoscope was inserted through this trocar. Additional 5 mm or 11 mm ports were placed in the right and left upper quadrants after inspection of the abdominal cavity. After thorough inspection of the abdomen for residual disease, adhesions were lysed. In one case, residual peritoneal tumor nodules were removed laparoscopically. Two inflow catheters were placed in the upper quadrants and one to two outflow catheters were placed in the lower quadrant and pelvis. Inflow and outflow temperature probes were inserted in the upper and lower abdomen, respectively (Fig. 1).

Once the peritoneal temperatures reached 40° C, mitomycin-C (30 mg/m^2) was infused over 90 minutes through the Viacirq (ThermaSolutions, Canonsburg, PA) peritoneal perfusion unit. This device records the flow rates, time of therapy, and the inflow and outflow temperatures. The operating table was rotated every 10 minutes, and the abdomen was agitated throughout the infusion to allow even exposure of peritoneal surfaces to the heated chemotherapy. After the infusion, the laparoscope was reinserted and the abdomen was examined for evidence of tissue injury or bleeding. The peritoneal cavity was irrigated with 2 L of normal saline, the port sites were closed in a standard fashion, and the patient was taken to the recovery room.

Patients were seen in clinic 1 to 2 weeks after surgery and then every 4 months after treatment. Abdominal/pelvic CT was performed every 4 months. Quality-of-life measurements were determined before treatment and at 4-month intervals by using the Functional Assessment of Cancer Therapy-Colon Subscale (FACT-C; version 4) instrument.[7] The FACT-C survey includes five subscales measuring physical well-being (PWB), social/family well-being (SWB), emotional well being (EWB), functional well-being (FWB), and a colon subscale (CCS). Higher scores indicate a higher quality of life. The Trial Outcome Index is PWB + FWB + CCS. FACT-General score is PWB + FWB + SWB + EWB. FACT-C score is PWB + FWB + SWB + EWB + CCS.

RESULTS

The subjects (Table 1) in this pilot study were selected from a larger group of patients with peritoneal metastases treated with cytoreductive surgery and HIPC at our institution. Patient 1 underwent a right hemicolectomy for a presumed cecal carcinoma at an outside institution; limited peritoneal metastases from an appendix tumor were identified and removed during the initial operation. The patient was later referred to our institution for laparoscopic HIPC. Patient 2 had a recent history of resected gallbladder cancer and subsequently developed isolated peritoneal nodules. We performed cytoreduction to remove these tumor nodules; HIPC was delayed to establish the final histologic diagnosis. Laparoscopic HIPC was performed after metastatic gallbladder cancer was confirmed. The final three patients

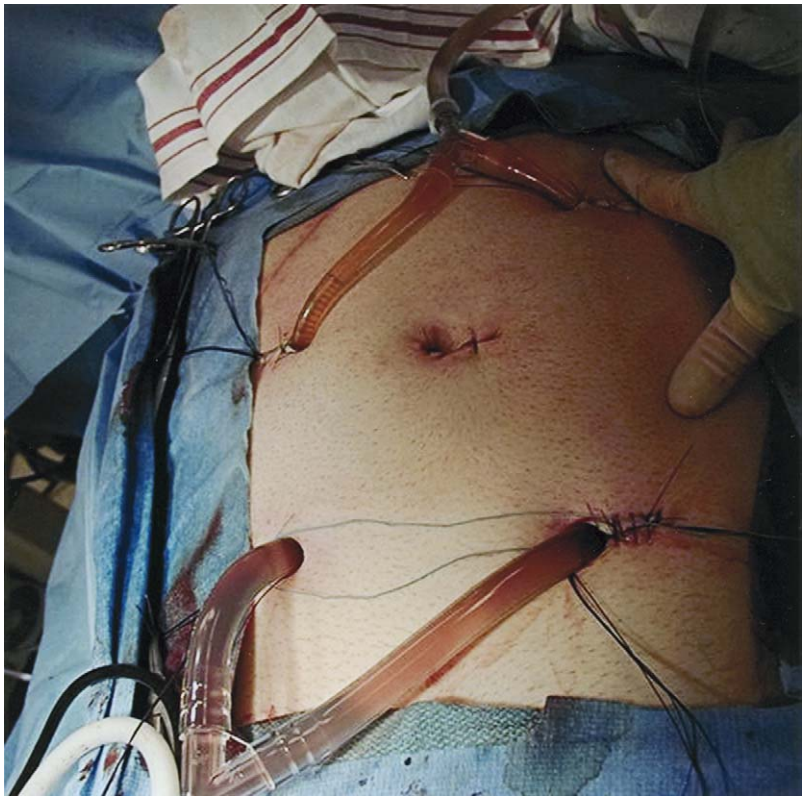

Fig. 1. Laparoscopic infusion of HIPC. Two inflow catheters and a temperature probe have been inserted in the upper abdomen. Two larger outflow catheters and a temperature prove have been placed in the lower abdomen and pelvis.

(patients 3, 4, and 5) were women who underwent cytoreductive surgery by gynecologic oncologists for presumed ovarian malignancies. The final histologic diagnosis was appendiceal malignancy in two patients and small bowel adenocarcinoma in one patient. One of the two patients with appendiceal malignancy had persistent tumor nodules that were removed during laparoscopy for HIPC.

Laparoscopic HIPC was successfully performed on all five patients. The inflow and outflow temperatures are recorded in Fig. 2. Mean inflow and outflow cannula temperatures were 42.1° C and 40.5° C, respectively. Intraperitoneal hyperthermia was maintained throughout the laparoscopic infusion. Mean peritoneal perfusion flow rates were 689.8 ml/minute. These temperature profiles and peritoneal perfusion flow rates are similar to those achieved in our larger series of patients undergoing open HIPC (data not shown).

The estimated blood loss was 100 ml or less for all patients. The only postoperative complication was cellulitis at a port site in one patient, requiring intravenous antibiotics. All patients were discharged home 1 to 2 days after laparoscopic HIPC.

Patient survival is shown in Table 2. One patient with peritoneal metastases from adenocarcinoma of the ileum died 7 months after treatment. The remaining four patients are alive 12 months to 39

Table 1. Patient characteristics

Patient	Malignancy	Age	Gender	LOS (day)	EBL (ml)	Complications	Operating time (min)
1	Appendix	65	Male	1	0	None	259
2	Gallbladder	60	Male	2	100	None	250
3	Appendix	45	Female	1	0	None	165
4	Appendix	52	Female	1	100	None	203
5	Ileum	70	Female	2	20	Cellulitis	204

LOS = length of stay; EBL = estimated blood loss.

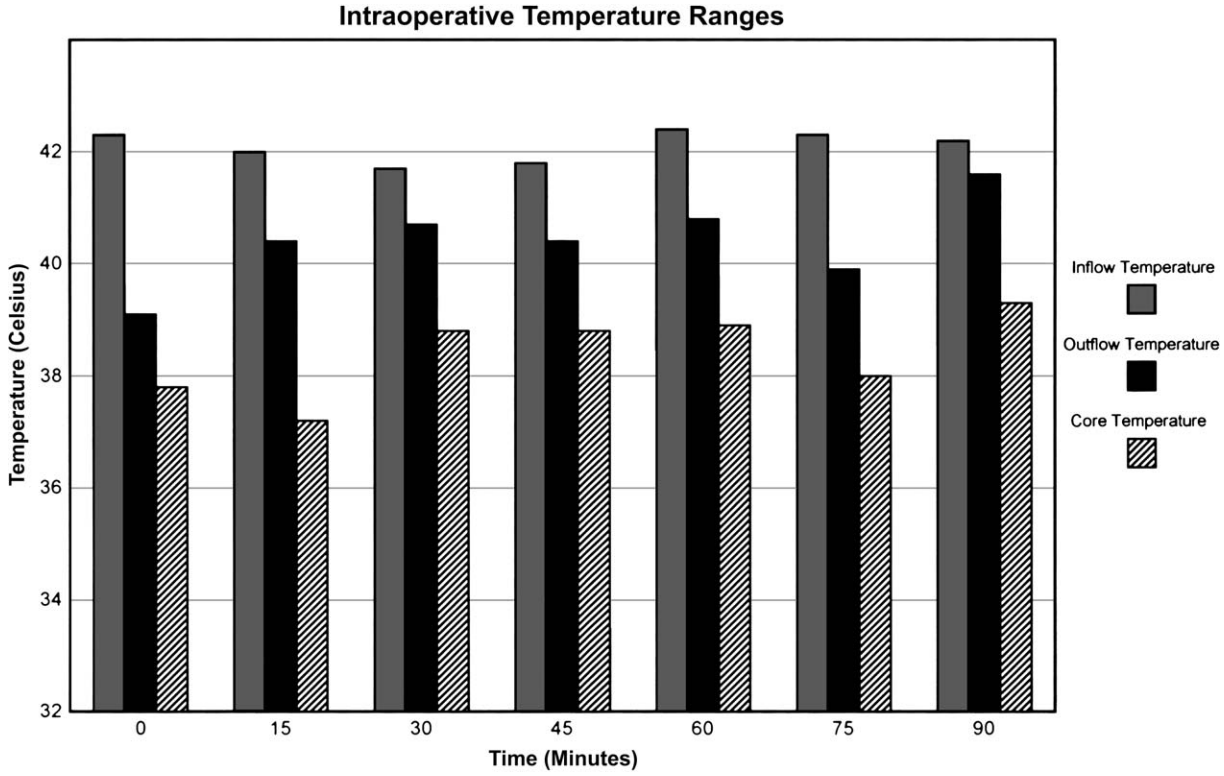

Fig. 2. Mean intraoperative temperature profiles of five patients during laparoscopic HIPC. Inflow temperatures were recorded under the hemidiaphragm near the inflow cannula. Outflow temperatures were recorded in the pelvis near the outflow cannula. Core temperatures were recorded from an esophageal temperature probe.

months after treatment. To date, surveillance abdominal/pelvic CT have not demonstrated recurrent tumor in these surviving patients.

All patients completed FACT-C quality-of-life surveys before treatment and at 4-month intervals. Higher scores indicate a higher quality of life. Trial Outcome Index, FACT-General, and FACT-C scores before and 4 months after treatment are listed in Table 3. Meaningful statistical comparisons between pretreatment and 4-month measurements were not possible because of small patient numbers. Nevertheless, quality-of-life measurements had noticeably returned to baseline levels 4 months after treatment.

DISCUSSION

The development of peritoneal metastases from gastrointestinal malignancies has generally been considered a terminal event with few treatment options. The outcome of patients with peritoneal metastases was evaluated in a multi-institutional prospective study from France called Evolution of Peritoneal Carcinomatosis.[8] In this study, researchers followed

Table 2. Patient survival after laparoscopic hyperthermic intraperitoneal chemotherapy

Patient	Malignancy	Alive	Length of follow-up
1	Appendix	Yes	25 months
2	Gallbladder	Yes	39 months
3	Appendix	Yes	12 months
4	Appendix	Yes	12 months
5	Ileum	No	7 months

Table 3. Quality of life scores using FACT instrument

Measurement	Pretreatment	4 months
TOI	61	58.2
FACT-G	83.7	81.8
FACT-C	103.3	102.8

Higher scores indicate higher quality of life.
TOI = Trial Outcome Index; FACT-G = Functional Assessment of Cancer Therapy-General; FACT-C = Functional Assessment of Cancer Therapy-Colon Subscale.

370 patients with nongynecologic metastases and reported a median overall survival of 3.1 months. For gastric, colorectal, pancreatic, and unknown primary tumors, median survival was 3.1 months, 5.2 months, 2.1 months, and 1.5 months, respectively.

Cytoreductive surgery plus HIPC may improve the survival of selected patients with peritoneal metastases. Sugarbaker and Chang[1] reported a 78% 5-year survival rate for patients with appendiceal malignancies after complete cytoreduction (removal of all peritoneal nodules > 2.5 mm) and HIPC. Glehen et al.[2] reported a median survival of 21.3 months for patients with peritoneal metastases from gastric cancer after complete cytoreductive surgery and HIPC. An international multi-institutional registry recently evaluated the survival of 506 patients with peritoneal metastases from colorectal cancer; median survival was 19 months and 5-year survival was 19% after cytoreductive surgery plus HIPC.[3] Feldman et al.[4] reported a median survival of 92 months for patients with peritoneal mesothelioma. For these various malignancies, cytoreductive surgery plus HIPC seems to improve overall survival as compared with palliative treatment.

However, these data were obtained from nonrandomized studies of highly selected patients. Recently, investigators at the Netherlands Cancer Institute reported the results of a randomized clinical trial involving 105 patients with colorectal cancer (including appendiceal malignancies) and peritoneal metastases or positive cytology from ascites.[5] Patients were randomly assigned to two arms: standard therapy or experimental therapy. Standard therapy included palliative surgery, if necessary, and systemic chemotherapy (5-fluorouracil, leucovorin). Experimental treatment included cytoreductive surgery, HIPC (mitomycin-C), and systemic chemotherapy. Median survival with the experimental treatment was significantly improved over standard therapy (22.3 vs. 12.6 months; $P = 0.032$). The actuarial 5-year survival rate in these patients was 20% after cytoreduction and HIPC. This well-designed study provides further evidence that aggressive regional treatment may improve overall patient survival.

Patients with peritoneal metastases suffer poor quality of life from debilitating ascites, bowel obstruction, abdominal pain, and early satiety. Thus, treatment strategies must address improvement in patient quality of life as well as long-term survival. McQuellon et al.[9,10] conducted a prospective evaluation of quality-of-life measurements by using the FACT-C and other quality-of-life instruments in 64 patients treated with cytoreductive surgery plus HIPC. Overall FACT-C scores decreased considerably in the early postoperative assessment period when compared with baseline scores, but had returned to baseline 3 months after treatment. The quality-of-life scores appear to plateau at 6 to 12 months and remain high for long-term survivors.

Despite the potential benefit in overall survival, the morbidity and mortality rates of cytoreductive surgery plus HIPC are high. In the prospective randomized trial from the Netherlands, the median estimated blood loss was 3.9 L, the median hospital stay was 29 days, and the postoperative mortality rate was 8%.[5] Shen et al.[11] reported a 12% operative mortality rate in a series of 77 patients undergoing cytoreductive surgery plus HIPC at a high-volume center. Stephens et al.[6] from the Washington Cancer Institute reported a 27% grade III/IV complication rate and a 1.5% operative mortality rate. Two of the 3 deaths in this series were attributed to neutropenic sepsis. Postoperative complications such as bleeding, pneumonia, intra-abdominal abscess, pancreatitis, and gastrointestinal fistula can be life threatening, especially when patients are immunocompromised and neutropenic from systemic absorption of intraperitoneal chemotherapy.

A pilot study of seven patients treated with laparoscopic HIPC was reported by Chang et al.[12] from the National Cancer Institute. Five patients had surgically resectable cancer (pancreas or gastric) without peritoneal metastases and underwent laparoscopic HIPC as a neoadjuvant treatment strategy. The remaining two patients had intractable ascites from peritoneal metastases and underwent laparoscopic HIPC without cytoreductive surgery. So, none of the patients in this series underwent staged laparoscopic HIPC after open cytoreductive surgery. Three laparoscopic ports were used to lyse adhesions and infuse cisplatin (250 mg/m^2) with or without mitomycin-C (8mg/L). Pharmacokinetic data demonstrated that the area under the curve values for cisplatin in the perfusate were 6.5-fold higher than that of the plasma; for mitomycin-C, the values in the perfusate were 22-fold higher than that of the plasma. The length of stay after the procedure was 3 to 8 days, and no treatment-related morbidities or mortalities were reported.

The rationale for staged laparoscopic infusion of HIPC after open cytoreductive surgery is to allow a period of healing (especially for gastrointestinal anastomoses) before rendering the patient immunocompromised with HIPC. Of course, this approach requires two separate operations with general anesthesia and may be limited by the development of early postoperative adhesions. In this pilot study, we have demonstrated that staged laparoscopic HIPC is feasible after cytoreductive surgery. The temperature profiles and peritoneal perfusion flow rates with laparoscopic HIPC are similar to those

achieved in our larger series of patients undergoing open HIPC. The hospital stay is short, bleeding is minimal, and patients are discharged early after laparoscopic HIPC. Moreover, short-term quality-of-life measurements after staged laparoscopic HIPC had returned to baseline levels 4 months after treatment.

An alternative strategy could involve placement of adhesion barriers and peritoneal catheters at the time of cytoreduction and then later delivery of staged intraperitoneal chemotherapy without laparoscopy. However, laparoscopy offers several important advantages over this approach: (1) identification and removal of persistent or recurrent tumor nodules, (2) lysis of adhesion to ensure uniform distribution of heat and chemotherapy, and (3) proper placement of inflow and outflow catheters.

CONCLUSIONS

To the best of our knowledge, this pilot study is the first report demonstrating the feasibility and safety of staged laparoscopic HIPC after cytoreductive surgery. Postoperative adhesions after open surgery were successfully managed laparoscopically and did not negatively impact peritoneal perfusion or temperatures. Although adhesion barriers (SepraFilm) were not used in this study, these products may facilitate staged laparoscopic HIPC. This staged approach may reduce complications, especially neutropenic sepsis, associated with cytoreductive surgery and HIPC. Other potential advantages include less bleeding, shorter hospital stay, and decreased treatment costs. Staged laparoscopic HIPC may also shorten overall recovery and allow earlier initiation of systemic chemotherapy.

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The Vascular Nature of Hemorrhoids

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The arterial blood supply of the internal hemorrhoidal plexus is commonly believed to be associated with the pathogenesis of hemorrhoids. Ultrasound-supported proctoscopic techniques with Doppler-guided ligature of submucosal rectal arteries have been introduced for the therapy of hemorrhoids. The present investigation focuses on caliber and flow changes of the terminal branches of the superior rectal artery (SRA) supplying the corpus cavernosum recti (CCR) in patients with hemorrhoids. Forty-one outpatients (17 female, 24 male; mean age 48 years) with hemorrhoids of Goligher grades I–IV were compared with 17 healthy volunteers (nine female, eight male; mean age 29 years) by means of transperineal color Doppler ultrasound. The mean caliber of the arterial branches in the study group with hemorrhoids was 1.87 ± 0.68 mm (range, 0.6 to 3.60 mm) and 0.92 ± 0.15 mm (range, 0.6 to 1.2 mm) in the control group ($P < 0.001$). The arterial blood flow was significantly higher in patients with hemorrhoids than in the control group (mean 33.9 vs. 11.9 cm/second, $P < 0.01$). Our findings demonstrate that increased caliber and arterial blood flow of the terminal branches of the SRA are correlated with the appearance of hemorrhoids. We suggest that the hypervascularization of the anorectum contributes to the growth of hemorrhoids rather than being a consequence of hemorrhoids. Transperineal color Doppler ultrasound (CDUS) is an appropriate method to assess these findings in patients with hemorrhoids. (J GASTROINTEST SURG 2006;10:1044–1050)

KEY WORDS: Hemorrhoids, color Doppler ultrasound, caliber, arterial blood flow, corpus cavernosum recti

Clinical-anatomical studies of the anorectal region support the various hypotheses on the pathogenesis of hemorrhoids.[1–5] The role of vascular hyperplasia, muscle tone, and tissue elasticity in the development of hemorrhoidal cushions is still controversially discussed. However, it is difficult to measure each of these contributing factors separately because they form an undistinguishable unity in clinically apparent hemorrhoids.[6]

Several studies have already demonstrated changes in anal physiology by determining, before and after hemorrhoidectomy, the anal resting pressure in patients with hemorrhoids. Overactivity of the internal anal sphincter muscle in patients with hemorrhoids is believed to be a consequence rather than a primary cause for the development of symptomatic hemorrhoids.[7–10] On the other hand, hyperplasia of the arteriovenous network within the anorectal submucosa (corpus cavernosum recti [CCR])[11] results in an increased vascular pressure within hemorrhoids that might account for the increased anal resting tone.[12] The link between increased muscular pressure and vascular hyperplasia has not been proven, however.

Traditional therapeutic strategies focused on resection of the dilated blood vessels with the covering anoderm (Milligan-Morgan,[2] Parks[13]) and ligature of the main supplying blood vessels. In contrast, newer techniques either aim to reduce the vascularity of hemorrhoidal tissue,[14,15] or aim for permanent reposition of the prolapsed hemorrhoidal tissue and amelioration of the venous drainage, rather than reduction of the arterial inflow, by clipping rectal mucosa and submucosa.[16] These modern techniques (e.g., hemorrhoidal artery ligation [HAL][14]) and the stapled hemorrhoidopexy (Longo procedure[16]) are increasingly accepted by clinicians. Nevertheless, the anatomical background of these procedures is not fully elucidated.

We have recently shown that transmural branches of the superior rectal artery (SRA) play a crucial role in the arterial blood supply of the CCR.[17] By means of standardized transperineal ultrasonography, the

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vascularity of the CCR and the rectal wall can be well visualized, thus enabling a precise assessment of the blood supply of the distal anorectum before planning the surgical technique.[18]

The aim of this investigation was to determine morphological and physiological alterations of the terminal branches of the SRA in patients with various grades of hemorrhoids by means of transperineal color Doppler ultrasound (CDUS). In addition to previous studies,[19] we performed measurements of the caliber of the terminal branches of the SRA and the arterial blood flow associated with hemorrhoids. The outcome in the patient group undergoing stapled hemorrhoidopexy was analyzed with regard to the arterial blood supply of the CCR before and after the procedure.

PATIENTS AND METHODS

Forty-one outpatients (17 female, 24 male; mean age, 48 ± 12 years) with symptomatic hemorrhoids of Goligher grades I–IV[3] of several years duration (mean, 6.8 years), who were treated at the Department of General and Transplant Surgery, Innsbruck Medical University, Innsbruck, Austria were included in this study. The medical history of all the patients was carefully studied, and all patients underwent inspection, digital exploration, proctoscopy, and transperineal CDUS before intervention. Clinical symptoms of these patients according to the grade of hemorrhoids are listed in the Table 1.

Therapy was adapted according to the individual grade, including conservative treatment for grade I, rubber band (Barron) ligature for symptomatic grade II, and HAL, stapled hemorrhoidopexy, or Milligan-Morgan[2] for grades III–IV. Patients with anorectal disorders such as colorectal cancer, chronic inflammatory bowel disease, or perineal trauma were excluded from this study.

Seventeen healthy volunteers served as control group (n = 17; nine female, eight male; mean age, 29 ± 15 years). Symptomatic hemorrhoids and other anorectal disorders were excluded by physical examination. Both groups underwent standardized transperineal CDUS examination (HDI 5000, Philips, Hamburg, Germany) as for preoperative assessment, which was performed by a single radiologist who was blinded to whether the subject was in the study group or was one of the controls. Informed consent was obtained before inclusion in the study.

Table 1. Clinical symptoms of different grades of hemorrhoids

	Symptoms (ranked by prevalence)
Grade I	Bleeding, anal pruritus
Grade II	Bleeding, anal pruritus, burning, moistening, anal pain
Grade III	Bleeding, burning, anal pruritus, anal pain, moistening
Grade IV	Anal pain, bleeding, anal pruritus

A broadband linear transducer working at 4–7 MHz (HDI 5000, Philips) was positioned in a longitudinal scan at the perineum, with the patient or volunteer in a lateral position. Using CDUS, the distal rectum and the anal canal were investigated for arterial vessels in and around the rectum based on the following anatomical criteria: All vessels draining into the CCR, including submucosal branches and the previously described external arterial branches perforating the rectal wall right above the levator ani muscle,[17] were included in this study. The extremely variable middle rectal artery and the inferior rectal artery, which do not contribute to the blood supply of the CCR, were excluded. Arterial blood flow was measured in centimeters per second. In addition, calibers of the respective terminal branches of the SRA were determined. In the subgroup of patients who underwent stapled hemorrhoidopexy (n = 9), the outcome of the technique with regard to the arterial blood supply of the CCR before and after the procedure was analyzed. Patients were reinvestigated at 4 weeks postoperatively.

Statistical Analysis

Values are expressed as range and means with standard deviations (SD). Differences in caliber and arterial blood flow between patients and the control group were tested by the two-tailed unpaired Student's *t* test for continuous, normally distributed data. The nonparametric Kruskal-Wallis test was applied whenever indicated. Statistical significance was defined as $P < 0.05$. SPSS for Windows 11.0 software (SPSS Inc., Chicago, IL) was used for all analyses.

RESULTS

In both groups, arterial flow measurements corresponded in a linear way with the caliber of the arterial vessels as shown in Fig. 1. Both the flow and the caliber of the vessels increased with age in the study group (Fig. 2, *A*, *B*).

Healthy individuals (control group, n = 17) showed the following findings, which were used as gold standard. The mean caliber of visible arterial branches of the SRA in the control group was 0.92 ± 0.15 mm (range, 0.6 to 1.2 mm), and the mean arterial blood flow was 11.9 ± 4.0 cm/second (range, 5.0 to 21.0 cm/second). In 75% of the volunteers, a clearly

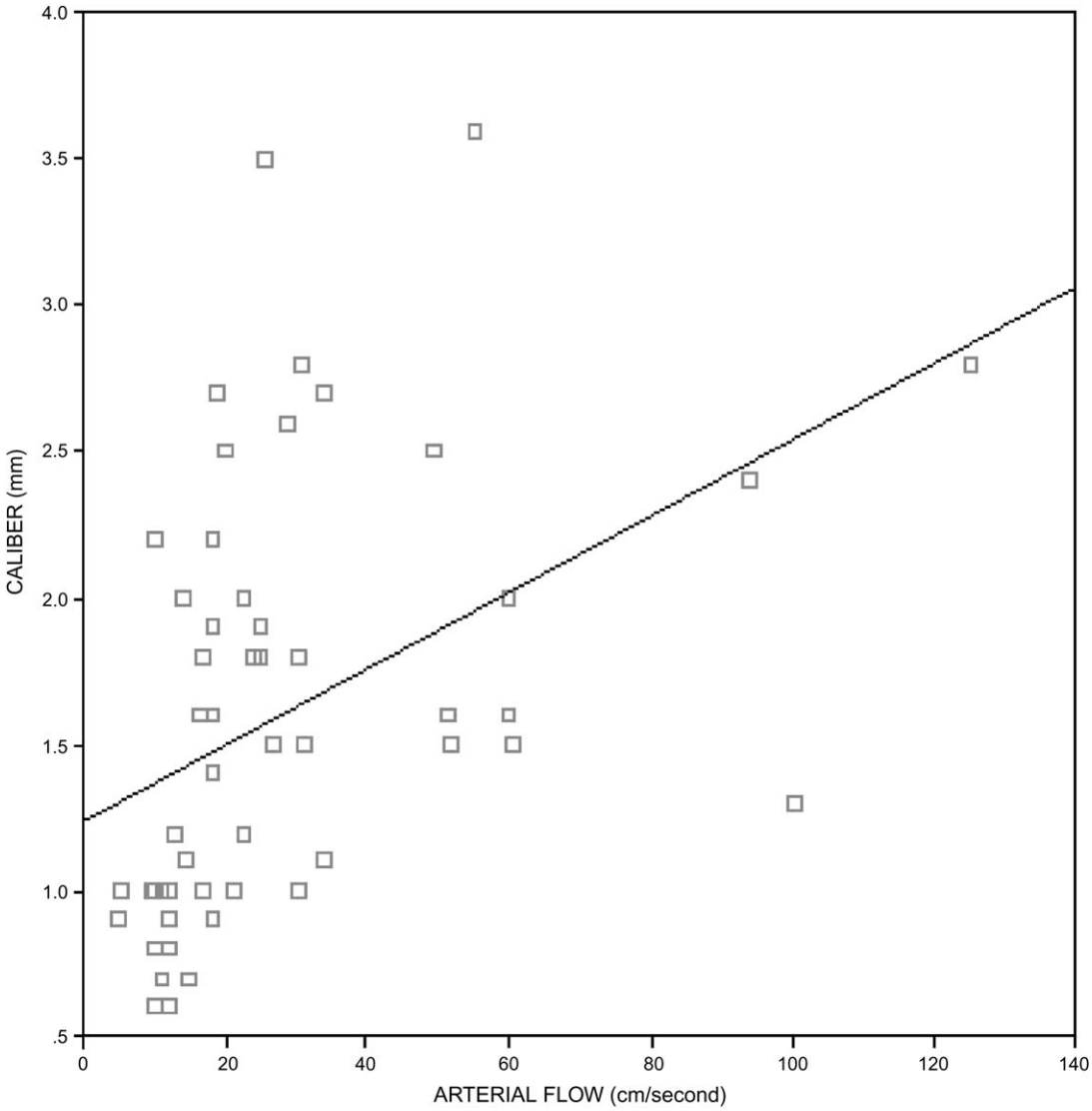

Fig. 1. Graph showing a positive correlation between the caliber of the terminal branches of the SRA and the increasing arterial blood flow.

definable artery with corresponding branches to the CCR was found (Fig. 3, *A*, *B*).

In most patients, a single artery was detectable. It has to be assumed, however, that some arteries remained undetectable with the methods applied. In a minority of these individuals, the blood supply was found to be originating from multiple small arteries with only low flow. All analyzed arterial branches described a transmural or submucosal course within the rectal wall entering the CCR.

All patients with hemorrhoids showed at least one clearly identifiable major artery deriving from the SRA, with branches after the above-described topographical course in each individual (Fig. 4 *A*, *B*). The mean caliber of the terminal branches was 1.87 ± 0.68 mm (range, 0.6 to 3.60 mm), and the mean arterial blood flow was 33.9 ± 25.2 cm/second (range, 10 to 125 cm/second). A statistically significant difference in caliber was found between patients and control group ($P < 0.001$), as well as significantly higher arterial blood flow in the terminal branches of the SRA within the patient group as compared with the control group ($P < 0.01$). Fig. 5 shows arterial flow and caliber according to the various grades of hemorrhoids as compared with healthy controls (grade 0). Patients were divided in two age groups with a cutoff at 25 years.

The patients undergoing stapled hemorrhoidopexy showed no postinterventional changes in the arterial caliber (2.28 ± 0.7 mm vs. 2.38 ± 0.7 mm), $P = 0.7$ (Wilcoxon assay), and no significant difference in the arterial blood flow at 3 weeks after the

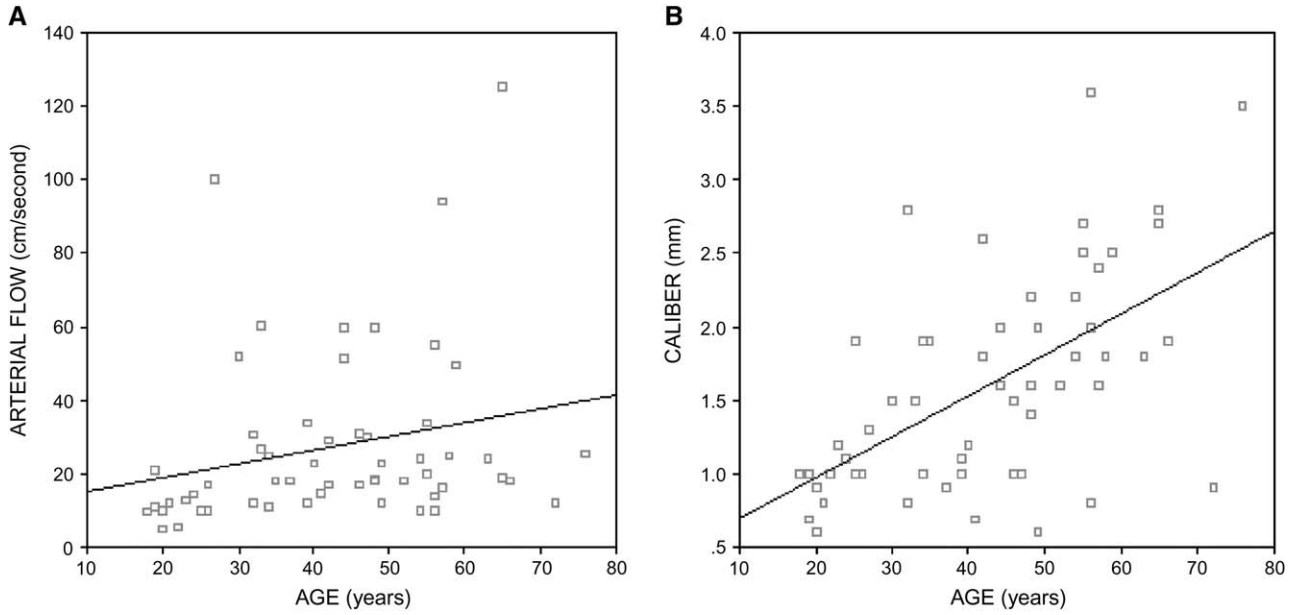

Fig. 2. Graphs demonstrating an increase of arterial blood flow (**A**) and caliber (**B**) with age in individuals with and without hemorrhoids (grades 0–IV).

procedure (59.6 ± 31.8 vs. 41.4 ± 15.5 cm/second, $P = 0.2$ (Wilcoxon assay).

DISCUSSION

Our study provides strong evidence that the arterial blood supply of the CCR is of relevance in the development of hemorrhoidal cushions. Vascular dilation and increased blood flow suggest that there might exist an increased arterial inflow rather than a venous stasis or outflow problem supporting the development of hemorrhoids.

Demographic data of the control group were different, in particular with regard to age. However, when excluding individuals younger than 25 years from the analysis in the three groups of defined grades of hemorrhoids (group A including grade 0, group B including grades I/II, and group C including grades III/IV), the data still show an enlarged caliber of the investigated blood vessels and an

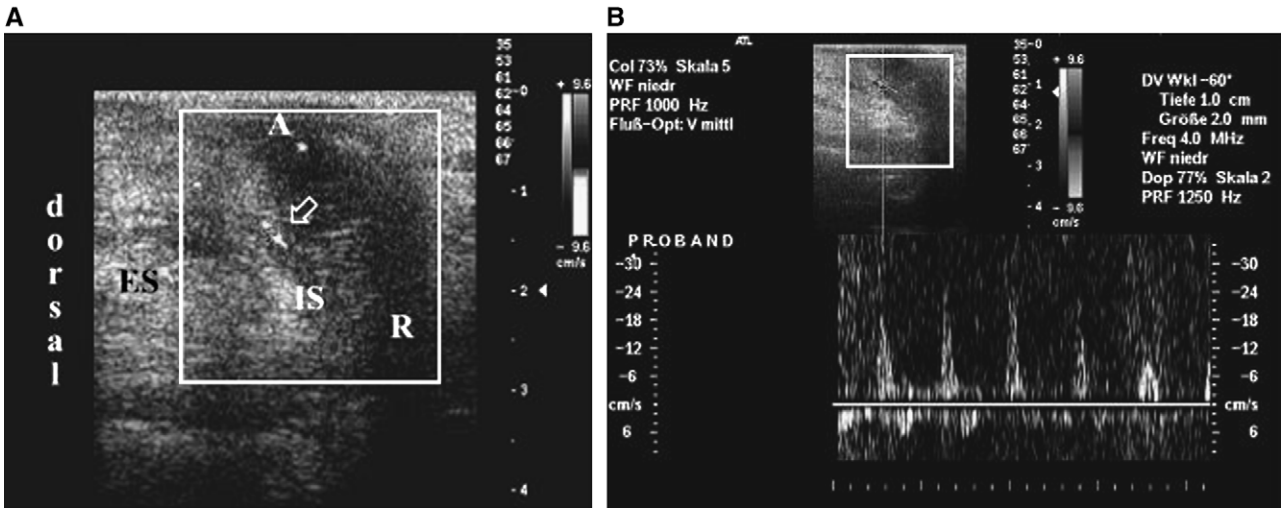

Fig. 3. A 19-year-old male patient with no anorectal abnormalities; transperineal color Doppler ultrasound, longitudinal scan. (**A**) A dorsally situated terminal branch of the SRA is shown (*open arrow*) running through the rectal submucosa (caliber 1 mm). A, anal canal; R, rectum; IS, internal anal sphincter; ES, external anal sphincter. (**B**) Same patient and arterial vessel within the dorsal rectal wall. Doppler measurement of the arterial blood flow (21.0 cm/second).

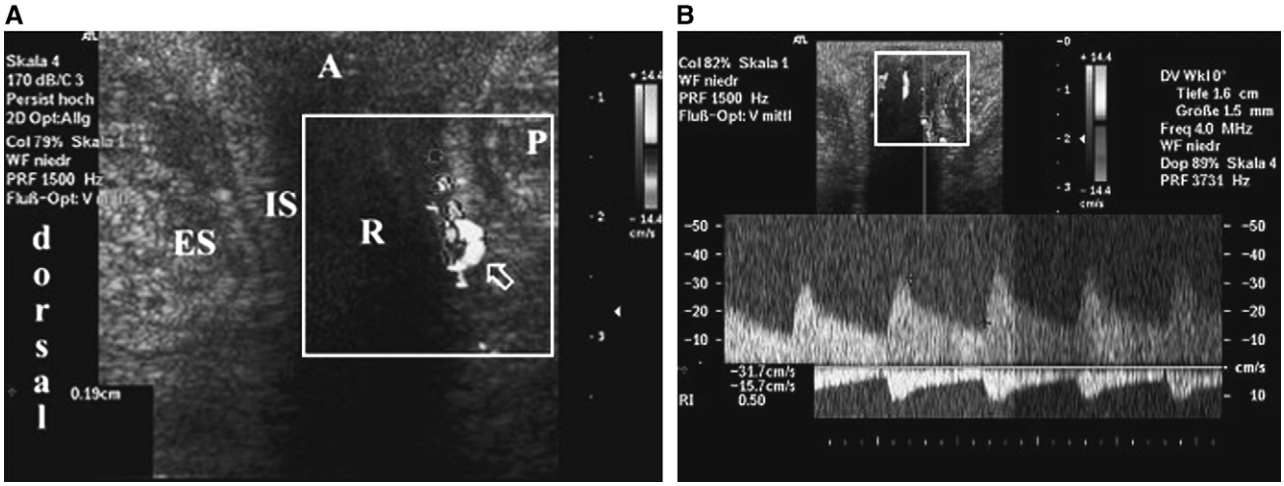

Fig. 4. A 33-year-old male patient with hemorrhoids, Goligher grade II[3]; transperineal color Doppler ultrasound, longitudinal scan. (**A**) A dilated terminal branch of the SRA (*open arrow*, caliber 1.9 mm) is shown within the rectal submucosa connecting to the CCR (dark grey colored vascular signals). A, anal canal; R, rectum; P, prostate; IS, internal anal sphincter; ES, external anal sphincter. (**B**) Same patient and arterial vessel within the ventral rectal wall. Doppler measurement of the arterial blood flow (31.7 cm/second).

increased blood flow in patients with hemorrhoids (Fig. 5). Within the study group, patients with grade III/IV hemorrhoids had significantly larger vessels with a higher flow than those with grade I/II.

Several anatomical and clinical studies demonstrated an influence of vascular hyperplasia on the development of hemorrhoids.[4,5,20,21] It is certainly plausible that hypervascularization within the CCR

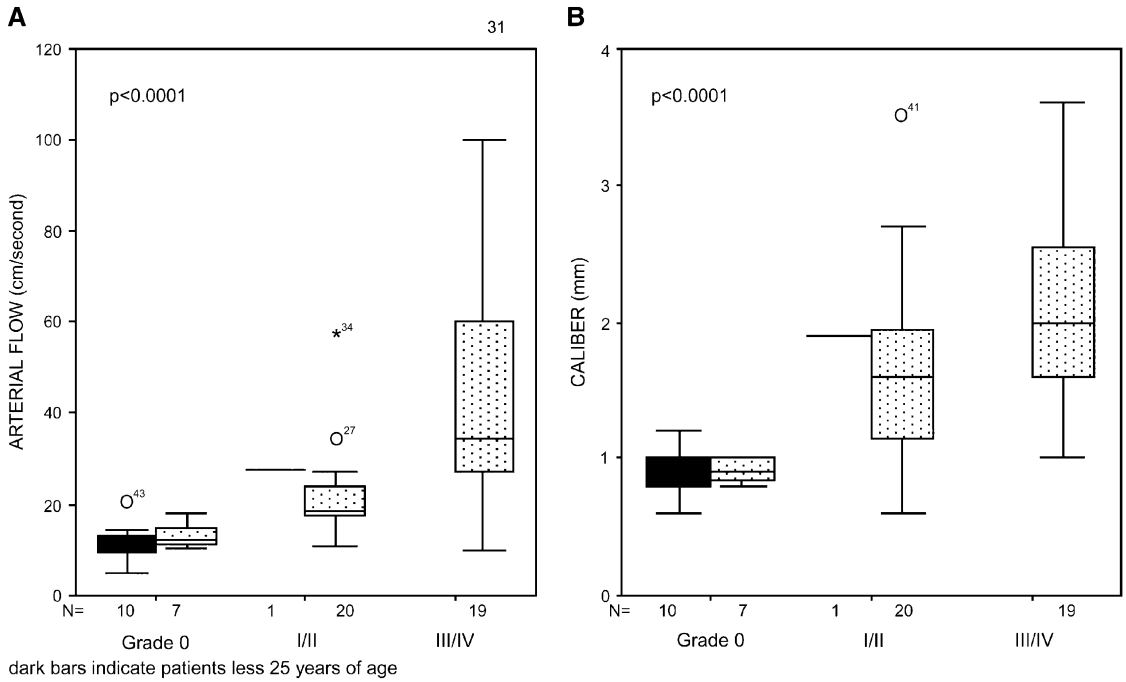

Fig. 5. Arterial blood flow (**A**) and caliber (**B**) of the terminal branches of the superior rectal artery significantly increase in higher hemorrhoidal grades (right bars indicate grade III and IV hemorrhoids). The morphological and physiological changes of the arteries according to the different grades of hemorrhoids are still obvious after exclusion of individuals less than 25 years of age (black bars on the left). Grade 0 is defined as group A, grades I/II are defined as group B and grades III/IV are defined as group C in the text. Outliners are indicated with patient/volunteer numbers.

plays an important role. Thus far it is not clear, however, if this is due to an increased inflow or decreased outflow. The impact of chronic inflammation of the anorectal submucosa or persistent irritation of the anal mucosa in patients with hemorrhoids leading to hypervascularization within and around the rectum remains a matter of debate and warrants further elucidation.

Abnormal relaxation of the internal anal sphincter with hindered venous drainage should lead to an increase in the size of the arteriovenous cushions within the rectal submucosa, and subsequently, to the prolapse of hemorrhoidal tissue into the anal canal. Resting pressure measurements of the anal sphincter in patients with hemorrhoids provided strong evidence for an existing correlation between the development of hemorrhoids and the increased internal anal sphincter pressure.[7–10,12,21–24] The raised pressures have been discussed controversially as being an etiologic factor rather than a consequence of hemorrhoidal piles.[7,24]

Our investigation of the vascularity of the CCR revealed significant changes in the arterial inflow of the CCR in patients with hemorrhoids. Terminal branches of the SRA and additional transmural branches of the SRA, the course of which is not exactly definable within the rectal submucosa,[17] showed identical morphological changes when compared with healthy individuals. The venous hyperplasia theory in the pathogenesis of hemorrhoids has mainly been evolved by authors referring to the vascular anatomy as being unchanged in patients with hemorrhoids.[8] It has been claimed that dilated veins of the CCR are constant and normal structures, from birth on, and are found in every adult.[5] Moreover, the vascular architecture of the arteriovenous network within the rectal submucosa has been proven to be already developed in early fetal stages,[17] which might explain the presence of strong terminal branches of the SRA (mean diameter, 0.9 mm), even in healthy individuals without any history of symptomatic hemorrhoids in 75% of the investigated cases. Previous works on the prevalence of hemorrhoids have confirmed that 70% of individuals 30 years of age or older show hemorrhoids that are not automatically associated with clinical symptoms (bleeding, moistening, burning, and more),[25] such as our controls who were totally free of symptoms. The morphological changes increased significantly with age (Fig. 2, *A*, *B*), although the difference was still obvious after division of the study group and exclusion of individuals less than 25 years of age (Fig. 5).

In our study, the terminal branches of the SRA detected in the rectal wall showed a clear diastolic flow with a low resistance index (RI $<$ 0.6, data not shown), indicating the existence of an arteriovenous shunt system or sinusoidal-type portal vein system. More data will be required to demonstrate a connection between changes in this shunt system and the increasing prolapse of hemorrhoidal cushions.

The arteriovenous network of the CCR without interposition of a capillary system that contributes to the gas-tight closure of the anal canal—according to the original description by Stelzner[4]—can be easily dissected into its arterial and venous portion by means of transperineal CDUS (Fig. 4, *A*). This technique facilitates preoperative assessment of the anorectal vascularization status in patients with hemorrhoids and should help with the choice of the appropriate surgical technique for the treatment of various grades of hemorrhoids.

At least one arterial vessel (mean, two arteries) with increasing caliber in patients with hemorrhoids was detected within and closely around the rectal wall. We assume that these vessels, which contribute to the blood supply of the CCR, might explain the bleeding complications or recurrence of hemorrhoidal prolapse in over 30% after ligature techniques such as HAL[26] or stapled hemorrhoidopexy,[27] because of their course and caliber.

Several studies revealed a postinterventional decline of the anal resting tone after hemorrhoidectomy techniques, thus outlining the increased anal resting tone as a result of enlarged hemorrhoidal piles.[7–10]

Consequently, hyperplasia of the described vessels is seemingly not resulting from an increased anal resting tone, according to our observation of patients undergoing stapled hemorrhoidopexy who did not show postinterventional changes in either the caliber or in the blood flow after a short-term follow-up (preliminary data).

The postoperative outcome after stapled hemorrhoidopexy therefore does not depend on the complete interruption of the arterial inflow of the hemorrhoids,[28] which cannot be achieved by means of hemorrhoidopexy regarding extramural branches of the SRA that we have described elsewhere.[17] Reposition of the prolapse and the improvement of the venous drainage of the CCR might be more important.

As with an increase in the grade of hemorrhoids, the caliber and the blood flow also rises; adapting the therapy according to the grade of hemorrhoids becomes even more rational.

In summary, the presented data shows that the SRA is basically involved in the pathogenesis of hemorrhoids according to the observed morphological changes of its terminal branches in patients with hemorrhoids. Our morphological data provide

strong evidence that the hyperplasia of the arterial branches of the CCR might be an explanation for the remarkable recurrent rate and postoperative hemorrhage after hemorrhoidopexy procedures.[26]

Our observations confirm that hemorrhoids are not anatomical abnormalities, as evidenced in our findings in the control group. Morphological changes are clearly detectable with the use of transperineal CDUS in patients with symptomatic hemorrhoids. This method should help the coloproctologist to assess the vascularization status of the anorectum before intervention and to choose the appropriate surgical technique. Vascular hyperplasia, not only of the submucosal rectal arteries but also of additional transmural branches of the SRA, is significantly correlating with the appearance of hemorrhoids.

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Fecal Stream Is Essential for Adaptive Induction of Glucose-Coupled Sodium Transport in the Remnant Ileum After Total Proctocolectomy

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Our previous studies demonstrated that sodium glucose cotransporter 1 (SGLT-1) was induced in the remnant ileum of total colectomized rats via the action of factors other than hyperaldosteronism. The aim of the present study was to clarify whether fecal stream is required for the enhancement of SGLT-1–mediated sodium transport. Twenty-seven pairs of ileal tissues were obtained from the proximal and distal side, respectively, of loop ileostomy after total proctocolectomy. Mucosae were mounted in an Ussing chamber to evaluate glucose-coupled sodium transport. Levels of SGLT-1 mRNA in proximal and distal mucosae were compared by Northern blotting. Villous height and crypt depth were measured to test for correlations between mucosal structure and SGLT-1–mediated sodium transport or mRNA expression levels. Both glucose-coupled sodium transport and expression of SGLT-1 mRNA were significantly lower in distal mucosae relative to proximal mucosae. In distal mucosae, villous height, but not crypt depth, was significantly lower than in proximal mucosae, demonstrating a positive correlation between villous height and SGLT-1 function and expression. Comparative studies of proximal and distal mucosae demonstrated that in addition to hormonal changes, fecal stream is required for full induction of the sodium transport system (which includes SGLT-1-mediated transport) in the remnant ileum following total proctocolectomy. (J GASTROINTEST SURG 2006;10:1051–1059)

KEY WORDS: Total proctocolectomy, sodium glucose cotransporter 1, ileostomy, intestinal adaptation

In recent decades, patients with uncontrolled ulcerative colitis and familial adenomatous polyposis have frequently been subjected to total proctocolectomy (TPC) followed by ileoanal (canal) anastomosis. The advantage to this procedure is that patients are cured of their diseases by the removal of the entire colon without receiving a permanent ileostomy. However, complete removal of the colon causes symptoms such as watery diarrhea, frequent bowel movements, and dehydration soon after closure of the covering loop ileostomy in almost all patients. Dehydration is often facilitated by a preexisting chronic deficit of body fluid and sodium.

Although the significance of subsequent renal compensation to maintain homeostasis has been well addressed clinically[1] and experimentally,[2] there is no doubt that frequent bowel movements, watery diarrhea, and subclinical sodium deficits can substantially impair a patient's quality of life after surgery.

Because the renin-angiotensin-aldosterone system primarily regulates water homeostasis and electrolyte balance in the gastrointestinal tract as well as the kidney,[3] we focused on this system after surgery. In our previous reports where a rat TPC model was used, we demonstrated molecular induction of all three subunits of epithelial sodium channel (ENaC), prostasin, and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in epithelial cells of the remnant ileum.[4–6] ENaC plays a major role in amiloride-sensitive sodium absorption from the apical side of epithelial cells.[7] Prostasin is a novel serine protease that substantially increases the activity of coexpressed ENaC.[8]

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11β-hydroxysteroid dehydrogenase type 2 is an enzyme that confers specificity on nonselective mineralocorticoid receptors, enzymatically converting abundant glucocorticoids to their receptor-inactive metabolites and allowing selective binding of aldosterone to mineralocorticoid receptors.[9] Induction of sodium glucose cotransporter 1 (SGLT-1), which is responsible for glucose absorption that is coupled to the electrochemical gradient of sodium transport in the plasma membrane,[10–13] was also seen in the remnant ileum of TPC rats.[6] In aldosterone-infused rats that function as nonsurgical models for hyperaldosteronism, induction of ENaC, prostasin, and 11β-HSD2 was detected.[6] However, steady-state levels of SGLT-1 mRNA did not increase in aldosterone-infused rats. These data strongly suggest that, unlike ENaC, prostatin, and 11β-HSD2, factors in addition to circulating aldosterone are required for adaptive induction of SGLT-1.

As was initially described, watery diarrhea and frequent bowel movements (more than 10 per day), which are very common immediately after closure of the covering loop ileostomy, improve to pastelike stool and less frequent movements with time. Although such "adaptation" is not necessarily adequate, this improvement in bowel habits implies that increased sodium and water absorption in the distal ileum can occur. Nonetheless, molecular mechanisms underlying this improvement have not been addressed.

In the present study, we focused on the significance of fecal stream on SGLT-1 mRNA expression in epithelial cells and the activity of the sodium glucose cotransport system in mucosae of the ileum. We used paired samples obtained from the proximal and distal mucosae of the loop ileostomy to maximize the similarity of experimental tissue samples in the presence or absence of fecal stream. Our results clearly demonstrate that fecal stream is required for adaptive SGLT-1-mediated sodium absorption after TPC.

MATERIAL AND METHODS

Patients and Tissue Sampling

Twenty-five ulcerative colitis and two familial adenomatous polyposis patients were subjected to this study (Table 1). All had previously undergone TPC, ileoanal (canal) anastomosis, and diverting loop ileostomy on the proximal side, approximately 40–60 cm from the ileal end. Paired ileal tissues were obtained from the proximal and distal sides of loop ileostomy at surgery for ileostomy closure. All patients were corticosteroid-free at the time of tissue sampling. This study protocol was approved by the Ethics Committee of Tohoku University School of Medicine.

Table 1. Patients' outline

	UC	FAP
Number of patients	25	2
Sex (male:female)	15:11	2:0
Mean age, years (range)	33.6 (16.5–60.5)	42.1 (32.8–51.4)
Mean duration of FD, months (range)	5.5 (3.0–12.5)	8.5 (4.1–12.8)

UC = ulcerative colitis; FAP = familial adenomatous polyposis; FD = fecal diversion.

Measurement of Glucose-Coupled Sodium Transport in the Proximal and Distal Mucosae

Short-circuit current (abbreviated as Isc) was measured in vitro using an Ussing chamber.[5,14] An isolated 8 cm ileal segment that included both proximal and distal sides of the loop ileostomy was opened and rinsed, and the muscle layer and submucosal tissue were removed. Proximal and distal mucosae were mounted vertically between acrylic resin chambers with an internal surface area of 0.1 cm^2 (Physiologic Instruments, Inc., San Diego, CA). The volume of the bathing solution in each chamber was 8 ml, and its temperature was kept at 37° C in a heat-warmed chamber holder. The mucosal solution contained 119 mmol/L NaCl, 21 mmol/L $NaHCO_3$, 2.4 mmol/L K_2HPO_4, 0.6 mmol/L KH_2PO_4, 1.2 mmol/L $CaCl_2$, 1.2 mmol/L $MgCl_2$, and 8.5 mmol/L mannose. The serosal solution was identical except that it contained 2.5 mmol/L glutamine in the place of mannose, 5 mmol/L glucose, 0.5 mmol/L β-hydroxybutyrate (sodium salt), and 3×10^{-4} mmol/L tetrodotoxin. Each solution was bubbled with 95% O_2 and 5% CO_2 (pH 7.4). Tissues were continuously short-circuited using a voltage-clamping amplifier (CEZ9100; Nihon Kohden, Tokyo, Japan), with compensation for fluid resistance between the two potential-sensing bridges. The transepithelial potential was measured using 1M-KCl agar electrodes, while transepithelial current was applied across the tissue through a pair of Ag/AgCl electrodes in contact with mucosal and serosal bathing solutions. Isc was positive when current flowed from the mucosal side to the serosal side. Transepithelial resistance was calculated according to Ohm's law from the change in current in response to voltage pulses. Serial amounts of glucose were added to the mucosal-side buffer to achieve concentrations of 4, 8, 16, 32, and 48 mmol/L, and Isc

was continuously recorded. Glucose-coupled Na transport was estimated from the increase in Isc (ΔIsc) and maximal ΔIsc (ΔIsc^{max}).

Northern Blot Analysis

Proximal and distal mucosae (6 paired samples) were homogenized individually in guanidine thiocyanate solution immediately after tissue sampling. Total RNA was extracted using cesium chloride gradients. Twenty micrograms of total RNA from each sample were resolved by electrophoresis on a single 1% agarose gel and then transferred onto a Hybond-N + nylon membrane (Amersham Pharmacia Biotech, Bucks, England). The quality and quantity of RNA was determined by absorbance at 260 nm. Complementary DNAs of β-actin and SGLT-1 were prepared by polymerase chain reaction cloning using the following primer sets: β-actin (sense: 5′-GAGCATCCCC-CAAAGTTCAC-3′, antisense: 5′-GGCAAGG-GACTTCCTGTAACAA-3′), SGLT-1 (sense: 5′-TTGACCTGGATGCGGAAGA-3′, antisense: 5′-CTGTCCTCCACAAAGGCTTCTC-3′).

Protocols for probe labeling and hybridization have been previously described.[5] Hybridization signals were visualized using a BAS imaging analyzer system (Fuji Film, Kanagawa, Japan), and the intensity of each band was measured using imageJ 1.32j (NIH, http//rsb.info.nih.gov/ij/).[15] The ratio of SGLT-1 to β-actin signal intensities was calculated for each track lane.

Morphometric Analysis of the Proximal and Distal Mucosae

Mucosal structure was morphometrically assessed from an ileal segment (approximately 1 cm in length) from each patient that included the proximal and distal sides of the loop ileostomy to investigate whether mucosal structure affects sodium-coupled glucose transport and SGLT-1 mRNA expression. Tissues were fixed in 10% buffered formalin and mounted in paraffin wax. Three micrometer-thick sections were stained with hematoxylin-eosin, and villous height and crypt depth were then measured using an ocular micrometer. Mean villous and crypt lengths were calculated from measures of 10 villi and 10 crypts selected at random from each sample.[16–23]

Statistics

Values of each measure were presented as mean + SE or median and percentile. The significant difference of data between proximal and distal mucosae

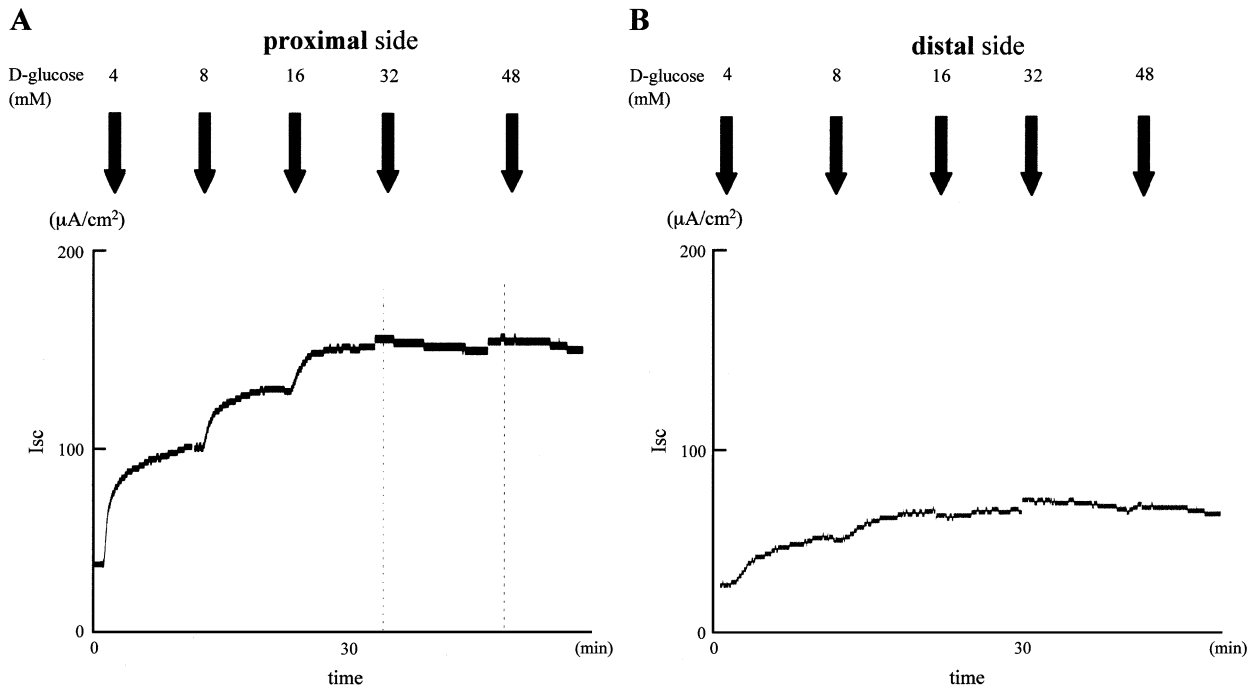

Fig. 1. A typical tracing of short-circuit current (Isc) changes following addition of D-glucose to proximal (**A**) and distal (**B**) mucosae of the loop ileostomy. Short-circuit current was continuously recorded. D-glucose concentrations were increased serially (0, 4, 8, 16, 32, 48 mmol/L) in the bathing medium. *Arrows* mark the times when D-glucose stock solution was added.

was tested using the Mann-Whitney *U* test. Correlations between mucosal structure and sodium-coupled glucose transport or levels of SGLT-1 mRNA expression were evaluated statistically using Spearman's correlation coefficient by rank test. *P* values < 0.05 were considered significant.

RESULTS

Measurement of Glucose-Coupled Sodium Transport in Proximal and Distal Mucosae

When serial amounts of glucose were added to the mucosal side of the Ussing chambers, Isc increased in a dose-dependent fashion to an ultimate plateau (Fig. 1, *A*, *B*). Among all patients, both ΔIsc between each dose glucose concentration and the maximal ΔIsc (ΔIsc^{max}) were less in the distal mucosa than in the proximal mucosa (Fig. 2), with mean ΔIsc^{max} of the distal side (67 μA/cm^2) approximately 70% of the proximal side (95 μA/cm^2). When pairs of samples were compared individually, ΔIsc at all glucose concentrations and ΔIsc^{max} were, without exception, lower on the distal side than on the proximal side.

Expression of SGLT-1 mRNA on the Proximal and Distal Sides of Loop Ileostomy

SGLT-1 mRNA expression was examined in six paired samples from proximal and distal sides of the loop ileostomy. Using SGLT-1 cDNA as a probe, a single 2.3 kb band was detected in all samples (Fig. 3, *A*). Densitometric analysis showed that the intensity of this band in RNAs from distal mucosae was significantly lower than proximal mucosal samples, demonstrating that steady-state levels of SGLT-1 mRNA are higher on proximal mucosae (Fig. 3, *B*). No significant correlation was seen between the duration of fecal diversion in covering loop ileostomies and the steady-state level of SGLT-1 mRNA in either proximal or distal mucosae (data not shown).

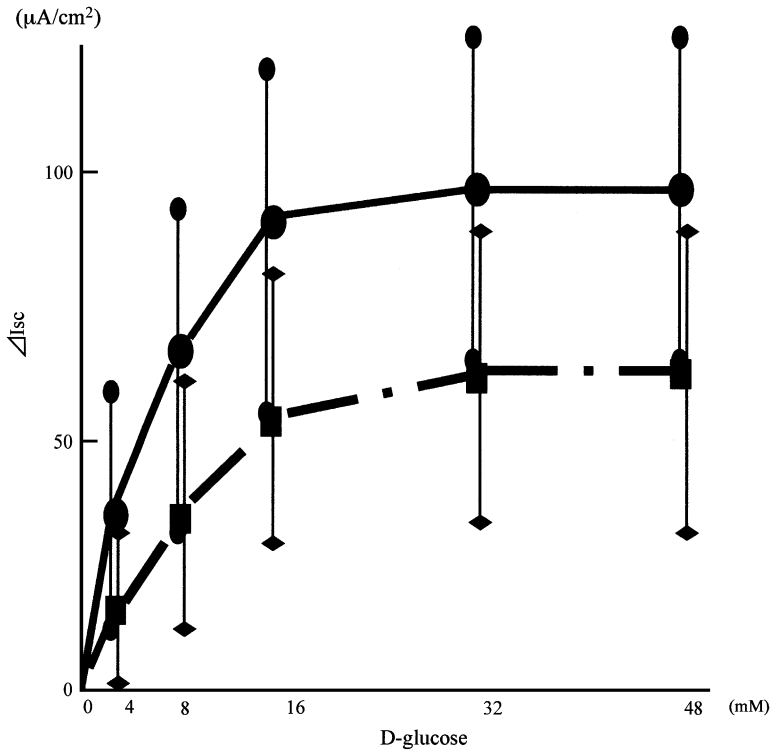

Fig. 2. Average difference in short-circuit current (ΔIsc) between five pairs of proximal (closed circles) and distal (closed squares) mucosae of loop ileostomies. D-glucose was added serially to the bathing medium to final concentrations of 4, 8, 16, 32, and 48 mmol/L. The differences seen in Isc (ΔIsc) were considered to represent changes in glucose-coupled Na transport. Data is presented as mean ΔIsc + SE. Note that ΔIsc at each concentration of glucose and ΔIsc^{max} were greater for proximal mucosae than distal mucosae.

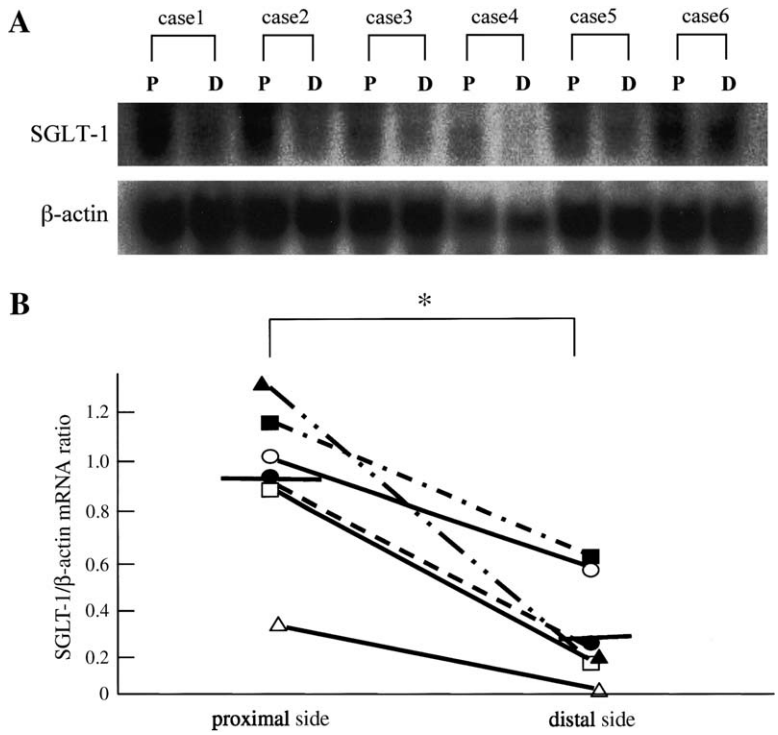

Fig. 3. Steady-state levels of mucosal SGLT-1 mRNA in 6 pairs of proximal (P) and distal (D) mucosae samples from the loop ileostomy. (**A**) Northern blot analysis. Blots were hybridized with radiolabeled SGLT-1 (upper lanes) or β-actin (lower lanes) cDNA probes. Note that signal intensities from proximal mucosae were higher than the distal mucosae for all sample pairs. (**B**) Semiquantification of SGLT-1 mRNA expression in six pairs of mucosae RNA samples. Closed or open circles, triangles, or squares indicate individual pairings. The ratio of signal intensities from SGLT-1 relative to β-actin bands was significantly higher in proximal-side RNA than distal-side RNA. $^*P < 0.05$.

Morphometric Analysis

Villous height and crypt depth were measured in 16 pairs of mucosal samples (Fig. 4). As shown in Fig. 5, *A*, mean villous height was significantly lower in the distal mucosa (358 μm) than the proximal mucosa (520 μm) in all sample pairs. However, there was no significant difference in length of crypt depth from distal and proximal samples (130 and 139 μm, respectively). Furthermore, no significant correlations were seen between the duration of fecal diversion with covering loop ileostomies and villous height in proximal or distal mucosae (data not shown).

Correlations between Villous Height and ΔIsc^{max} or SGLT-1 mRNA Levels

There was a significant positive correlation between villous height and ΔIsc^{max}, regardless of the mucosal site of the loop ileostomy (Fig. 6). Positive correlations were also seen between villous height and SGLT-1 mRNA expression in distal and proximal mucosae (Fig. 7).

DISCUSSION

Removal of the entire colon affects multiple functions of the gastrointestinal tract, including intestinal motility, absorption of nutrients, bile acid metabolism, composition of enteric flora, sodium, and water absorption.[1,14] The remnant small intestine is an essential organ for "adaptive" changes (so-called intestinal adaptation), in particular, altered electrolyte and water absorption.[1,24,25] Water and electrolyte transport in the ileal mucosa are affected by hormones,[26] microcirculation,[27] neural signals and transmitters,[28] and mucosal immune and mesenchymal cells[27] and the humoral factors secreted from them.[13]

In the present study, we focused on the function of glucose-coupled sodium transport and SGLT-1 expression levels in intestinal epithelia. Several electrogenic and electroneutral sodium transport

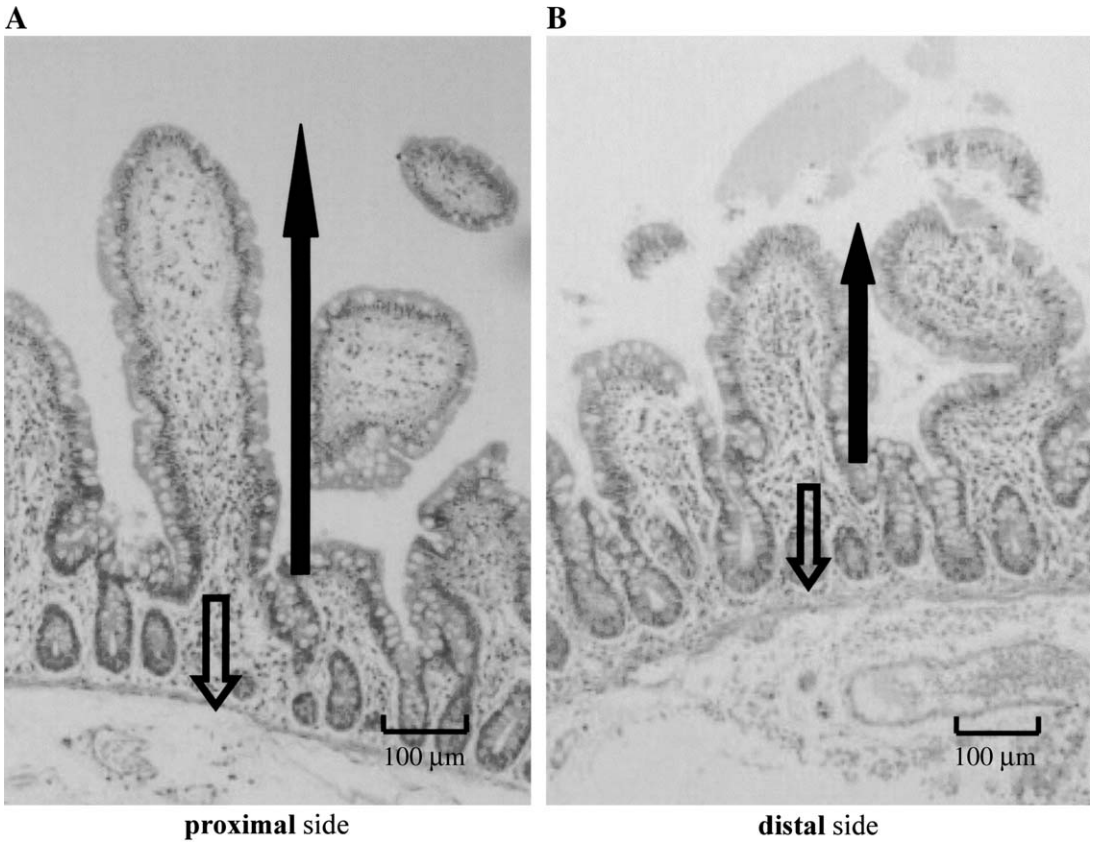

Fig. 4. Morphometric analysis of 16 pairs of mucosae from the proximal (**A**) and distal side (**B**) of loop ileostomy. *Closed arrows* and *open arrows* demonstrate measures of villous height or crypt depth, respectively. Ten villi and 10 crypts were randomly selected from each section. Bar indicates 100 μm.

systems function in intestinal epithelia, including glucose-coupled sodium transport, sodium-dependent amino acid transport, Na + −Cl cotransport, Na + /H + exchangers, and Na + /K + −ATPases.[10,29,30] Among these, glucose-coupled sodium transport is quantitatively the most prevalent absorptive ion transport system. An electrochemical gradient cues sodium absorption from the lumen into epithelial cells via SGLT-1 at the brush border membrane, and sodium is then extruded across the basolateral membrane of epithelial cells to the blood via Na + /K + −ATPase.[31,32]

Comparative analysis of sodium transport in paired samples of proximal and distal mucosae from the loop ileostomy was ideal for this study because these mucosae share features such as original disease, medication history, patient age, genetic background, hormonal condition, and very close location of the sampling site. In addition, the fact that none of the patients in the study excreted feces from the anus following covering loop ileostomy made fecal flow on the distal side of loop ileostomy unlikely. This enabled us to investigate the role of fecal stream on glucose-coupled sodium transport and SGLT-1 expression after TPC in high levels of circulating aldosterone 1.

We clearly demonstrated that fecal stream is required for high levels of both glucose-coupled sodium transport and SGLT-1 mRNA expression. However, it is difficult to obtain "control" mucosa from the future site of loop ileostomy before TPC or control subjects and to compare them between "control" mucosa and proximal mucosa of loop ileostomy. Therefore, we cannot prove that "induction" of glucose-coupled sodium transport and SGLT-1 mRNA occurs in the remnant small intestine of human patients that have undergone TPC. In other words, altered hormonal environment after TPC is not adequate to induce and/or maintain highly functional glucose-coupled sodium transport system.

As was expected, villous height was significantly lower in distal mucosae than relative to proximal mucosae, demonstrating that the presence or absence of fecal stream affects mucosal morphology. Oh et al.[33] recently demonstrated that in humans, villous atrophy occurs in distal mucosae of the loop

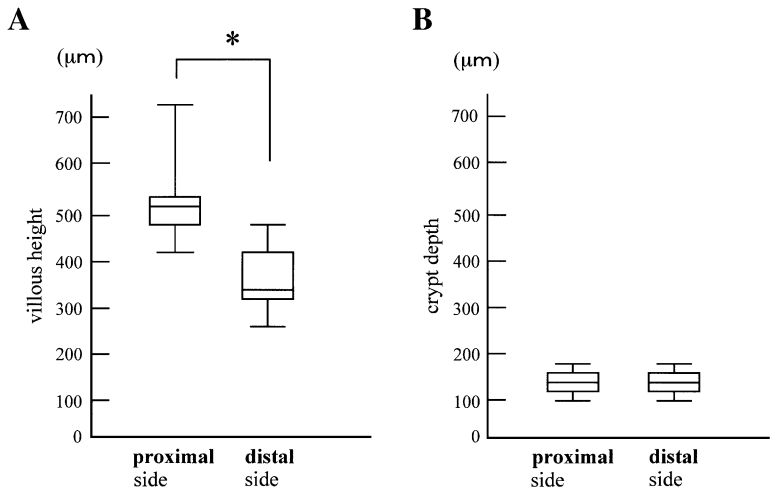

Fig. 5. Morphometric analysis of 16 pairs of mucosae from the proximal and distal side of the loop ileostomy. Villous height (**A**) and crypt depth (**B**) were individually measured from 16 sample pairs, and the median and percentile values from these samples are shown. Median villous height on the proximal side (513 μm) was significantly higher than the distal side (369 μm). In contrast, there was no difference in the crypt depths (130 vs. 139 μm, respectively). $^*P < 0.05$.

ileostomy relative to "control" mucosae. Development of mucosal atrophy is also well known to occur in intestinal mucosa during total parenteral nutrition combined with prohibition of proximal intake,[23] and decreased glucose transport has also been observed in the ileal mucosa of patients receiving total parenteral nutrition. Thus, the environment of mucosae subjected to this regime differs substantially from the distal mucosae of loop ileostomy due to the presence of bile and pancreatic juices in the lumen, hormonal conditions, and many other unspecified factors. Nonetheless, the fact that a decline in both glucose-coupled sodium transport activity and steady-state levels of SGLT-1 mRNA occurred concomitant with short villi in the distal mucosae of loop ileostomy suggests that down-regulation of SGLT-1 mediated transport may be relevant to villous atrophy as a secondary effect following this morphological change. Villous atrophy was also present, together with declined activity and/or mRNAs of digestive enzymes, in intestinal biopsies from patients with malnutriation.[34,35]

This hypothesis is supported by the fact that there is a significant positive correlation between villous height, maximal changes of glucose-induced short-circuit current, and SGLT-1 mRNA expression levels. However, it is possible that unknown factor(s) in feces modulate the function of SGLT-1 expression and function directly in intestinal epithelia. Carbohydrates in the diet stimulate intestinal crypt cells, leading to an increased density of glucose transporters in matured villous enterocytes.[36] Moreover, luminal factors may also influence mucosal immune and mesenchymal cells, which could modulate SGLT-1 expression in epithelia by secreted humoral factors.

Colorectal surgeons generally notice that patients suffer particularly from watery diarrhea and frequent bowel movement just after closure of covering loop ileostomy. The present study provides some clues

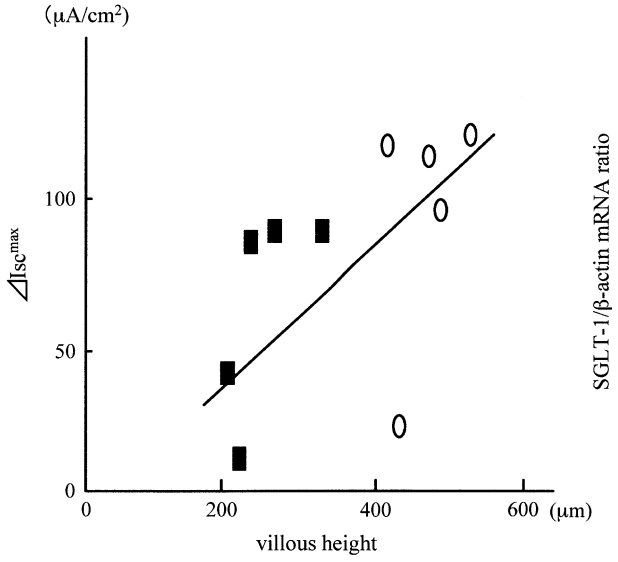

Fig. 6. Correlation between villous height and ΔIsc^{max} from five sample pairs. Open circles and closed squares indicate proximal or distal mucosae, respectively, of the loop ileostomy. The correlation coefficient was 0.66 with statistical significance. $^*P < 0.05$.

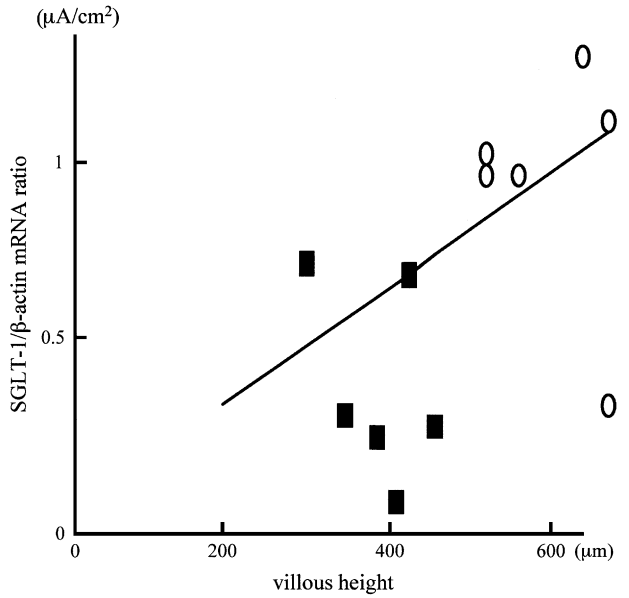

Fig. 7. Correlation between villous height and the ratio of SGLT-1 to β-actin mRNA signals from six sample pairs. Open circles and closed squares indicate the proximal or distal mucosae, respectively, of the loop ileostomy. The correlation coefficient was statistically significant at $^*P < 0.05$.

toward explaining these symptoms. Terminal ileal mucosae separated from fecal stream at the distal side of loop ileostomy show decreases in the activity and mRNA levels of SGLT-1, the most important protein in absorptive ion transport. Time-dependent improvement of watery diarrhea following TPC may depend, at least in part, on the absence or presence of feces guiding the activity of SGLT-1-mediated sodium transport in the lumen.

In conclusion, this comparative study clearly demonstrates that alterations of the hormonal environment of the ileum after TPC are not sufficient to induce or maintain waning glucose-coupled sodium transport, and that fecal stream is essential for adaptive activation of this ion transport system in the remnant ileum after TPC. We propose that a better understanding of the mechanisms of "intestinal adaptation" could ultimately contribute—in the near future—to better management of persistent diarrhea following TPC.

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Anti–*Saccharomyces cerevisiae* Antibodies Are Associated With the Development of Postoperative Fistulas Following Ileal Pouch-Anal Anastomosis

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Although serologic testing for perinuclear antineutrophil cytoplasmic antibodies (pANCA) and anti–*Saccharomyces cerevisiae* antibodies (ASCA) is reportedly useful in distinguishing ulcerative colitis (UC) from Crohn's disease (CD), there are few and conflicting reports assessing their utility in predicting postoperative complications after ileal pouch-anal anastomosis (IPAA). We examined the associations between postoperative complications such as pouchitis or fistulas and pANCA and ASCA antibodies in a group of patients who underwent IPAA for UC. We conducted a retrospective chart review of 34 patients initially diagnosed with UC (four of these patients had a diagnosis of indeterminate colitis) who underwent IPAA by a single surgeon, and who had pANCA and ASCA antibody levels measured during their clinical course. Study patients were assigned to four groups based on the pattern of antibody reactivity: pANCA+/ASCA− (16 patients), pANCA−/ASCA+ (nine patients), pANCA+/ASCA+ (five patients), and pANCA−/ASCA− (four patients). The median length of follow-up was 16 months (3–144 months). None of the patients (0 of 16) who were pANCA+/ASCA− had their preoperative diagnosis of UC changed after a median follow-up of 14 months (3–118 months). Of the nine patients with a preoperative diagnosis of UC who were pANCA−/ASCA+, four patients (44%) had their diagnosis changed postoperatively to CD based on clinical findings, with a median follow-up: 15 months (5–98 months). Of 16 patients who underwent IPAA and who were pANCA+/ASCA−, 15 of 16 (93.75%), were free of fistulas postoperatively, with a median follow-up of 14 months (3–118 months). Of nine patients with a preoperative diagnosis of UC who underwent IPAA and who were pANCA−/ASCA+, four of nine (44%; $p = 0.04$) developed fistulas postoperatively, with a median length of follow-up of 55 months (15–67 months). No relationship between serologic profiles or antibody titer levels and the development of pouchitis was identified. In a cohort of patients undergoing IPAA for UC, serologic profiles may be useful in identifying patients at risk of postoperative fistula formation. Patients who were pANCA−/ASCA+ were at increased risk for the development of fistulas postoperatively compared to patients who were pANCA+/ASCA−, and were also more likely to have their diagnosis changed postoperatively to CD. A larger study is needed to validate these observations. (J GASTROINTEST SURG 2006;10:1060–1064)

KEY WORDS: pANCA, ASCA, ileal pouch-anal anastomosis, fistulas

The vast majority of patients with inflammatory bowel disease (IBD) can be classified as having either ulcerative colitis (UC) or Crohn's disease (CD). However, the diagnosis remains indeterminate in 10–15% of patients.[1,2] In some patients who require urgent colectomy, pathologic examination of the resected colon may not yield a definitive diagnosis of either UC or CD. For most patients without clinical, endoscopic, or radiologic evidence suggestive of CD and in whom the final pathology is indeterminate, it is a common surgical practice to perform an ileal pouch-anal anastomosis (IPAA).[3] Total proctocolectomy with IPAA has become the surgical treatment of choice for most patients with refractory ulcerative

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colitis.[2,4] Approximately 5–13% percent of IPAA procedures are performed in patients whose primary diagnosis is revised at some point after surgery from UC to CD.[2,5] These patients are thought to be at increased risk for postoperative complications, such as pouchitis, fistula formation, and pouch failure.[5–8]

The reported cumulative risk of developing pouchitis in all UC patients undergoing IPAA approaches 25–50% after 10 years.[2,6] The reported pouch failure rate (resulting in a permanent stoma) in patients with postoperative fistulous complications associated with the diagnosis of CD approximates 30%.[2,8]

Since clinical, endoscopic, radiologic, and histological evidence is sometimes still not enough to differentiate between UC and CD, attempts have been made to utilize specific serologic markers. In 1990, an IBD-specific anti-neutrophil cytoplasmic antibody with perinuclear highlighting (pANCA) was first described and has been associated with UC.[2] Antibodies to baker's and brewer's yeast, anti−*Saccharomyces cerevisiae* antibody (ASCA), have been described in patients with Crohn's disease.[2,9–15] The sensitivity and positive predictive value for combining the pANCA−ASCA assay to differentiate between UC and CD have been reported as approximately 60–70% and 94–96%, respectively.[9–15]

Some studies have suggested that the pANCA test might be useful in predicting pouchitis postoperatively in UC patients undergoing IPAA and that high titer levels of pANCA might be predictive of chronic pouchitis, although other studies have contradicted these findings.[16–19] To date, there have been no studies looking at the combination of the pANCA and ASCA serology with regard to their association with postoperative complications. Thus, the aim of this study was to examine the utility of pANCA and ASCA antibodies in a group of patients who underwent IPAA for UC to determine if there were associations with postoperative complications, such as pouchitis or fistulas.

MATERIAL AND METHODS

From the patient records of a single general surgeon at our institution (J.M.B.) with more than 20 years of experience in performing over 750 IPAA operations, 34 patients were identified for this study. All patients in the study had a preoperative diagnosis of UC (30 patients) or IC (four patients), underwent IPAA for refractory ulcerative colitis, and had serologic testing for pANCA and ASCA antibodies performed by Prometheus Laboratories (www.prometheus-labs.com). Thirteen patients had the serologic testing performed preoperatively and 21 patients had the testing postoperatively.

Study Subject Data Collection

In reviewing each patient's chart retrospectively, the following information was gathered: date of original diagnosis of UC; results of serologic testing; date of colectomy or date of bowel resection; date of IPAA; pathologic diagnosis; the incidence and frequency of pouchitis, and fistula formation; and, the final clinical diagnosis based on last follow-up visit. Information regarding the serologic results that was recorded included ASCA IgG, ASCA IgA, and pANCA antibody titer levels, the date of the test, and the Prometheus interpretation of the serologic pattern of reactivity.

Serum ANCA presence was determined by Prometheus Laboratories using a fixed ELISA assay. Levels were determined relative to a Prometheus laboratory standard consisting of pooled sera obtained from well-characterized pANCA+ UC patients. Sera with circulating antineutrophil cytoplasmic IgG antibody exceeding the normal reference range value were termed "ANCA positive" (ANCA+). Numeric values below the normal reference range were termed "ANCA negative" (ANCA−). ANCA+ sera were further subtyped via indirect immunofluorescence staining to determine the ANCA neutrophil binding pattern. Sera exhibiting the characteristic perinuclear highlighting which then lost this characteristic staining pattern when first treated with DNase were termed "pANCA+."[20]

Serum ASCA expression was performed by Prometheus Laboratories using a fixed ELISA assay. Results were expressed as ELISA units (EU/ml). Levels were determined and results expressed as ELISA units (EU/ml) relative to a Prometheus Laboratory standard that were derived from a pool of patient sera with well-characterized CD found to have reactivity to this antigen. Sera exhibiting ASCA reactivity (IgG and/or IgA) exceeding the normal reference range were termed "ASCA positive" (ASCA+).[21]

We used the diagnosis established at the time of the last postoperative office visit, which was based on symptoms, endoscopy, and histology, as our gold standard. This diagnosis was associated with the results of the serologic testing for each patient. This study was reviewed and approved by the Institutional Review Board at the Boston Medical Center.

Patient Population

Patients included in this study underwent IPAA from 1980 to 2002 and had serologic studies drawn

during their clinical course. Our study identified 34 patients (M/F, 15/19; mean age, 38 years [range, 17–66 years]) who were seen by a single general surgeon for the surgical management of presumed UC or IC. The 34 patients in the study included all the IPAA patients in our patient population who had serologic testing performed. The indication for the drawing of the serologies was not apparent from the chart review in all cases. In the majority of the cases, the markers appear to have been drawn in patients who were having symptoms related to their pouch function (i.e., pain, increased bowel movements, documented pouchitis, or fistula formation).

Statistics

Differences between groups were tested for statistical significance using a χ^2 test or Fisher's Exact Test, as appropriate.

RESULTS

Our study examined 34 patients (30 patients with UC and four patients with IC) who underwent IPAA for refractory UC and had serologic testing drawn during their clinical course. These patients were categorized into the following four groups based on serologic testing: (1) pANCA+/ASCA− (16 patients), (2) pANCA−/ASCA+ (nine patients), (3) pANCA+/ASCA+ (five patients), and (4) pANCA−/ASCA− (four patients).

The diagnosis was changed postoperatively on clinical grounds from UC/IC to CD more frequently in patients who had the serologic pattern of pANCA−/ASCA+, which is typically associated with CD. Postoperatively, none of the patients (0 of 16) who were pANCA+/ASCA− had their diagnosis changed from UC/IC to CD, with a median follow-up time of 14 months (3–118 months). Postoperatively, four of nine patients (44%) who were pANCA−/ASCA+ had their diagnosis changed from UC (three patients)/IC (one patient) to CD, after a median follow-up time of 15 months (5–98 months) ($p = 0.04$).

Our study further showed that a relationship between the antibody reactivity patterns and the development of fistulas postoperatively after IPAA. Of 16 pANCA+/ASCA− patients who underwent IPAA, 15 of 16 (94%) remained fistula-free throughout the follow-up period. One pANCA+/ASCA− patient developed a pouch-vaginal fistula that was thought to be a complication of the surgery and not a manifestation of CD, by report of the patient's surgeon. Of nine pANCA−/ASCA+ patients who underwent IPAA, four of nine (44%) developed fistulas postoperatively (one enterocutaneous, one perianal, two pouch-vaginal fistulas). Of 13 ASCA+ patients in any combination who underwent IPAA, 7 of 13 (54%) developed fistulas postoperatively (three pouch-vaginal, one rectovaginal, one enterovaginal, one enterocutaneous, and one perianal fistula) ($p = 0.04$). Patients who developed a fistula had been followed for a median of 50 months (15–144 months); patients who were fistula free at the time of the study had been followed for a median of 14 months (3–120 months).

Our study did not suggest a relationship between the serology patterns and the development of pouchitis in patients undergoing IPAA (see later). We also examined the relationship between the development of pouchitis and the antibody titer level, but again did not find a relationship (data not shown). Of patients who were pANCA+/ASCA− and underwent IPAA: 9 of 16 (56%) patients developed pouchitis, after a median follow-up time of 17 months (4–112 months). Patients who were pANCA+/ASCA− and did not develop pouchitis were followed up for a median of 8 months (3–118 months). Of patients who were pANCA−/ASCA+ and underwent IPAA: seven of nine (77%) patients developed pouchitis, after an average follow-up time of 55 months (7–98 months). The patients who were pANCA−/ASCA+ and did not develop pouchitis were followed up for a median of 8 months (5–15 months). All patients who developed pouchitis were followed up for a median of 42 months (4–120 months); those who had not developed pouchitis at the time of the study had been followed for a median of 8 months (3–144 months).

In the two smaller groups of patients (pANCA+/ASCA+ and pANCA−/ASCA−), the associations with fistula formation and change in diagnosis were as follows. Of five patients who were pANCA+/ASCA+, three patients developed fistulas. Two of those patients had their diagnosis changed to CD. Of the four patients who were pANCA−/ASCA−, one patient developed a fistula and had the diagnosis changed to CD. Further analysis of these groups was not performed because of the small numbers in each cohort (see Tables 1 and 2).

DISCUSSION

Our study is the first to examine the association between the serologic markers, pANCA and ASCA, and fistula formation in post-IPAA patients. This study also suggested an association between the serologic identity of these patients and a final diagnosis of CD postoperatively. Existing literature, including

Table 1. Rates of postoperative fistula formation in 34 IBD patients who underwent IPAA for presumed UC and who had serologic markers measured

Serologic pattern	No. of patients	Median follow-up (mo)	Range of follow-up (mo)
ANCA+/ASCA−			
Total	16	14	3–118
Fistula	1	48	48
No fistula	15	14	3–118
ANCA−/ASCA+			
Total	9	15	5–98
Fistula	4	55	15–67
No fistula	5	8	5–98
ANCA+/ASCA+			
Total	5	18	5–144
Fistula	3	35	18–144
No fistula	2	6.5	5–8
ANCA−/ASCA−			
Total	4	48	14–120
Fistula	1	72	72
No fistula	3	23	14–120

These patients were divided into four groups based on their serologic patterns of reactivity.

the most recent and largest retrospective study, imply that pouch failure is most often seen in patients who have their diagnosis changed to CD or with complications often associated with CD (pelvic sepsis, perianal disease, fistula formation).[5]

Table 2. Rates of pouchitis in 34 IBD patients who underwent IPAA for presumed UC and who had serologic markers measured

Serologic pattern	No. of patients	Median follow-up (mo)	Range of follow-up (mo)
ANCA+/ASCA−			
Total	16	14	3–118
Pouchitis	9	17	4–112
No pouchitis	7	8	3–118
ANCA−/ASCA+			
Total	9	15	5–98
Pouchitis	6	55	7–98
No pouchitis	3	8	5–15
ANCA+/ASCA+			
Total	5	18	5–144
Pouchitis	1	35	35
No pouchitis	4	13	5–144
ANCA−/ASCA−			
Total	4	48	14–120
Pouchitis	4	48	14–120
No pouchitis	—	—	—

These patients were divided into four groups based on their serologic patterns of reactivity.

The role of serologic testing (pANCA or ASCA) has not been evaluated in predicting the risk of postoperative fistula formation or diagnosis change to CD in IPAA patients. Based on the results of this study, patients who fall into the pANCA−/ASCA+ pattern of serologic reactivity may be at increased risk for these complications. While this had not been examined previously in the IPAA population, there is mounting evidence in the general CD population that ASCA positivity is predictive of a disease phenotype that is usually confined to the ileum and that also exhibits structuring and penetrating phenotypic presentations.[22–24] This lends support to our findings that those patients who are ASCA positive in the population of UC patients post IPAA are more prone to fistula formation and change in diagnosis to CD. If such an association could be borne out in a larger study, it would add value to the serologic testing in that the results of the tests could lead to a change in treatment decisions for patients who develop persistent complications post IPAA surgery.

Previous studies have examined the utility of pANCA in predicting pouchitis in IBD patients undergoing IPAA; the conclusions from the various studies have been contradictory at times.[16–21] Our study did not find an association between the serologic profile and the development of pouchitis. In the patients who had a serologic pattern typically associated with CD, (pANCA−/ASCA+), there was a trend toward developing pouchitis.

There are several limitations to our study. The sample size is small, which makes it difficult to draw conclusions when no association was found, as in the case of the serologies with pouchitis. Our study is also limited by the potential for selection bias. Many of the samples were drawn postoperatively from patients who were having symptoms such as increased frequency of bowel movements, bloody bowel movements, and abdominal pain. This group, therefore, would not represent a random sample of post-IPAA patients. Consequently, the association between ASCA/ANCA pattern and the likelihood of postoperative fistulas or change in diagnosis to CD may have been overestimated. The potential for selection bias is suggested by the high rate of fistula formation and pouchitis in the study population, compared to the surgeon's cohort of about 750 patients undergoing the IPAA surgery. The rate of fistula formation in the study population was 26%; in the general cohort, it was 1.5%. The rate of pouchitis in the study population was 63%; in the general cohort, it was 10–15%.

In conclusion, in a cohort of patients undergoing IPAA for presumed UC, serologic profiles may be useful in identifying patients at risk of postoperative

fistula formation. Patients who were pANCA−/ASCA+ were at increased risk for the development of fistulas postoperatively compared to patients who were pANCA+/ASCA− and were also more likely to have their diagnosis changed postoperatively to CD. A larger prospective study is needed to validate these observations.

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Factors Affecting the Bowel Function after Proctocolectomy and Ileal J Pouch–Anal Anastomosis for Ulcerative Colitis

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The aim was to study determinants of postoperative bowel function after restorative proctocolectomy for ulcerative colitis. Medical records of patients who underwent proctocolectomy with ileal J pouch–anal anastomosis (IPAA) in two- or three-stage operations and whose status of defecation was known via a questionnaire were retrospectively reviewed. Bowel function, including stool frequency, stool consistency, and degree of nighttime soiling, was correlated with age at the time of surgery, time after ileostomy closure, mean resting anal pressure, longitudinal length of ileal J pouch, and duration of fecal diversion by using univariate and multivariate analyses. Stool frequency decreased significantly with time after ileostomy closure in both univariate and multivariate analyses. Stool frequency tended to be less in patients having a long J pouch, but the correlation was not significant ($P = 0.071$) in univariate analysis. Nighttime soiling ameliorated with time after ileostomy closure in multivariate, but not univariate, analysis. Deterioration of nighttime soiling was seen in patients whose duration for fecal diversion was long, both in univariate ($P = 0.068$) and multivariate ($P = 0.052$) analyses. Stool consistency was related to none of the five factors investigated. These results indicate that as the time after surgery increases, stool frequency decreases and nighttime soiling ameliorates. Delaying ileostomy closure because of anticipated postoperative incontinence does not significantly alter postoperative continence. (J GASTROINTEST SURG 2006;10:1065–1071)

KEY WORDS: Ileal J pouch–anal anastomosis, nighttime soiling, stool consistency, stool frequency, ulcerative colitis

Restorative proctocolectomy with ileal J pouch–anal anastomosis (IPAA) or ileal J pouch–anal canal anastomosis are the most common operative procedures for patients with ulcerative colitis (UC). We have been performing mainly IPAA in two- or three-stage operations. Although the level of patient satisfaction after IPAA is generally high, bowel function, including postoperative incontinence, stool frequency, and stool consistency, influence patients' postoperative quality of life.

The five quantitative factors that could affect patients' bowel function after IPAA should be considered. Because anal sphincter function is impaired in elderly patients,[1] age at the time of surgery should be considered among the factors that affect functional outcome after IPAA. Bowel function is generally believed to improve with increasing time after ileostomy closure.[2] Anal manometry is the most frequently used method to evaluate anal sphincter function.[3] Volume of the J pouch can also affect functional results after IPAA, because greater volume of the pouch could lead to less frequent stools.[4] Duration for fecal diversion, defined as the time between IPAA with defunctioning loop ileostomy and ileostomy closure, is also likely to alter the recovery of the anal sphincter function after ileostomy closure.

The aim of the present study was to investigate whether these five factors—age, time after ileostomy

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closure, anal manometry, volume of the J pouch, and duration of fecal diversion—could be determinants of bowel function (stool frequency, stool consistency, and nighttime soiling estimated by a questionnaire) after IPAA, by using univariate and multivariate analyses.

PATIENTS AND METHODS

Patients

Among patients with UC who underwent proctocolectomy with rectal mucosectomy, ileal J pouch–anal anastomosis with two- or three-stage operations in our department from 1993–2002, there were 73 patients whose functional status of defecation was provided by a questionnaire mailed to and returned by each patient. We reviewed the charts of these 73 patients and excluded six who had complications that affected postoperative bowel function: three patients with cuff abscess, two patients with dehiscence of IPAA that became evident with contrast-medium enema, and one patient with pouch-vaginal fistula who underwent transanal repair.

Surgical Procedures

We performed proctocolectomy with rectal mucosectomy, hand-sewn IPAA in two- or three-stage operations. Three surgeons performed this procedure during the years 1993–2002. Basically, three-stage operations were indicated for patients who necessitated emergency operation because of severe colitis or uncontrollable bleeding with conservative treatment, whereas two-stage operations were indicated for patients in whom elective surgery was feasible. In the first operation of three-stage operations, the patient underwent subtotal colectomy and construction of rectal mucous fistula and end-ileostomy. After several months, the second operation was carried out; after performing transanal rectal mucosectomy for 5–7 cm length, we performed transabdominal resection of the remnant rectum and created the ileal J pouch by using a stapler. Defunctioning loop ileostomy was made 40–50 cm orad to the pouch after performing transanal IPAA with 22–25 stitches. In two-stage operations, the first and second operations in the three-stage operations were performed as the first operation. In the final operation, loop ileostomy was excised and closed with a side-to-side ileo-ileostomy by using a stapler.

Methods

Among those 67 patients, we determined each patient's age at the ileostomy closure, the duration of time since ileostomy closure, the results of anal manometry before closing loop ileostomy, the longitudinal length of the ileal J pouch, and the duration of fecal diversion. Anal manometry was measured twice in each patient, one time before performing IPAA and one time before closing loop ileostomy with a nonperfusion-type microtransducer (MMS Co., Tokyo, Japan). A catheter was introduced into the J pouch at the left decubitus position and then pulled automatically with a speed of 6 cm/minute to measure the maximal resting pressure (MRP) of the anus. Correlation was studied between MRP before IPAA and before ileostomy closure. The longitudinal length of the ileal J pouch that was measured during surgery was used as an indicator of J pouch volume. Overall incidence of pouchitis was determined whether or not patients who complained of symptoms suggesting pouchitis (melena, increase in stool frequency, lower abdominal pain, general fatigue) had inflammation (redness, ulcer, bleeding) on the pouch endoscopy.

Questionnaire

In our questionnaire, we asked for information about the following factors: daily stool frequency, stool consistency, and degree of nighttime soiling. Stool consistency could be ranked as one of four classes: solid, semisolid, semiliquid, and liquid. Similarly, five levels of nighttime soiling were established: everyday, frequent (4 times or more a week), often (1–3 times a week), occasionally (only when stool gets watery), and never.

Statistical Analysis

Student's t test was used for comparison of the mean MRP before IPAA and before ileostomy closure. For univariate analysis, correlations between each factor (age, time after ileostomy closure, MRP before ileostomy closure, longitudinal length of the J pouch, and duration for fecal diversion) and stool frequency, stool consistency, or degree of nighttime soiling were analyzed. Pearson's correlation coefficient test was used for analyzing correlation between MRP before IPAA and before ileostomy closure, and comparisons between each factor and stool frequency. Spearman's correlation coefficient by rank was used for comparisons between each factor and stool consistency or the degree of nighttime soiling. As multivariate analysis, multiple regression analyses were performed to study if stool frequency, stool consistency, or degree of nighttime soiling could be predicted using five factors: age, time after ileostomy closure, MRP, length of the J pouch, and duration of fecal diversion were set as independent

Table 1. Background of 67 patients

Total number of patients (male:female)	67 (33:34)
Two-stage: three-stage	14:53
Age	31 (11–62) years
Time after ileostomy closure	2.0 (0.2–8.8) years
MRP before IPAA	83 (36–204) cm H_2O
MRP before ileostomy closure	62 (21–120) cm H_2O
Length of the J pouch	15 (8–20) cm
Duration of fecal diversion	5 (2–24) months
Stool frequency	7 (4–18) per day
Stool consistency	Solid: 1 (1.5%)
	Semisolid: 23 (34.5%)
	Semiliquid: 40 (59.7%)
	Liquid: 3 (4.5%)
Degree of nighttime soiling	Never: 24 (35.8%)
	Occasionally: 27 (40.3%)
	Often: 9 (13.4%)
	Frequently: 5 (7.5%)
	Everyday: 2 (3.0%)
Incidence of pouchitis	11/67 (16.4%)

Values are median (range).

variables, whereas stool frequency, stool consistency, or degree of nighttime soiling were set as dependent variables. *P* values less than 0.05 were regarded as significant. All values are expressed as mean + SEM.

RESULTS

The backgrounds and bowel function of 67 patients are summarized in Table 1. There was no significant difference in sex distribution. Fifty-three of 67 (79%) patients underwent three-stage operations because most patients had severe colitis. Median time after ileostomy closure was 2 years, with a mean value of 2.5 + 0.2 years, and follow-up time was longer than 1 year in 49 of 67 patients. Median MRP before IPAA and before ileostomy closure was 83 and 62 cm H_2O, respectively, and mean MRP before IPAA (91.1 + 4.0 cm H_2O) was higher than that before ileostomy closure (60.5 + 2.5 cm H_2O; $P < 0.05$). No correlation was found between MRP before IPAA and before ileostomy closure ($P > 0.05$). J pouch lengths among patients varied from 8–20 cm. Although our goal was to create 10–15 cm of J pouch whenever possible, lengths varied to allow the tip of each ileal J pouch to easily reach the dentate line. Duration for fecal diversion varied from 2 to 24 months, with a median value of 5 months. Mean duration for fecal diversion was 5.7 + 0.4 months, and there were 16 patients whose duration for fecal diversion was longer than 6 months. Among these 16, ileostomy closure was delayed in eight patients (50%) whose symptoms suggested incontinence (soiling of mucus). Reasons for delaying the ileostomy closure in the other eight patients included patients' convenience in six (37%) patients, and severe stricture of the IPAA in two (13%) patients. Mean duration of fecal diversion was 4.5 + 0.1 months in 51 patients when 16 patients were excluded whose ileostomy closure was delayed. Median stool frequency was seven stools/day, with a mean value of 7.6 + 0.4, and more than half of the patients described their stool consistency as semiliquid. Although 24 patients were perfectly continent, two patients complained of daily nighttime soiling. Overall incidence of pouchitis was 16.4%, as there were 11 patients who fulfilled our criteria. Seventeen (25%) patients were taking an antidiarrheic drug loperamide (2 mg/day) when their stool was loose, whereas the remaining 50 patients were free from drugs. Stool frequency/day in patients taking loperamide (7.8 + 0.7) was not different from patients free from drugs (7.5 + 0.4). The use of loperamide did not correlate with stool consistency or degree of nighttime soiling, either.

Correlations between each factor and stool frequency are shown in Table 2. Time after ileostomy closure was the only factor that correlated with stool frequency in univariate and multivariate analyses (Fig. 1, Table 2). Length of the J pouch showed a trend toward correlation with stool frequency

Table 2. Correlation between stool frequency and five factors

	Univariate analysis*		Multivariate analysis		
	Correlation coefficient	***P* value**	**Coefficient**	**Standard coefficient**	***P* value**
Age	0.09	0.47	0.0040	0.017	0.90
Time after ileostomy closure	−0.36	0.0027	−0.74	−0.41	0.0070
MRP before ileostomy closure	−0.06	0.65	−0.016	−0.11	0.27
Length of J pouch	−0.231	0.071	−0.047	−0.044	0.76
Duration of fecal diversion	0.052	0.68	0.097	0.058	0.66

*Pearson's correlation coefficient test.

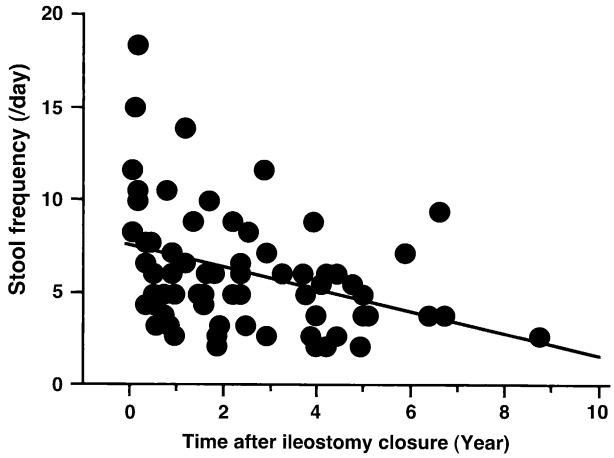

Fig. 1. Time after ileostomy closure and stool frequency. Stool frequency decreased as time after ileostomy lengthened. *P* values were less than 0.05 in both univariate and multivariate analyses.

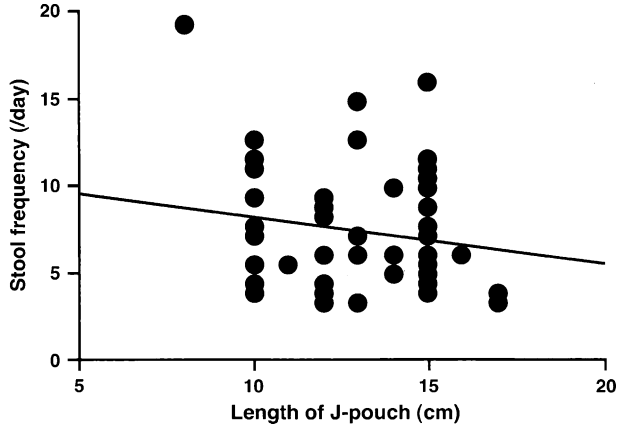

Fig. 2. Length of J pouch and stool frequency. Stool frequency tended to decrease as the volume of the J pouch increased, but the correlation did not reach statistical significance.

that did not reach statistical significance in univariate analysis (Fig. 2, Table 2). Age, MRP, and duration of fecal diversion did not correlate with stool frequency (Table 2). Stool consistency did not relate to any of these factors, either univariate or multivariate analysis (Table 3). Reductions in the frequency of nighttime soiling were dependent on the time after ileostomy closure in multivariate analysis, but not in univariate analysis (Table 4); time after surgery was 2.4 + 0.4 years in patients who never had soiling, 3.2 + 0.4 in patients with occasional soiling, 1.8 + 0.7 in patients who often have soiling, 1.8 + 0.7 in patients with frequent soiling, and 0.3 in patients who have soiling every day (Fig. 3). Nighttime soiling tended to increase in frequency in patients whose duration of fecal diversion was long, but the correlation was not significant in univariate and multivariate analyses; duration of fecal diversion was 5.1 + 0.4 months in patients who never had soiling, 5.4 + 0.3 for occasional soiling, 5.8 + 0.4 in patients who often had soiling, 10.4 + 3.9 in patients with frequent soiling, and 5.5 in patients who had soiling everyday (Fig. 4, Table 4). Age, MRP, and J pouch length did not correlate with degree of nighttime soiling (Table 4).

DISCUSSION

Among the factors that were predicted to affect bowel function after IPAA, we found that time after ileostomy closure is more important than age, MRP, pouch volume, or time for fecal diversion. Results in the present study indicate that the frequency of stool and of nighttime soiling both decrease with time after ileostomy closure. This observation does not conflict with a previous report stating that the frequency of defecation decreased between the fourth month and 1 year after ileostomy closure.[4] Three mechanisms for this phenomenon should be considered: recovery of absorptive function of the ileum distal to the loop ileostomy, increase of ileal reservoir capacity, and recovery of anal sphincter function.[3] Changes in the hormonal environment after

Table 3. Correlation between stool consistency and five factors

	Univariate analysis*		Multivariate analysis		
	Correlation coefficient	*P* value	Coefficient	Standard coefficient	*P* value
Age	−0.17	0.17	−0.0060	−0.13	0.36
Time after ileostomy closure	0.064	0.34	0.015	0.046	0.77
MRP before ileostomy closure	0.034	0.79	−0.0010	−0.051	0.72
Length of J pouch	0.17	0.19	0.032	0.16	0.30
Duration of fecal diversion	−0.0040	0.98	−0.044	−0.15	0.30

*Spearman's correlation coefficient by rank.

Table 4. Correlation between nighttime soiling and five factors

	Univariate analysis*		Multivariate analysis		
	Correlation coefficient	*P* value	Coefficient	Standard coefficient	*P* value
Age	−0.075	0.54	−0.0050	−0.049	0.70
Time after ileostomy closure	−0.17	0.17	−0.22	−0.36	0.017
MRP before ileostomy closure	−0.14	0.27	−0.0080	−0.157	0.24
Length of J pouch	−0.0060	0.96	0.039	0.10	0.47
Duration of fecal diversion	.23	0.068	0.15	0.26	0.052

*Spearman's correlation coefficient by rank.

IPAA must also be associated with this postoperative adaptation.[5] Other investigators reported opposing results that stool frequency did not change with time.[2,6] This difference may be associated with different analytical methods, because the same patient was followed at certain intervals in those reports, whereas our study analyzed stool frequency in multiple patients at different postoperative times.

There was a tendency toward less frequent stools in patients with a long J pouch, although the correlation was not statistically significant. This finding corresponds with previous reports of an inverse correlation between stool frequency and pelvic pouch volume.[4,7,8] Differences of several centimeters in the length of the pouch might not significantly alter pouch capacity, which may explain why significance was not obtained between pouch length and stool frequency. In hindsight, the volume rather than length of the pouch could have been measured for a more precise comparison.

The degree of nighttime soiling tended to deteriorate with increasing time for fecal diversion, but the correlation was not significant. This could be due to the fact that we postponed the ileostomy closure in patients who complained of incontinence of mucus. Conversely, correlation between nighttime soiling and time for fecal diversion in the present study suggests that nighttime soiling does not improve if ileostomy closure is delayed. Functional outcome after IPAA did not differ between patients whose ileostomy closure was delayed for various reasons and those who had earlier closure.[9]

We found that age at the time of surgery and MRP had no significant effects on postoperative bowel function. It is generally accepted that a patient's age is not related to the functional outcome

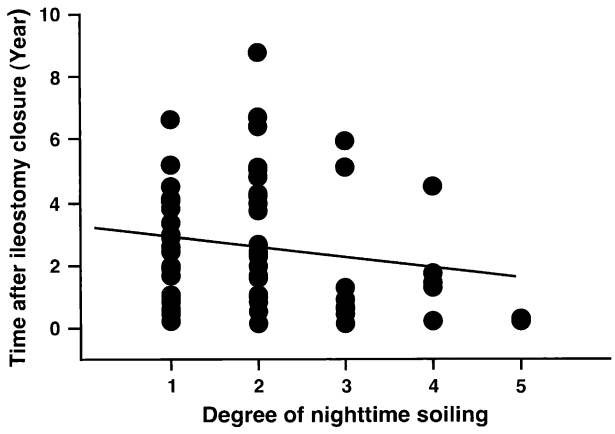

Fig. 3. Time after ileostomy closure and degree of nighttime soiling. Degree of nighttime soiling tended to ameliorate as the time after ileostomy closure increased, and statistical significance was seen in multivariate analysis. Degree of nighttime soiling: 1, never; 2, occasionally (only when stool gets watery); 3, often (1–3 times a week); 4, frequently (4 times or more a week); and 5, everyday.

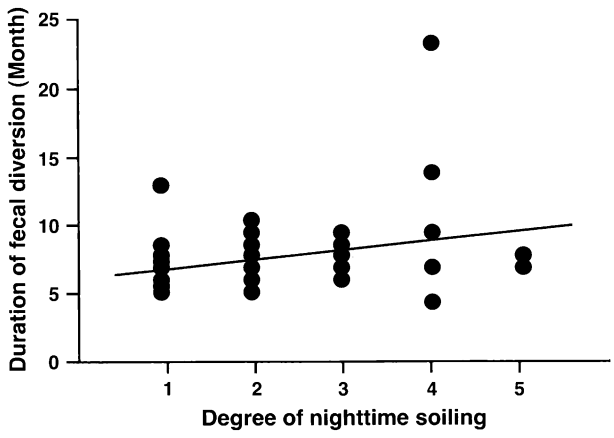

Fig. 4. Duration of fecal diversion and degree of nighttime soiling. Degree of nighttime soiling tended to deteriorate as the duration for fecal diversion increased, but the correlation did not reach statistical significance. Degree of nighttime soiling: 1, never; 2, occasionally (only when stool gets watery); 3, often (1–3 times a week); 4, frequently (4 times or more a week); and 5, everyday.

after IPAA, and that IPAA should be performed in relatively old patients.[10–12] Anal manometry is the most common method to measure the function of the anal sphincter. Although several parameters can be measured by anal manometry, we employed MRP, which is believed to represent the function of the internal anal sphincter and therefore should associate closely with the degree of nighttime soiling. There are reports that preoperative MRP cannot predict degree of soiling after surgery,[13–15] whereas ambulatory anal canal pressure is higher in continent patients than incontinent patients.[16] In view of our findings and those of these reports, it seems that MRP before ileostomy closure measures a parameter of the anal sphincter that cannot be used to predict postoperative incontinence.

Stool consistency did not correlate with any of the five parameters investigated, suggesting that stool consistency must be affected by other factors such as composition of food intake and volume of water intake. Although measurement of water content in the stool could function as a more objective indicator for stool consistency, asking patients about stool consistency in a questionnaire permits information about the patients' overall impression of stool consistency.

Pouchitis, nonspecific and idiopathic inflammation of the ileal reservoir, is the most common long-term complication after IPAA for UC and is believed to affect patients' bowel function.[17] Although we evaluated overall incidence of pouchitis as 16.4% in our study, we did not exclude those patients from our study nor did we correlate bowel function with degree of pouchitis, because diagnosis of pouchitis cannot be made based on only clinical symptoms.[18] Precise diagnosis or grading of pouchitis necessitates endoscopic and histologic examinations as well as clinical evaluation.[19] It is impossible to perform those examinations in all patients, including those without symptoms suggesting pouchitis. An antidiarrheic drug loperamide is reported to decrease daily stool frequency.[20,21] In our study, bowel function, including stool frequency, did not differ between patients taking loperamide and those free from drugs. This difference was considered to be associated with the fact that the dose of loperamide in previous reports (8–12 mg/day) was much greater than that in our patients (2 mg/day).

In conclusion, in patients undergoing IPAA, the frequencies of both stool and nighttime soiling decrease significantly with time after ileostomy closure. Furthermore, greater volume of the pelvic pouch may lead to less frequent stools. Finally, delaying loop ileostomy closure in patients with symptoms that suggest future incontinence, such as soiling of mucus, may not alter the occurrence of postoperative incontinence.

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Open Pancreaticogastrostomy After Pancreaticoduodenectomy: A Pilot Study

Claudio Bassi, M.D., Giovanni Butturini, M.D., Roberto Salvia, M.D., Ph.D., Stefano Crippa, M.D., Massimo Falconi, M.D., Paolo Pederzoli, M.D.

Management of the pancreatic stump following pancreaticoduodenectomy (PD) has always been a main source of concern among pancreatic surgeons. The present pilot study describes the reconstructive technique of anterior transgastric pancreaticogastrostomy (PG) after pylorus-preserving PD. Outcome in 50 patients with "soft" residual parenchyma treated with this technique is also reported. The average duration of the intervention was 351 minutes (range, 240–360); only two patients needed intraoperative transfusion with 2 units of blood. The postoperative period involved complications in 15 cases (30%). In particular, four patients developed pancreatic fistulas (8%), which were grade C in three cases (6%) and grade B in one patient (2%). Two patients (4%) presented with enteric fistula from erosion from a drain. Two patients experienced perianastomotic fluid collections associated with delayed gastric emptying (4%) and a clinically silent 5-cm abdominal collection was observed in an additional case. Bleeding of a gastric ulcer was treated in one case and four patients developed bronchopneumonia. None of the complications required a second surgical intervention and there were no deaths. One patient with a symptomatic fluid collection was treated by ultrasound-guided cutaneous drainage. The mean hospitalization time was 11.1 days (range, 8–25 days). The results obtained in this pilot study appear encouraging and merit further analysis in a randomized comparative trial. (J GASTROINTEST SURG 2006;10:1072–1080)

KEY WORDS: pancreaticoduodenectomy, pancreaticogastrostomy, pancreaticojejunostomy, pancreatic surgery

Reconstruction of pancreatic-intestinal continuity following pancreaticoduodenectomy (PD) is a surgical problem that has remained unresolved.[1,2] Pancreatic anastomosis is the Achilles' heel that is responsible for the high frequency of postoperative complications and, in particular, pancreatic fistulas.[3] Many reconstruction techniques have been proposed,[2,4–11] although only a few prospective and randomized studies aimed at comparing different techniques have been carried out.[12–21] Moreover, the available studies do not allow the surgeon to determine which technique is superior to another and thus the pancreatic surgeon must possess a large amount of reconstructive "fantasy." In the present report, we describe the reconstructive technique of anterior transgastric pancreaticogastrostomy (PG) after pylorus-preserving PD (PPPD). Outcome in 50 patients treated with this technique is also reported.

METHODS

Once the resection phase of PPPD is completed, the pancreatic stump is mobilized for about 5 cm with respect to the underlying splenoportal axis. The stomach is approximated to the residual

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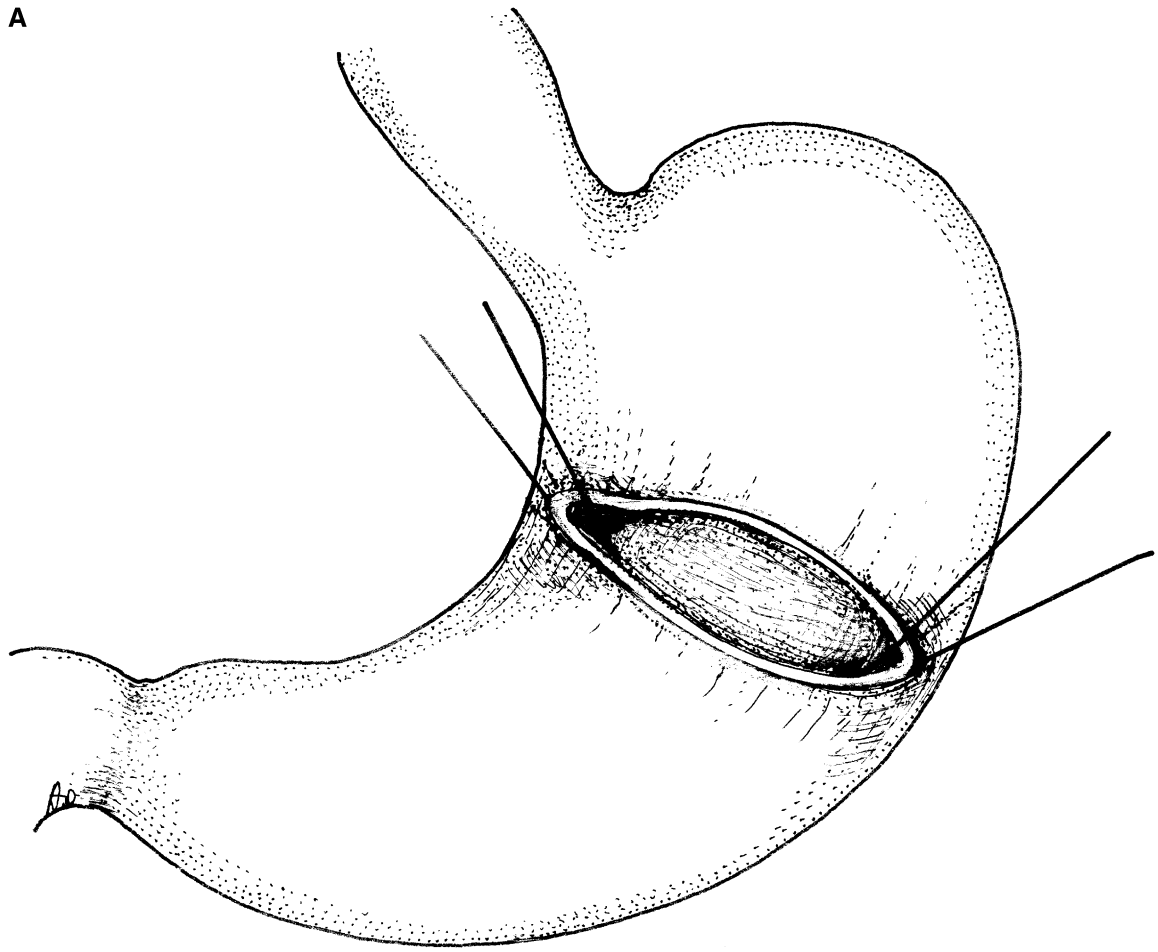

Fig. 1. Open anterior gastrostomy (**A**) and posterior gastrostomy with insertion of the pancreatic gland in the gastric cavity (**B**).

pancreas, and calculating the distance between the different planes until adequate tension is achieved, an ample anterior gastrostomy is performed. At the same level, posterior gastrostomy is performed, the extension of which is adapted to the dimensions of the gland (Fig. 1, *A*, *B*); using the two gastrostomies, the pancreas is brought first into the gastric cavity and then taken out anteriorly, applying slight traction on the sutures positioned at the superior margin and inferior to the glandular section. The anterior gastrostomy is spread open using two small Farabeuf retractors, and the posterior wall of the stomach is pushed posteriorly until the pancreas, lightly pushed upward and thus toward the gastric cavity, is abundantly invaginated by at least 3–4 cm. At this point, the anterior and posterior margins of pancreas are fastened with PDS sutures (3-0 or 4-0). Anastomosis of the superior rim is then initiated with button sutures between the gastric serous muscle and glandular parenchyma, taking care to load the mucosa with a Shapeaux. The gastric mucosa is attached to the parenchymal gland with a second row of sutures between itself and the capsule gland. The Wirsung is incannulated in order to avoid being sutured and the inferior rims of the pancreas and stomach are anastomosed using the same technique described above (Fig. 2, *A*, *B*). This portion of the anastomosis is reinforced on the outside by turning the stomach upwards and positioning sutures between the posterior gastric serous and the glandular capsule. The final result is shown in Figure 3. The pancreas amply invaginated in the stomach by at least 3–4 cm, and the entire anastomotic rim can be controlled and reinforced if necessary using additional sutures. The endo-Wirsung stent is removed, and a nasogastric tube is placed avoiding direct contact with the anastomosis. The anterior gastrostomy is then closed in a single layer. The biliodigestive and submesocolic duodenojejunal anastomoses are then performed, and the pancreatogastric anastomosis is drained with an intraperitoneal drain positioned in the subpancreatic, retrogastric region.

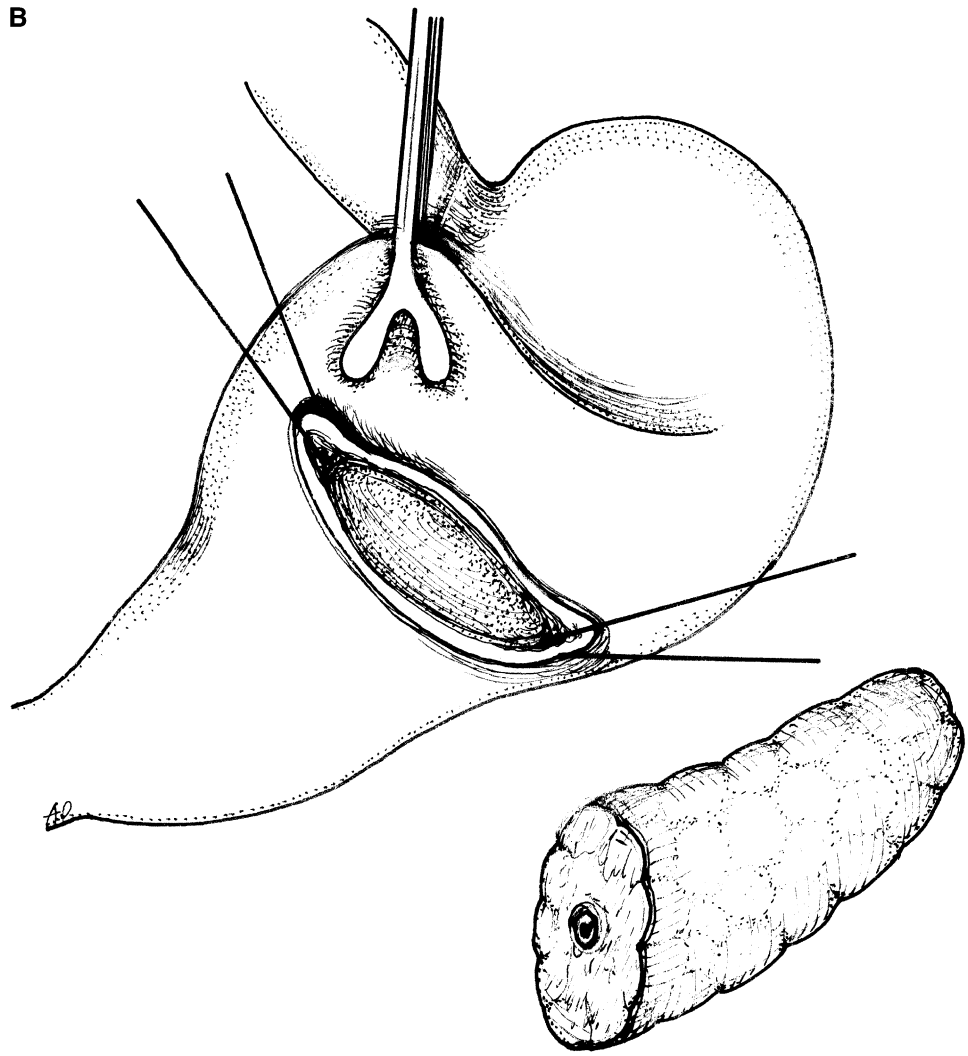

Fig. 1 (Continued).

RESULTS

We prospectively used anterior transgastric PG in 50 consecutive patients with "soft," "high-risk" pancreatic tissue subjected to PPPD. This group of patients consisted of 30 men and 20 women, with an average age of 67 years (range, 35–69) and an average body mass index of 23.9 kg/m^2 (range, 20.8–27.8). Only three patients underwent gastric resection. The mean duration of the intervention (all carried out or supervised by the first author) was 351 minutes (range, 240–360). Only two cases needed intraoperative transfusion with 2 units of blood. Complications were observed during the postoperative recovery period in 15 patients (30%). In particular, four patients developed a pancreatic fistula (8%), which was grade C in 3 cases (6%) and grade B in one patient (2%) according to the classification of the International Study Group on Pancreatic Fistula.[21]

Two patients (4%) developed enteric fistula from erosion from a drain. Two experienced a perianastomotic leak associated with delayed gastric emptying (4%), and a clinically silent 5-cm abdominal collection was observed in an additional patient. Bleeding from a gastric ulcer was treated in one patient, and four patients developed bronchopneumonia. None of the complications required a second surgical intervention, and there were no deaths. One patient with a symptomatic fluid collection was treated by ultrasound-guided percutaneous drainage. The mean hospitalization time was 11.1 days (range, 8–25 days).

Histological examination led to a diagnosis of 22 ductal adenocarcinomas (44%), 7 intraductal papillary mucinous tumors (14%), 6 neuroendocrine tumors (12%), 5 papillary of Vater carcinomas

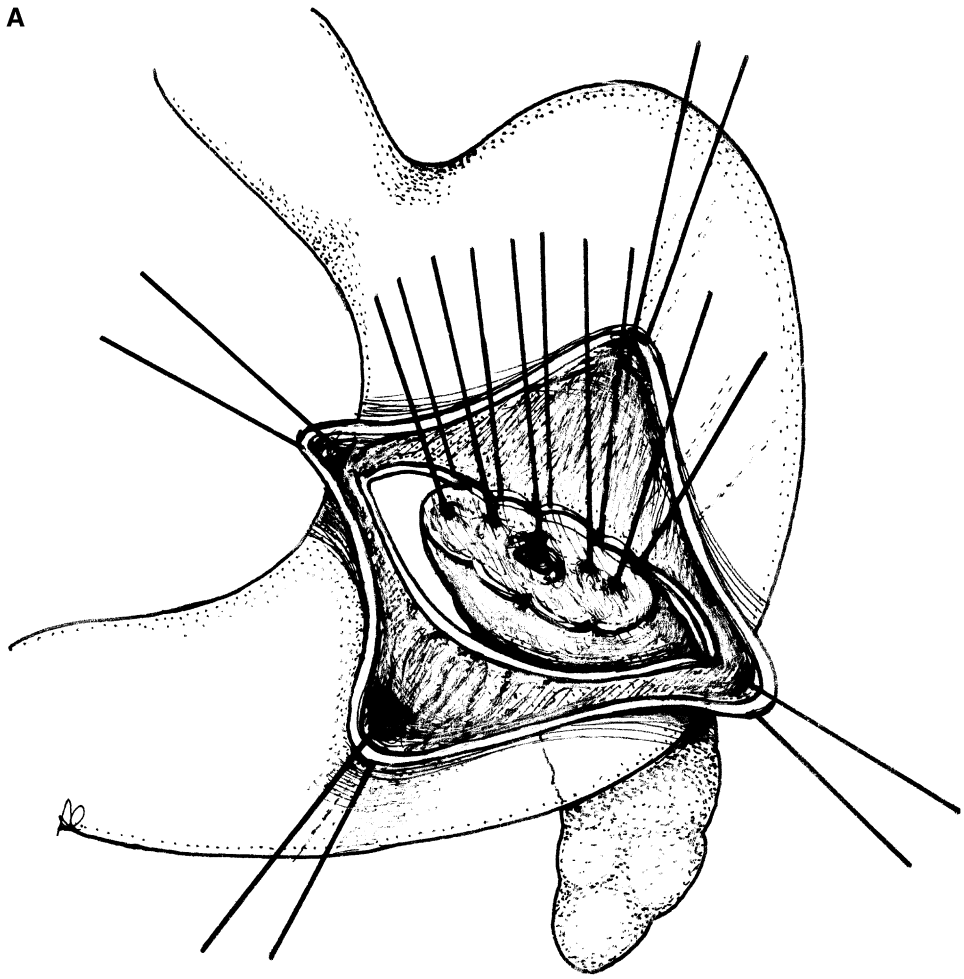

Fig. 2. Intragastric invagination of the pancreas and wrapping of the superior (**A**) and inferior (**B**) anastomoses.

(10%), 4 distal bile duct neoplasms (8%), 2 duodenal tumors (4%), and 4 rare pancreatic tumors (8%).

DISCUSSION

Management of the pancreatic stump after PD has always been a main source of concern for pancreatic surgeons.[1–3] None of the available reconstructive techniques assessed in randomized trials have demonstrated a frank superiority in prevention of postoperative complications, with particular reference to leakage, subsequent fluid collection, and fistulas.[12–20] Despite these limitations, reconstruction with PG seems to be gaining popularity as shown by a small number of noncontrolled studies showing favorable results[22–27] as well as by some considerations coming from the only two controlled clinical trials on the topic.[13,20] In fact, it has been stressed that with respect to pancreatojejunal anastomosis (PJ), PG is both easier and faster to perform. The stomach lies in front of the pancreatic stump and there is no luminal discrepancy in terms of size with the pancreatic remnant. This allows a wide, "tension-free" anastomosis with more than adequate tissue to telescope the stump. Moreover, without doubt PG foresees, obviously, a single loop of jejunum for gastric and biliary anastomosis with two, and not three, anastomoses to a single loop with a lower chance of kinking.

Additionally, from a theoretical standpoint, PG should be less prone to ischemia considering the gastric vascular supply; the exocrine secretions, entering in an acid environment with a low pH, do not become activated, avoiding the enzymatic activity and alkaline pancreatic secretion that could protect the development of marginal ulcer in duodenojejunostomies or gastrojejunostomies.[28] In our clinical context, as already stressed, PG did not show any significant differences in the overall postoperative

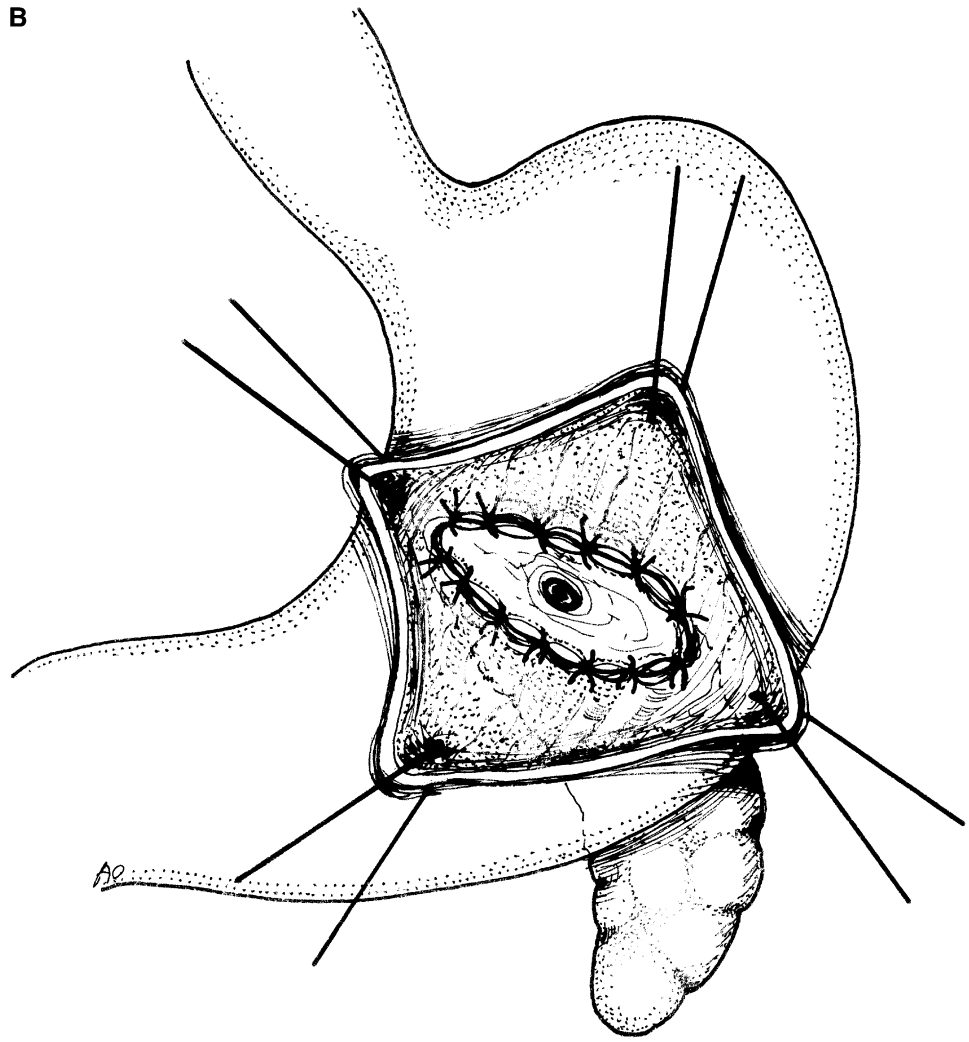

Fig. 2 (Continued).

complication rate or incidence of pancreatic fistula compared to PJ. However, biliary fistula, postoperative collections, and delayed gastric emptying were significantly reduced by PG. In addition, PG has been associated with a significantly lower frequency of multiple surgical complications versus PJ.[20]

All of the theoretical and demonstrated advantages, or "conveniences," of PG versus PJ are in contrast only with the consideration that in PPPD, the closeness of the anastomosis to pyloric activity might pose a risk for containment during the stasis phase in the immediate postoperative period. Nonetheless, such a possibility has never been clearly observed. The "standard" PG involves anastomosing the pancreas to the posterior wall of the stomach from the outside.[13,20,22–27] In 1975, Mackie et al.[29] proposed for the first time direct visualization of the area from the inside for the pancreaticojejenostomy after hemigastrectomy. To the best of our knowledge, this is the first report of an "open PG" after PPPD.

This technique of performing the PG, with respect to a classic approach, is even easier to carry out. It is safe, it can put the pancreas into the gastric cavity in an optimal manner (only on the condition that the pancreas is rendered highly mobile), and it provides excellent vision of the surgical field and, consequently, more accurate performance of the anastomosis. The outcome in this pilot study is encouraging in that no deaths were observed and no second open interventions were needed with the exception of one minimally invasive procedure involving ultrasound-guided drainage of a peripancreatic collection. Abdominal morbidity (30%) was less than previously reported by us in other recent publications in 2001 (38%), 2003 (36%), and 2005 (34%).[3,12,20] In particular, the reduced incidence of

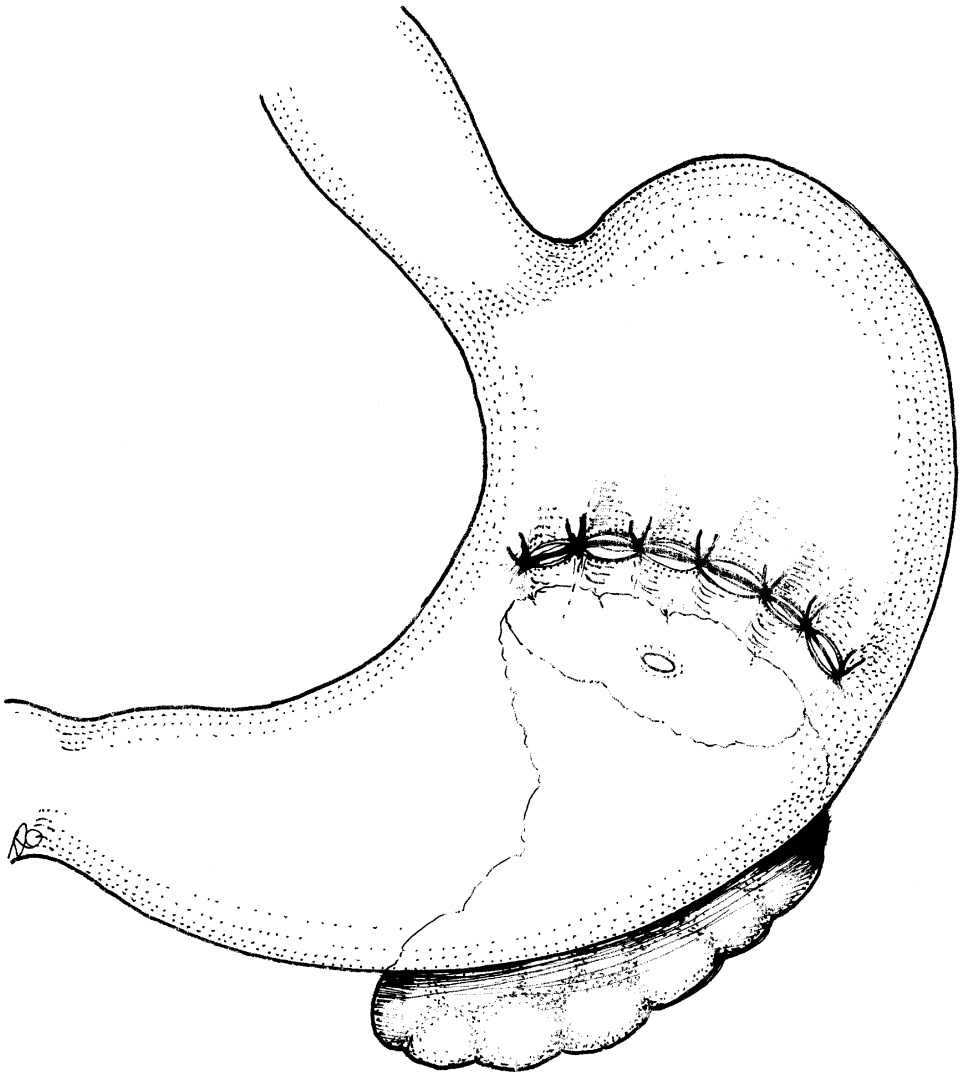

Fig. 3. Final pancreaticogastric anastomosis.

pancreatic fistulas also decreased to 8%, compared to a previous mean of 13.8%.[3,12,20]

With regard to the four pancreatic fistulas observed in the present series, it is worthwhile to underline that they were resolved with conservative therapy. One was classified as grade B according to the criteria established by the International Study Group on Pancreatic Fistula,[21] which determined a delay in drainage removal, and was treated on an outpatient basis for 1 month. The other three cases were more challenging: one patient (the only one with a positive methylene blue test per os) needed percutaneous drainage because of signs of sepsis, while the remaining two required prolonged enteral and parenteral nutrition. The trend toward a particularly benign postoperative recovery period in the pancreatic fistulas following PG is also confirmed by recent reports with all cases showing conservative recovery.[30,31]

PG is a promising technique that should be part of the knowledge of surgeons in high-volume centers. This is implicit not only for the advantages already described but also considering that specific pathologies, such as intraductal papillary mucinous tumors, are increasing in incidence in reference centers. In the follow-up of this disease, as suggested by Gigot et al.,[32] the role that endoscopic controls play following cephalic resection with reconstruction by PG should not be underrated.

In the future, the definitive choice of the type of standard reconstruction, comparing PJ and PG, including the variant described here, should be evaluated in a randomized prospective trial. While there are some data available from animal models,[28]

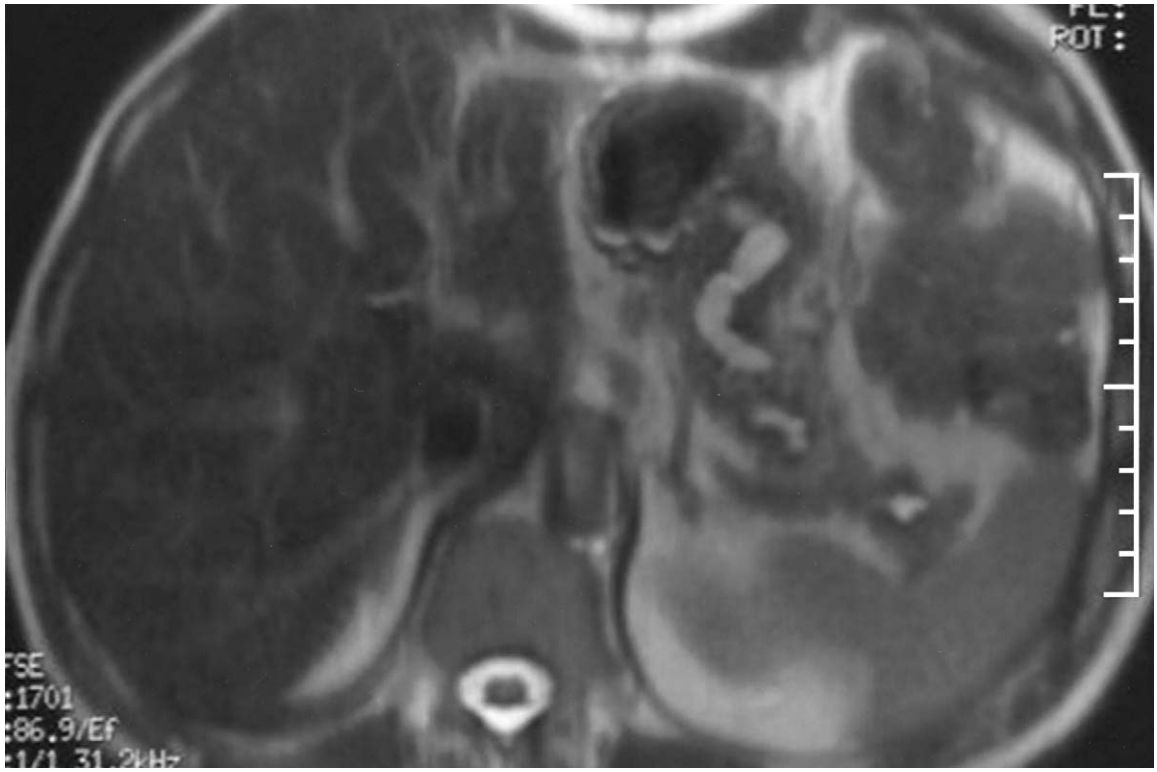

Fig. 4. Magnetic resonance cholangiopancreatography after nonfunctioning pancreogastroanastomosis: the main duct is dilated.

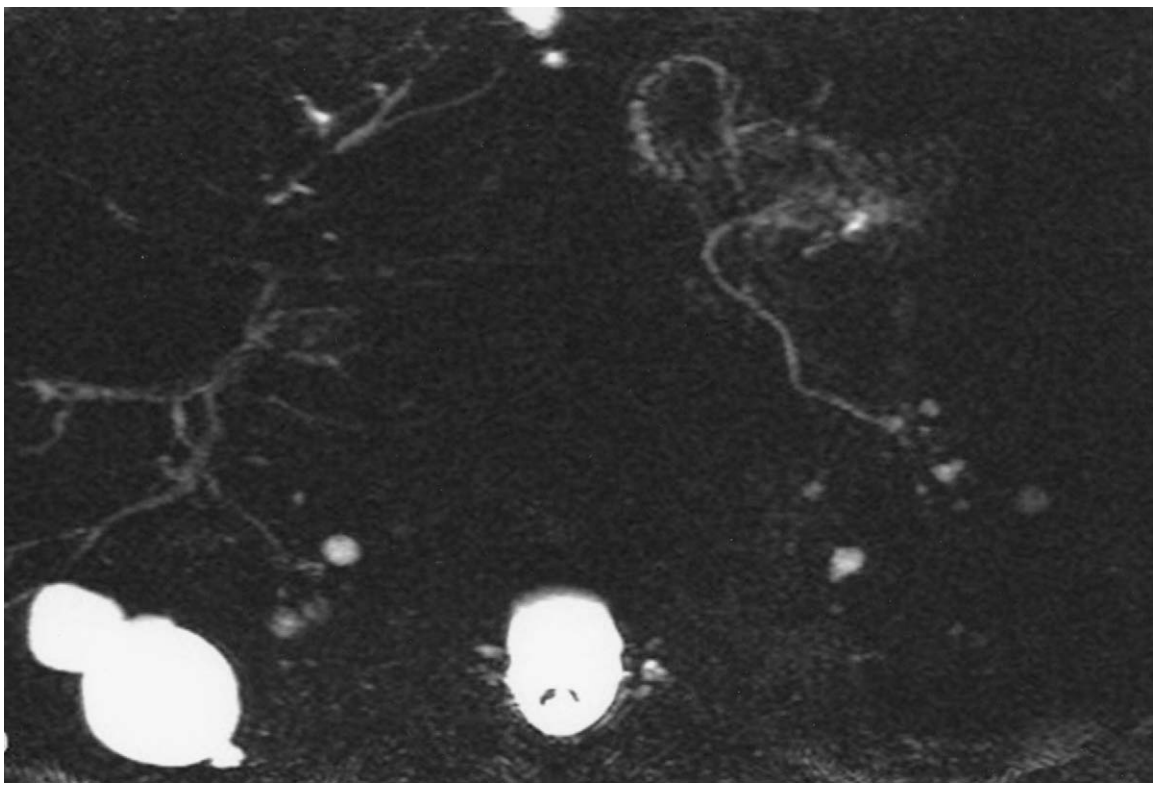

Fig. 5. Magnetic resonance cholangiopancreatography with functioning pancreatic anastomosis into the stomach.

almost no data are available for humans. The choice between PJ and PG does not influence the development of glucose intolerance.[33] After PG, gastric pH circadian rhythms are comparable to healthy controls, while stool amylase levels are significantly higher in controls with respect to patients after PG or PJ; no significant differences were observed between the two reconstructive techniques. These data show that patency of the duct is maintained and secreted enzymes are active following eating.[34]

Today, the patency of the anastomosis in PG can be easily analyzed in a noninvasive manner using magnetic resonance cholangiopancreatography (MRCP) (Figs. 4 and 5).

In 10 patients, Aube et al.[35] studied the patency of the PG by secretin MRCP. Patency was classified into four grades, from 0 (obstruction) to 3 (good patency). Pancreatic exocrine function was assessed by fecal elastase-1 concentrations. MRCP grades were 0 in two patients, 1 in four, 2 in five, and 3 in eight. There were statistically significant differences between the secretin MRCP permeability grade and fecal-1 elastase concentration ($p < 0.03$) and between the secretin MRCP permeability grade and pancreatic atrophy ($p < 0.005$). In contrast, fecal elastase-1 concentrations were lower than reference values in all but one case. The authors conclude that secretin MRCP may indicate PG stenosis or dysfunction, but it is not the only factor suggesting exocrine insufficiency.

The Wirsung duct has a well-known tendency to dilate during follow-up, and this phenomenon is not necessarily a sign of functional insufficiency[36]; it appears to be minimized by adopting duct to mucosa reconstructive techniques.[37]

Le Blanc et al.[38] analyzed the pattern of motility of the upper digestive tract after both PJ and PG. As expected, despite differences in the location of entry of the pancreatic and biliary secretions into the gastrointestinal tracts, no patients with normal jejunal motility were observed. Surprisingly, PG yielded a more "normal-like" tracing.

Considering all reported studies, it can be concluded that PG provides long-term patency of the main duct with a reduced secretion of enzymes that can nonetheless be activated without determining devastating effects on the motility of the upper digestive tract.

Following the first PG performed in 1946,[39] this reconstructive technique has continued to intrigue pancreatic surgeons.

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Review Article

Conversions During Laparoscopic Cholecystectomy: Risk Factors and Effects on Patient Outcome

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In view of the substantial, at times conflicting, literature on conversion to open surgery during laparoscopic cholecystectomy (LC), we have considered it timely to review the subject to identify the risk factors for conversion and its consequences. The review is based on a complete literature search covering the period 1990 to 2005. The search identified 109 publications on the subject: 68 retrospective series, 16 prospective nonrandomized studies, 8 prospective randomized clinical trials, 5 prospective case-controlled studies, 5 reviews and 7 others (3 observational, 2 population-based studies, 1 national survey, and 1 editorial). As the majority of reported studies are retrospective, firm conclusions cannot be reached. Single factors that appear to be important include male gender, extreme old age, morbid obesity, cirrhosis, previous upper abdominal surgery, severe/advanced acute and chronic disease, and emergency LC. The combination of patient- and disease-related risk factors increases the conversion risk. In the training of residents, the number of cases needed for reaching proficiency exceeds 200 cases. The value of predictive scoring systems is important in the selection of cases for resident training. Conversion exerts adverse effects on operating time, postoperative morbidity, and hospital costs, especially when it is enforced. There appears to be no absolute contraindication to LC that is agreed upon by all. There is consensus on certain individual risk factors and their additive effect on the likelihood of conversion. Predictive systems based on these factors appear to be useful in selection of cases for resident training.
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Gallstone disease occurs in 3%–20% of the population worldwide. Cholecystectomy remains the most effective treatment for this disease when symptomatic. Since the introduction of laparoscopic cholecystectomy (LC) 15 years ago,[1–3] this approach has all but replaced traditional open cholecystectomy, as indicated by reported estimates that between 75% and 95% of all cholecystectomies in different health care systems are nowadays performed by the minimal access surgery approach.[4,5] The issue of conversion during this very common laparoscopic operation remains unsettled, with widely varying conversion rates. Perhaps more important than actual conversion rates is the appreciation of the important circumstances and factors underlying the need for conversion, aptly described as the Achilles heel of laparoscopic surgery by Leon Morgenstern in 1995.[6] Ten years on from this seminal editorial, and following the publication of vast experiences of LC, we have considered it important to revisit the literature in an attempt to identify (i) the risk factors for conversion and (ii) its consequences on patient outcome and health care systems. Such information should enable surgeons to give improved informed consent to patients at high risk for conversion, and recognize earlier the need for conversion, avoiding longer operating times and unnecessary morbidity.[7]

METHODS

The review is based on a complete literature search by PubMed indexed for Medline, covering

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the period 1990 to 2005, in which all articles with titles including laparoscopic cholecystectomy, conversion, risk and predictive factors, complications and bile duct injury, gender, age, obesity, previous abdominal surgery, acute cholecystitis, gangrenous cholecystitis, biliary pancreatitis, cirrhosis, Mirizzi syndrome, and gallbladder cancer were used in the search directive. All publications identified by this search (N=109) were read, analyzed, and classified according to the Oxford Levels of Evidence (Table 1). The majority of these publications (n=68) consisted of retrospective series; in addition, 16 were prospective nonrandomized studies, 8 were prospective randomized clinical trails, 5 were prospective case-controlled trials, 5 were reviews, and 7 were others (3 observational studies, 2 population-based studies, 1 national survey, and 1 editorial). Abstracts were not included in the review.

The data obtained from this source were analyzed to determine influence of factors (patient and disease-related variables, surgeon-related variables, and circumstances) that necessitate conversion. In addition, the impact of conversion (elective or emergency) on patient outcome was assessed, and finally, an attempt was made to determine the value of a reported predictive system for conversion of LC to open surgery.

Reported Variability in Conversion Rate for Laparoscopic Cholecystectomy

Although the range of conversion rate of LC to the open approach is commonly reported as 1.5%–10%,[4,8–10] actual reported rates in different series can vary from 1%–74%, depending on the target patient populations and the associated risk factors for conversion.[11,12] Realistically, conversion rates are subject to specific factors, the most important of which are the experience of the surgeon in performing specific operations and patient/disease-related variables (disease severity, difficult anatomy, comorbid disease, etc.). In this respect, overall reported conversion rates have to be regarded as approximate norms and no more, as in the end, the surgeon faced with a difficult intraoperative situation has to rely exclusively on assessment of the operative situation and his or her ability to cope when reaching a decision to proceed endoscopically or to convert.

Table 1. Oxford System of Levels of Clinical Evidence

Levels of evidence	Study design
Ia	Systematic review of randomized controlled trials
Ib	Individual randomized controlled trial
Ic	All-or-none case series
IIa	Systematic review of cohort studies
IIb	Individual cohort study
IIc	"Outcome" research
IIIa	Systematic review of case-control studies
IIIb	Individual case-control study
IV	Case series
V	Expert opinion, bench, or animal research

Individual Factors Influencing Conversion

Individual risk factors for conversion have been identified by several published reports. Brunt et al.[13] reported that the conversion rated in extremely elderly patients (>80 years) was four times higher than in those younger than 80. Male gender with severe symptomatic cholelithiasis is also associated with higher conversion.[14] Patients with previous upper abdominal surgery have a higher conversion rate, as much as 19%.[15] Gangrenous cholecystitis is associated with a conversion rate of 35%.[16,17] Patients with cirrhosis average a conversion rate of 10% and incur a morbidity of 13%–20%.[18–21] In patients with difficult pathology such as the Mirizzi syndrome, the conversion rate can reach 74%.[12]

Thus, several factors influence the conversion from LC to open cholecystectomy (OC), but some appear to have more impact as risk factors.[4,22,23] However, many surgeons remain unconvinced and believe that the need for conversion can only be established with certainty during surgery, and that the value of these individual predictors is relative rather than absolute.[24,25] There is undoubtedly published evidence to support this viewpoint. Thus, in a study of patients with multiple risk factors but without a clear-cut contraindication to the laparoscopic approach, uneventful LC was performed in two thirds of the patients.[4]

Ideologically, laparoscopic surgeons fall into two groups: those who practice patient selection for the laparoscopic approach based on clinical variables, and those who always favor the laparoscopic approach in the first instance. Given equivalent experience, the former are more likely to incur a lower conversion rate during LC. However, we could not substantiate this probability from the reported literature.

Nonetheless, it is important to consider the important factors related to the risk of conversion during LC (Table 2). These can be categorized as (i) patient-related, (ii) disease-related, and (iii) surgeon-related variables.

Table 2. Risk factors for conversion of laparoscopic cholecystectomy

Patient-related	Disease-related	Surgeon-related
Male gender	Thickened gallbladder wall	Caseload of surgeon
Older age	Elevated C-creative protein, white blood cell count, and serum alkaline phosphatase	Proficiency gain curve
Obesity	Acute cholecystits	Human factors
Previous upper abdominal surgery	Gangrenous cholecystitis and empyema	Perceptual and technical errors
Emergency LC	Cirrhotic portal hypertension	Serious intraoperative complication: bile duct injury, bowel injury, and other complications
Comorbid cardiopulmonary disease	Biliary pancreatitis	Errors: technique of LC, equipment and instrument failure
	Mirizzi syndrome	
	Gallbladder cancer	
	Common bile duct stone	

Patient-Related Variables

The general condition of the patient based on assessment of the ASA status and accurate assessment of functional reserve in the presence of comorbid disease has to be the initial preoperative assessment. In this respect, patients with significant ischemic heart disease are at risk from the positive-pressure capnoperitoneum associated with conventional laparoscopic surgery because of the adverse effect on the stroke volume and cardiac index.[25–28] In such patients, if the collective decision (by surgeon, patient, and relatives) is in favor of the laparoscopic approach, this should be undertaken by the isobaric (gasless) technique using some form of abdominal wall lift, preferably of the planar variety.[28]

Male gender. Several reports have identified the male gender as a risk factor for conversion, probably because of the more frequent association with severe, acute, and chronic disease.[4,9,14,29–31] The more recent national American survey by Livingston et al.[4] demonstrated that obese males with both acute and chronic cholecystitis incurred more than twice the risk of conversion (12.9%) than female patients (5.3%). In the report by Lee et al.,[31] the high conversion in males with acute cholecystitis was associated with more severe disease and longer operating times. The higher conversion rate in males with acute cholecystitis due to more frequent associated severe disease is confirmed by two additional reports.[14,30]

Age. The influence of age on the conversion rate remains controversial. Although many reports have indicated that older patients have a higher conversion rate,[7,16,22,23,30,32–35] others have observed little or no correlation between the two.[4,23,24] Nevertheless within this controversy, there is some consensus that LC in the very elderly (patients aged 80 years and older) appears to be associated with higher conversion rate, ranging from 9%–35%.[13,16,22,35–39] The conversion rate in this elderly subgroup is thus estimated to be twofold to fourfold higher than in patients younger than 70 years.[13,35] In many of these reports, conversion in the elderly was accompanied with longer operation times, increased postoperative morbidity and analgesic medication, a longer recovery time, and longer hospital stay.[13,16,35,37]

There are a number of possible underlying reasons for these observations of increased conversion and morbidity in the very elderly: higher incidence of severe acute or even gangrenous cholecystitis, comorbid cardiopulmonary disease, common bile duct stones, and previous abdominal surgery.[13,16,38] Undoubtedly, the incidence of choledocholithiasis increases markedly with age, with rates as high as 43% in patients over 80 years.[13] In patients older than 65 years, severe complicated biliary disease results in a tenfold increase in conversion rate when it is associated with an increased morbidity (27%).[35] In this series, the main reason for conversion (accounting for 63%) was the failure to identify the anatomy correctly because of severe acute cholecystitis.

There are two practical considerations relating to the treatment of elderly patients with acute disease. The first is that the surgeon should make an early decision to convert in the presence of any difficulties.[23] Secondly, very elderly patients (80 years and above) with symptomatic ductal stone disease should

be managed with endoscopic stone extraction in the first instance, followed by an expectant policy with clinical review at 12 months prior to discharge from further follow-up or decision to proceed with LC.[40]

Obesity. Morbid obesity is generally considered a risk factor for conversion mainly because it requires longer operative time and is associated with respiratory complications, higher conversion rate, and higher morbidity.[22,33,41–45] In a retrospective study,[43] the operative time in morbidly obese patients was 20–30 minutes longer than that in nonobese patients, whereas postoperative morbidity was double (4.7% and 11.8%). A predictive factors study has confirmed that obese patients with acute cholecystitis incur an increased conversion rate, as do morbidly obese patients with chronic cholecystitis and a thickened gallbladder wall in the elective situation.[44]

However, a retrospective report on 1804 patients undergoing LC showed no correlation between patients body mass index and the conversion rate, and postoperative morbidity. In this study, the only difference observed was that the obese patients required longer operative time.[45] Similar findings (longer operative time without increase in conversion and postoperative morbidity) have been reported by other retrospective studies[46,47]

Previous abdominal surgery. Previous abdominal surgery is generally regarded as one of the risk factors of conversion from LC to OC, particularly after upper abdominal operations. Previous upper abdominal surgery is also accompanied by a higher intraoperative and postoperative morbidity.[15,41,48,49] In one of these studies, relevant adhesions were found in 62% of patients after previous abdominal surgery, and 28% of these required adhesiolysis for the LC to proceed. This study documented that patients undergoing LC after previous upper abdominal surgery had a higher conversion rate (19% vs. 5.2%) and postoperative wound infection compared with patients with previous lower abdominal surgery (3.3% vs. 0.7%) or no prior surgery (5.4% vs. 1.2%).[15] Similar findings were also reported by others.[48] In contrast, other reports indicate that previous abdominal surgery has little impact on the feasibility and safety of LC.[9,50,51]

The main problems in patients with previous upper abdominal surgery include obtaining safe access to the abdomen and exposure of the surgical field. Several solutions to the initial access for closed laparoscopy using a Veress needle have been proposed, including preoperative ultrasonography to identify the abdominal quadrant free of adhesions or use of an alternative access point in the left upper quadrant—Palmer's point[11,15] or adoption of Hasson's technique of open laparoscopy.[52–54]

Emergency LC. Emergency LC has a higher conversion rate compared with the elective situation (16% vs. 2.5%, respectively). In addition, it has a higher mortality (1.8% vs. 0.16%, respectively) and morbidity (11.2% vs. 2.6%, respectively) and as expected, a longer hospital stay (5.4 vs. 2.1 days, respectively).[55] The higher conversion rate of emergency versus elective LC is confirmed by other reports.[44]

Disease-Related Variables

Severe acute cholecystitis, gangrenous cholecystitis, cirrhotic portal hypertension, gallstone-associated biliary pancreatitis, Mirizzi syndrome, gallbladder cancer, and thickened gallbladder wall are risk factors for both higher conversion rate and postoperative morbidity. However, none of these pathologies are nowadays regarded as absolute contraindications.[12,16,18,20,44,56–59] Early LC for acute cholecystitis should be performed within the first 48 hours of admission, as further delay increases the conversion rate, operative time, and hospital stay.[56,60]

Thickened gallbladder wall. The thickened wall of the gallbladder, measured preoperatively by ultrasound, is regarded by some as a risk factor for conversion. In two reported studies, a thickened gallbladder wall (>3.5 mm) incurred a sixfold increase in the conversion rate.[41] The higher conversion rate in patients with a thickened gallbladder wall on ultrasonography is confirmed by other reports.[44,61]

Elevated C-reactive protein, white blood cell count, and serum alkaline phosphatase. Elevated C-creative protein, white blood cell (WBC) count, and alkaline phosphatase (ALP) reflect the severity of the inflammation of cholecystitis and have been identified as risk factors for conversion in some.[9,62] The combination of elevated WBC count and raised ALP level increases this risk.[9] However, elevated C-creative protein level appears to be the most powerful predictor for conversion, followed by the preoperative duration of symptoms, male gender, and WBC count.[62]

Acute cholecystitis. Based on the findings of several randomized studies, nowadays LC is considered a safe and effective treatment for acute cholecystitis.[16,36,56,58–60,63–65] However, LC for acute cholecystitis does incur an overall increased incidence of conversion compared with elective LC. This risk, in reported studies, varies considerably from 8% to 25%.[4,56,58,59,65,66] The combination of cholelithiasis, ductal calculi, and acute cholecystitis carries

a conversion rate of 24%.[4] Gangrenous cholecystitis, which occurs in up to 30% of patients admitted with acute cholecystitis, has an even higher conversion rate, averaging 35%.[16,17,67,68]

Lower conversion rates comparable to those associated with elective LC have been reported if duration of symptoms is less than 96 hours.[69–71] All these reports provide a strong case for early LC at the time of admission rather than interval LC.[36,56,58–60,63,64,67–72] Interval LC that entails conservative treatment for the acute disease followed by delayed (interval) surgery increases the conversion rate, postoperative morbidity, and hospital stay.[56,59,72]

Conversion is most frequently needed for empyema and gangrenous cholecystitis, and once such pathologies are identified, excessive time should not be spent in laparoscopic trial dissection before converting to an open operation.[67] Others have suggested laparoscopic subtotal cholecystectomy in these difficult cases, although the reported experience with this procedure is limited.[73,74]

Cirrhotic portal hypertension. Cirrhosis is generally regarded a risk factor for conversion, higher morbidity (5%–23%), and mortality (7%–20%), and for this reason, some consider it an absolute contraindication to LC.[75] The average reported conversion rate in patients with cirrhosis is 10%.[18–21] More recent studies have indicated a better outcome in experienced hands,[20,76,77] and a randomized controlled trial of LC versus OC in patients with cirrhotic portal hypertension demonstrated that the LC is feasible, relatively safe, and superior to OC.[20] These findings are supported by a further comparative study between OC and LC in patients with cirrhosis.[77] The advantages of LC in this patient group demonstrated by these studies include reduced blood loss, lower morbidity, and shorter hospital stay.[18,20,77] Other retrospective reports have confirmed the safety of LC in selected patients with Child-Pugh A and B cirrhosis.[19,77,78] However, particular care is needed during the insertion of the umbilical port, especially in the presence of a collateral venous circulation, and meticulous hemostasis with obvious avoidance of large varices is essential. Blood transfusion is needed in 40% of patients with cirrhosis undergoing LC.[77]

Biliary pancreatitis. Several reports have indicated that patients undergoing LC for gallstone-associated biliary pancreatitis have a higher risk of conversion (averaging 12%) and longer postoperative hospital stay.[79–81] Despite these reported observations, the data from more recent studies indicate that patients with mild biliary pancreatitis should be offered LC during the same hospital admission if the pancreatitis is not severe.[57,82] The exact timing of the acute LC is not standardized. Taylor and Wong[82] proposed that patients with mild biliary pancreatitis should proceed to LC without any delay.

Some advocate early LC on the next available list[57,83] whereas others propose intervention as soon as serum amylase is decreasing and abdominal tenderness is improving.

Mirizzi syndrome. Compression of the common hepatic duct by an impacted stone first described by Mirizzi in 1948[84] is a rare cause (0.5%–1.4%) of partial obstruction of the common hepatic duct, with subsequent cholangitis or recurrent jaundice. The classification described by McSherry et al.[85] identifies two types: type I associated with external compression of the hepatic duct, and type II, which involves the existence of cholecystobiliary fistula.

Laparoscopic treatment of Mirizzi type I syndrome is technically feasible and safe. For Mirizzi type II syndrome, laparoscopic common bile duct (CBD) exploration is technically demanding and associated with higher conversion rate and postoperative morbidity.[12,86] One report has documented a conversion rate of 74% in patients with Mirizzi syndrome type I, and 100% in patients with type II disease undergoing LC.[12] In type I, subtotal or total LC is performed by the majority of the surgeons, with fundus-first dissection of the gallbladder, whereas type II cases are managed by CBD exploration with either T-tube insertion or biliary bypass procedures.[86–88] Combined endoscopic and surgical management (endoscopic stone extraction followed by LC) is a sensible option associated with a good outcome.[89]

Gallbladder cancer. Unsuspected gallbladder carcinoma is one of the major concerns in patients undergoing LC for symptomatic gallstone disease, as incidental gallbladder cancer is found in 0.29%–2.3% of these patients.[90,91] The incidence of gallbladder cancer is higher (up to 8%–9%) in elderly patients presenting with acute cholecystitis.[92] It is generally agreed that a conversion should be performed if gallbladder cancer is suspected during LC, and in addition, the port sites should be excised to prevent port site implantation and local recurrence.[91,93,94]

Surgeon-Related Variables

Increased laparoscopic experience is associated with reduction in conversion and complication rate. Surgeons with larger caseloads may have better outcomes than those who do the procedures less frequently.[8,11,66,95–97]

Learning curve versus proficiency gain curve. The suspect nature of the concept of the learning curve is exemplified by the conclusions that can be reached on the subject by analysis of reports from the early days of LC; comparing these with data from the more recent reports after LC became established as an integral part of surgical practice, and hence, they were incorporated within the surgical training curriculum. In the early reported series, the incidence of bile duct injury (BDI) ranged from 0.1%–0.7%.[2,3,8,96] The dramatic effect of the learning curve in these early studies is exemplified by the report from the Southern Surgeons Club that documented a high incidence (2.2%) of BDI during the first 13 cases operated by each surgical group, and which subsequently decreased to 0.1%.[2] The early reports indicated that the number of cases required for the surgeon to progress beyond the learning curve varied between 10–30 operations,[2,97,98] the range indicating an individual surgeon-related factor—which in our view—remains important. An analysis of 416 consecutive LCs demonstrated the effect of experience on conversion rates and morbidity. In this study, surgeons experienced a 17% conversion rate for the first 35 cases, and 75% of complications occurred in the first 30 cases.[97] Similar findings have been reported by others.[22] Deziel et al.[96] reported a significant difference in laparoscopic-related bile duct injuries between the hospitals that had performed more or less than 100 operations.

A different picture on the caseload needed to surpass the learning curve emerges when the findings of larger or more recent reports are analyzed. These studies were reported after training programs within the surgical curriculum were established and the practice of LC had matured. They indicate that the learning curve of trainees would appear to be substantially larger, ranging from 100–200 cases.[99,100] Reports from training centers suggest that the tail of the learning curve for LC is of the order of 200 operations, with continued steady improvement by 40%, before the plateau of proficiency is reached when the operating time and incidence of bile duct injuries decline to the level of experienced surgeons.[95,99,100] Aside from the training of residents, there is good evidence, based on data reported in large population-based studies, that the concept of the learning curve and its impact on BDI and morbidity is suspect, and with good reason, as these more recent studies have shown no direct relation between the incidence of bile duct injuries and experience of the surgeon. Indeed, a substantial cohort of BDIs documented in these reports was incurred by experienced surgeons.[101–103] Archer et al.[100] detected that at least one third of bile duct injuries are not related to inexperience, but reflect fundamental errors in the technique of LC as practiced by a broad population of surgeons in the United States. In this report, one third of surgeons reported BDIs after having performed 200 cases or more. Similar observations were reported by Savassi-Rocha et al.[102] in a multicenter study of 91,232 LCs performed in Brazil, and by Gentileschi et al.[103] in an audit of 13,718 operations in the area of Rome.

These considerations lend support to the view expressed by Cuschieri[104] that the phrase "learning curve" should be deleted from the surgical literature and replaced by "proficiency gain curve," defined as when a surgeon reaches the stage of consistent efficient and safe execution of any minimal access surgery operation. By virtue of the varying innate abilities that we all bring to manipulative tasks (laparoscopic surgery in this instance), the proficiency gain curve has to be individual-specific.[104,105] Thus, in addressing the issue of caseload required to reach proficiency, we need to consider both the norm and the outliers. The majority (perhaps some 80%) would fall within the caseload norm—others intrinsically more able will require less, whereas some will need more, and a few will never cope and thus fail to reach proficiency.

Bile duct injury detection, conversion, and etiology. Obviously the recognition of a BDI during LC is an indication for conversion, but regrettably, not all such injuries are recognized then. The findings of one report indicate that BDI is more likely to be discovered during surgery if an intraoperative cholangiography is performed (80.9% with cholangiography vs. 45.1% without cholangiography). The report by Savassi-Rocha et al.[102] demonstrated that 67.7% of BDIs are diagnosed during surgery and necessitate conversion. Similar findings have been reported by others.[103] In a retrospective nationwide cohort analysis of Medicare patients undergoing cholecystectomy, Flum et al.[106] found that the risk of CBD injury was significantly higher when intraoperative cholangiography was not used. This study indicates that the routine use of intraoperative cholangiography may decrease the rate of CBD injuries.

The human factor approach and the related technique of observational clinical human reliability assessment in etiology, and prevention of laparoscopic BDI and improvement of operative performance of LC, have been used by Way et al.[107] and Tang et al.,[108] respectively. The human factors study by Way et al. addressed the underlying causes and mechanisms associated with BDI during LC by analysis of 252 cases of laparoscopic BDI. This seminal work identified the primary cause in 97% of cases

as a visual perceptual illusion (misinterpretation of the perceived anatomy). Tang et al. used modified human reliability assessment for the analysis of technical errors enacted during LC. This study identified the dissection of a Calot triangle as the task in which most errors were committed (the hazard zone of LC). They also observed that the use of different dissecting instruments was associated with different error probabilities.[108]

Circumstances of Conversion

Conversion from LC to OC can be categorized as elective conversion or enforced (emergency) conversion. Elective conversion is defined as the decision by the surgeon, at any stage of the operation, to desist from the laparoscopic approach and to resort to laparotomy before being forced to do so because of a major intraoperative complication. The reasons can be difficult or obscure anatomy, advanced pathology, or lack of progress of the laparoscopic intervention for any reason. By contrast, enforced (acute) conversion is an intraoperative emergency (as distinct from a considered elective decision) when the surgeon has to resort to laparotomy because of a severe iatrogenic injury or severe laparoscopic uncontrollable bleeding.

Elective conversion. Based on the preoperative workup and known patient risk factors, there is a strong case for preoperative selection of patients with the appropriated consent into three categories: OC, LC, and borderline LC. Obviously, the more difficult and borderline cases should ideally be performed by the more experienced members of the surgical team to minimize conversion and morbidity.49 Some have argued that in borderline/difficult cases, if little or no progress in the dissection of the Calot's triangle is made within 15 to 30 minutes, the procedure should be converted,[9] although there is a lack of hard data to support this otherwise sensible statement. By and large, elective conversion—provided it is not unduly delayed such as to incur a long operating time—does not appear to increase postoperative morbidity beyond that expected after OC and commensurate with the ASA status of the patient.

The most common reasons for conversion to OC are difficult dissection and the inability to define the anatomy.[9,58,66,109] Difficult dissection is most commonly secondary to dense adhesions, severe inflammation, or obscure anatomy.[44,109] In one report, inability to correctly identify the anatomy at the porta hepatis accounted for 154 out of 310 patients undergoing conversion; anatomy obscured primarily by acute inflammation in 82%, by dense adhesions from prior abdominal procedures or chronic cholecystitis in 12%, and aberrant anatomy in 6%.[66] Another report confirmed that the most common reason for conversion (70% of cases) was inability to define anatomy in patients with inflamed contracted gallbladder.[61]

Enforced conversion. Enforced or emergency conversion is associated with higher postoperative morbidity and mortality than elective conversion.[4,9,44,61,62,109] In the series reported by Bingener et al.,[35] the most common cause for enforced conversion was intraperitoneal bleeding (14%), followed by the need for common bile duct exploration (11%), suspected bile duct injury (8%), and additional intraperitoneal pathology and misdiagnosis (4%). Intraoperative bleeding (50%) and bile duct injury (50%) accounted for all the cases in the series reported by Alponat et al.[9]

Impact of Conversion on Patient and Health Care

Patients who require conversion of LC tend to have a longer operating time, higher morbidity, and longer hospital stay compared with the electively successful LC.[4,9,59] Alponat et al.[9] reported that both wound and lung infection rates were significantly higher in the converted patients. In a prospective trial of patients with acute cholecystitis, the morbidity was 20% higher in the converted group, which had a longer hospital stay and more days on antibiotics.[71] The most disadvantaged group of patients were those in whom conversion was needed because of iatrogenic bile duct injury, which constitutes a major health problem with severe financial consequences.[110] Johansson et al.[59] provided health economic data confirming that the total cost per patient requiring conversion was 30% higher than patients who had undergone successful LC or elective OC.

Risk Scoring and Predictive Systems for Conversion of LC

There have been a number of scoring or predictive systems proposed for the prediction of probability for conversion.[4,9,44,61,62,110] Some of the preoperative factors included in these systems address the condition of the patient and the severity of the pathology of the gallbladder. The scoring system proposed by Kama et al.[61] incorporates the following as determinants, singly or in combination of the probability of conversion: male gender, abdominal tenderness, previous upper abdominal operation, thickened gallbladder wall on ultrasound scans, and age over 60 years. The combination of obesity and

Table 3. Details of randomized controlled prospective trials with data that impacts directly or indirectly on conversion rates during LC

Author reference	Randomized cohort	Important findings
Lo et al., 1996[36]	70	Elderly patients are at greater risk for conversion (23%), delayed recovery, and prolonged hospital stay.
Koo and Thirlby, 1996[70]	60	The conversion rate (30%), hospital costs, and convalescent times increased in patients with acute cholecystitis symptoms for more than 72 hours.
Kivilnoto et al., 1998[58]	63	LC for acute and gangrenous symptoms is associated with a moderately high conversion rate.
Lo et al., 1998[72]	99	Delayed LC for acute cholecystitis has a higher conversion rate (30%). Early operation (<72 hours from admission) is associated with lower conversion rate, lower complication rate and shorter hospital stay.
Madan et al., 2002[60]	45	Patients treated early (<48 hours from onset of symptoms) incur a lower conversion rate, shorter hospital operative time, and reduced hospitalization.
Johansson et al., 2003[63]	145	LC for acute cholecystitis is associated with higher conversion rate (early LC 31% vs. delayed LC 29%).
Ji et al., 2005[20]	80	patients with cirrhosis undergoing LC have higher postoperative morbidity.
Johansson et al., 2005[59]	70	LC for acute cholecystitis had a higher conversion rate (24%)

acute cholecystitis is associated with an increased chance of conversion.[44]

What is clear from all these studies is that a combination of risk factors increases the odds of conversion, and to a certain extent, this can be predicted. Thus, in one study, patients with none of four predictors (acute cholecystitis, thickened gallbladder wall on ultrasound scans, elevated WBC count, and alkaline phosphatase level) has a 1.5% conversion probability, whereas this rose to 9.3% in the presence of acute cholecystitis and elevated ALP, and to 27.5% if the acute cholecystitis was accompanied by an elevated WBC count and a high alkaline phosphatase level. Patients with all four predictors present incurred a conversion rate of 58.7%.[9] Livingston and Rege[4] devised a predictive system based on the major risk factors for conversion (male gender, obesity, cholecystitis, concurrent choledocholithiasis, and cholelithiasis with cholecystitis) identified from the National Hospital Discharge data base for 1998–2001. Similar systems have been reported by other authors.[7,9,16,22,33,61,62] Kologlu et al.[110] proposed a risk scoring system to predict the difficulty of LC as a basis for selection of cases suitable for resident training as opposed to the difficult cases that may be left to experienced surgeons.

CONCLUSIONS

It is difficult to draw firm conclusions, largely because the majority of reported studies are retrospective and uncontrolled, and thus, the overall quality of the available evidence is low. The 8 randomized clinical trials have some data that directly or indirectly impacts on conversion rates during LC (Table 3). Because of the multiplicity of variables involved, it is impossible to establish average conversion rates for this common operation. However, the single factors that appear to be important in influencing conversion rates are: male gender, extremely old age, morbid obesity, cirrhosis, previous upper abdominal surgery, severe/advanced acute and chronic disease, and emergency (as opposed to elective) LC. There is reasonable evidence that the combination of patient and disease-related risk factors increases the risk substantially in an incremental manner. The experience and technical ability of the surgeon is of paramount importance. In this respect, the learning curve concept appears to be flawed and should be replaced by the proficiency gain curve, which appears to be individually related but encompasses a norm. In the training of residents, the number of cases needed for reaching the plateau of this curve is likely to be in excess of 200 cases, that is, much higher than suggested by the early reports. The value of predictive scoring systems appears to be important in the selection of cases to be used for the training of residents. Conversion appears to exert a penalty in terms of increased operating time, postoperative morbidity, and hospital costs (level II evidence), especially when this is enforced (emergency conversion) because of an iatrogenic complication or injury. A better insight into the

problem of conversion will emerge from prospective studies on human factors research and clinical observational human reliability analysis.

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A Palpable, Obstructing Carcinoma of the Colon Incarcerated Within a Large Ventral Hernia

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Uncovering the etiology of a bowel obstruction in a patient with a hernia represents a diagnostic dilemma. Although the hernia is often initially the presumptive cause of the bowel obstruction, obstructive carcinoma or another pathological process hidden by the hernia are important considerations. Here we describe a case of a man with an obstructing neoplasm of the colon within a large ventral hernia, whose constipation was initially attributed to incarceration of the hernia. (J GASTROINTEST SURG 2006;10:1092–1094)

KEY WORDS: Obstructive carcinoma, colorectal cancer, hernia, incarceration

CASE REPORT

A 68-year-old man with a large ventral hernia and suffering from the early stages of Alzheimer's disease experienced worsening bouts of constipation for months before presenting to the Emergency Department at The Johns Hopkins Hospital (JHH) with constipation. He had seen several physicians near his home, but given the size of his hernia he was referred to JHH. The patient had no nausea or vomiting and complained only of vague abdominal discomfort. By report of his family, his symptoms had been attributed to his large, incarcerated ventral hernia, which measured approximately 25 × 15 cm. At JHH a mass was palpated within the hernia. Computed tomographic scan showed that the hernia contained multiple loops of small bowel, as well as the majority of the transverse colon. There was a mass within the transverse colon measuring 4 × 3 cm (Fig. 1).

The patient was initially scheduled for admission and evaluation in October of the same year, but cancelled his appointment for personal reasons. He eventually rescheduled and was admitted to the surgery service at JHH for evaluation and treatment of his condition. A colonoscopy was performed to rule out synchronous lesions. Although the endoscopist could not advance the colonoscope beyond the near-obstructing mass in his transverse colon, he was noted to have three sessile polyps within 5 cm distal to the obstructing mass during colonoscopy. These were stained with India ink to serve as an operative landmark.

After an appropriate bowel preparation, the patient was taken to the operating room, and after induction of general anesthesia, the large majority of the contents of the hernia sac were reduced, leaving a large redundant sac. A midline incision was made from the xiphoid to the hernia sac and then extended through the middle of the hernia sac to the midline of the abdomen below the sac. The incision was carried down to native fascia superiorly. The redundant sac was then incised, and the peritoneal cavity was entered. The underlying bowel was not densely adherent to the sac and was easily dissected free. The patient's colon was largely mobile because the majority of it had been within the hernia sac. The patient underwent an extended right hemicolectomy to include the inked polyps distal to the palpable, obstructing, apple core lesion. Primary end-to-side

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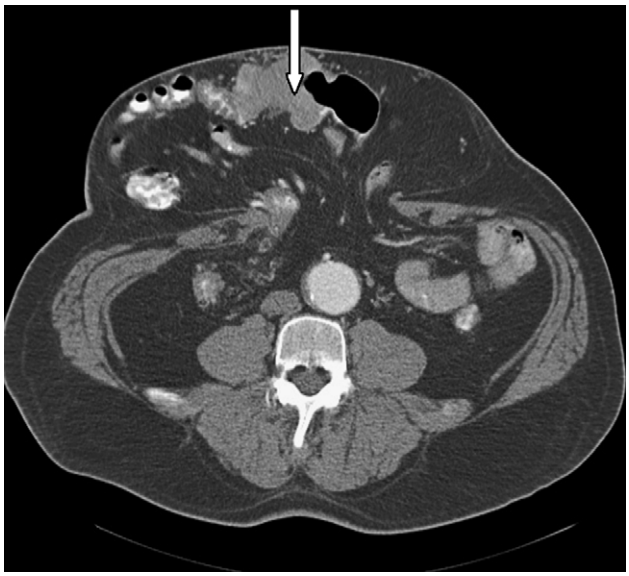

Fig. 1. Computed tomographic scan of the abdomen revealing a 4 × 3 cm mass (*white arrow*) in the transverse colon within a large ventral hernia. Right and left are reversed by radiological convention.

ileocolic anastomosis was performed. The fascial defect was approximately 10 × 15 cm; however, after removal of the colon and elevation of skin flaps, it was possible to reapproximate the fascia in the midline. There was a significant amount of redundant tissue and hernia sac after closure of the fascia. This was excised before closing the skin with deep dermal sutures and staples.

His postoperative course was unremarkable except for a low-grade fever with a negative workup on postoperative day 2. He was discharged on postoperative day 5. The pathology report on the surgical specimen revealed a T3N0 adenocarcinoma with mucinous features, arising in an 11.3 cm villous adenoma. There were two adjacent tubular adenomas distal to the mass, measuring 1.6 cm and 0.7 cm, and five tubular adenomas proximal to the mass, ranging in size from 0.2–0.7 cm.

DISCUSSION

In the months prior to his examination at JHH, the patient's worsening constipation and vague abdominal discomfort was presumed related to small bowel incarcerated within his ventral hernia. Indeed, small bowel obstruction can be attributed to hernia in approximately 25% of cases.[1] However, hernia is a "masquerader" of surgical disorders.[2] Whereas the patient with a hernia who presents to his local hospital with an acute bowel obstruction is likely to be evaluated until the cause of his condition is reasonably determined to be hernia or something else, the patient with a hernia who does not present acutely may not generate enough clinical concern to warrant further evaluation. Thus, a change in bowel habits and vague abdominal discomfort in a person with a hernia represents a diagnostic dilemma and should be further evaluated to rule out more ominous causes of bowel obstruction.

Because herniated contents are usually omentum and/or small bowel and rarely colon, hernia is a rare cause of large bowel obstruction.[3] Indeed, the majority of cases of large bowel obstruction are secondary to a neoplasm.[1] In one report, 24.9% occurred proximal to the splenic flexure.[4] Whereas there appears to be no association between colon cancer and the presence of a hernia,[5] screening colonoscopy is a useful tool in evaluating obstructive colon cancer as a potential cause of worsening constipation in any patient over 50 years old. Indeed, our patient, who had never had a colonoscopy before this admission, was long overdue for one.

Primary colon cancers found within herniated bowel are rare, and only a handful of cases are reported in the literature. In 1991, Hale and Solla[6] provided a literature review and discussion of 16 cases of colon cancer incarcerated within inguinal hernias, six of which were associated with large bowel obstruction.

Including the case presented here, there are four reports that describe colon cancers within ventral hernias.[7–9] Two of these cases appear to have been referred to surgeons because the hernia was no longer reducible[7] or because of a presumed strangulation,[8] and the other two came to attention because of symptoms of a bowel obstruction[9] (including the present case). In each ventral hernia, masses were palpable within the subcutaneous tissue. Whereas one obstructive carcinoma was discovered intraoperatively,[8] presumptive diagnoses that the masses were cancerous lesions of the large bowel were made preoperatively in three cases using barium enema[7] and CT scan/colonoscopy[9] (present case).

Many authors have suggested that a hernia with incarceration, obstruction, abdominal pain, or rectal bleeding ought to raise the suspicion of colorectal cancer,[3,5,6,8] but a hernia may also provide a window of opportunity to detect a mass in the absence of these symptoms. Almost certainly, the masses described within ventral hernias were palpable within the hernia long before the patients were evaluated for their acute condition. The patients may have reported that they had felt a persistent mass within the hernia when asymptomatic. These cancers may have been discovered and treated much earlier if a thorough history and physical exam of the hernia and its contents had been done. In addition to

earlier diagnosis and treatment, these patients may have been spared months of discomfort.

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Letter to the Editors

To the Editors:

I read with interest the paper by Mullen et al.[1] in the 2005 JOURNAL OF GASTROINTESTINAL SURGERY issue, entitled "Pancreaticoduodenectomy After Placement of Endobiliary Metal Stents," regarding a plea for the use of metal stents in the preoperative treatment of those with resectable pancreas cancer. No untoward events were noted with this protocol during the study period.

I have reviewed our experience with more than 100 patients who had pancreatic head resections after preoperative chemotherapy and radiation therapy. Eight of them had metal stents placed preoperatively. Three of those eight patients were seen to have stent exposures (through a necrotic bile duct wall) at operation: all grew out vancomycin-resistant enterococci from their bile, and all died in the hospital of ARDS and mutlisystem organ failure. Theoretically, the radiotherapy beams can superheat metal and also can be scattered by the metal. There is clear evidence from the radiation of metal esophageal stents that "hot spots" can occur. Perhaps the use of attenuated radiation therapy and lesser between radiation and surgery at M. D. Anderson have allowed the avoidance of these complications of radiation therapy and metal stents. We have used conventional radiation doses of 50.4 Gy, after which three of eight stents have been associate with stent exposure and subsequent lethal infection, we believe it is a risky proposition to advocate the placement of these metal stents in patients about to undergo preoperative radiation therapy with conventional radiation doses. Patients and treating physicians should be aware of the possibility and signs of plastic stent occlusion during preoperative therapy so that the stents can be exchanged easily and complications can be avoided.

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Letter to the Editors

Reply

Dr. Hoffman reports that in his experience with more than 100 patients who have received chemoradiation prior to pancreaticoduodenectomy, 8 patients had a metal endobiliary stent placed prior to the start of therapy. Intraoperative evidence of stent erosion through the wall of the bile duct was found in 3 of the 8 patients, all of whom died in the postoperative period. He infers that the stent was directly related to the cause of death in all 3 patients; the surgical outcomes of the other (> 100) patients was not provided. As a result of his experience, Dr. Hoffman recommends that plastic stents be used during neoadjuvant therapy whenever surgery remains an option and that stent occlusion be treated with (plastic) stent exchange. We offer the following comments in reply:

1. Space does not permit a complete review of the data or discussion on endobiliary stents and the risk of pancreaticoduodenectomy: we refer the reader to our previously published work on this topic by Pisters et al.[1] In brief, there in consensus on the association of bacterial colonization of bile (after placement of an endobiliary stent) and an increased risk for superficial wound infection following pancreaticoduodenectomy. Such bacterial colonization did not influence preoperative mortality, an experience largely limited to plastic stents.
2. When we increased the interval from diagnosis to surgery to allow for the delivery of additional, potentially beneficial, preoperative systemic therapy, plastic stent occlusion became more common, causing us to consider the use of metal stents. Metal endobiliary stents provide superior patency and a lower incidence of cholangitis when compared with plastic stents.[2,3] In addition, a strategy of plastic stent exchange in the event of stent occlusion incurs the inherent risks of stent occlusion (cholangitis), repeat ERCP, and procedure-related cost.[4] Importantly, when we place a metal stent, our endoscopists pay special attention to the location of the stent; the proximal end of the stent should lie at least 2 cm below the hepatic duct bifurcation so as to allow division of the hepatic/bile duct above the cephalad extent of the stent at the time of surgery.
3. Dr. Hoffman suggests that our favorable results may be due to the use of a lower dose of radiation (30 Gy). Although we have used 30 Gy in the protocol-based treatment of patients with potentially resectable pancreatic cancer, we have recently completed a large phase I trial in patients with locally advanced pancreatic cancer.[5] In this trial, patients received concurrent bevacizumab and 50.4 Gy of external-beam radiation in 28 fractions. Metal stents were present in 39 of 48 patients at the time of the radiotherapy. Since the safety of concurrent bevacizumab and radiotherapy was unknown at the start of the trial, patients were monitored extremely closely and no stent-related complications other than stent occlusion were observed.
4. Dr. Hoffman raises the concern the radiation beams may "superheat" metal. In fact, megavoltage radiation is a very inefficient way to heat metal or tissue. The energy deposited from megavoltage radiation produces a biologic effect through molecular excitation and ionization events rather than through heating tissue. The delivered dose of radiation is defined as absorbed energy per unit mass (J/Kg or Gray [Gy]). Even if one assumes that all of the absorbed energy from radiotherapy is deposited in the form of heat, there would be only a minute increase in temperature in any irradiated material. The increase in temperature of a metal stent would be approximately 10-fold greater than the increase in tissue, but this translates into less than one-hundredth of a degree Celsius per radiation fraction—a change in temperature of no clinical significance.
5. Finally, Dr. Hoffman raises the concern that metal stent–induced radiation scatter can result in "hot spots" and increased tissue damage. Megavoltage X-rays cause ionization of atoms (usually hydrogen atoms) in tissue, which generates unpaired electrons (or free radicals) that in turn cause ionization of DNA leading to cell death. Since metals are more electron-dense than human tissue, the effect of megavoltage radiation on metal may lead to potential forward scatter of electrons (and dose inhomogeneities, or "hot spots") at the interface of metal and tissue. The forward scatter from modern endobiliary stents has not been measured directly in

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experimental models, but studies have been conducted evaluating the amount of forward scatter due to various metals placed in tissue-equivalent material at different photon energies. The amount of forward scatter at these interfaces depends on the energy of radiation and the thickness of the metal. With 18-MV photons entering a tissue-steel interface, the increase in dose due to forward scatter varies from 5% at 1-mm thickness to 10% at 2-mm thickness and then reaches a plateau of 15% at 5-mm thickness. Beyond a thickness of 5 mm, there is actually a shielding effect of the metal, limiting the amount of forward scatter.[6] Since the walls of most endobiliary stents are less than 1 mm thick, the dose to the bile duct wall is increased by no more than 5%. Because metal stents have a mesh configuration rather than a solid configuration, the forward scatter due to the stent is probably much lower than 5%. At the commonly used radiation dose of 50.4 Gy, a 5% increase in dose due to forward scatter would result in a small localized area of the duct, 1- to 2-mm-thick, receiving approximately 53.0 Gy, when the surrounding tissues receive 50.4 Gy. We routinely irradiate patients with primary unresectable cholangiocarcinoma (who have indwelling metal stents) to a dose of 68.4 Gy and have not seen perforation, necrosis, or even clinically relevant fibrosis of the bile duct wall.

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