Pancreaticoduodenectomy With or Without Distal Gastrectomy and Extended Retroperitoneal Lymphadenectomy for Periampullary Adenocarcinoma—Part 3: Update on 5-Year Survival

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The study objective was to update the survival analysis at the 5-year mark of patients undergoing standard versus radical (extended) pancreaticoduodenectomy (PD) for periampullary adenocarcinoma (cancers of the pancreas, ampulla, common bile duct, and duodenum). A prospective randomized trial was performed (April 1996 through June 2001) comparing survival after pylorus-preserving PD resection (standard) to survival after PD with distal gastrectomy and retroperitoneal lymphadenectomy (radical). An interim report (Ann Surg 1999;229:613) and report after closing the trial (Ann Surg 2002;236:355) showed no differences in survival between the standard and radical groups. Two hundred ninety-nine patients were randomized to either the standard or radical group. Five patients were excluded from final analysis because final pathology failed to reveal adenocarcinoma. The 5-year survival of the two groups was evaluated. The median live patient follow-up is now 64 months (5.33 years). For all periampullary cancer patients, those undergoing standard resection had 1- and 5-year survival rates of 78% and 25%, respectively, compared with 76% and 31% ($P = 0.57$) for those patients in the radical group. For pancreatic adenocarcinoma patients, the 1- and 5-year survival rates in the standard group were 75% and 13%, respectively, compared with 73% and 29% in the radical group ($P = 0.13$). The increased morbidity rate, longer operative time, and similar survival for radical PD led us to conclude that pylorus-preserving PD without retroperitoneal lymphadenectomy should be the procedure of choice for most patients with resectable periampullary adenocarcinoma. While there is an intriguing trend toward improved survival in patients with pancreatic adenocarcinoma in the radical group, this trend may be largely accounted for by the higher incidence of microscopically margin positive resections in the standard resection group (21%) compared with a 5% incidence in the radical group ($P = 0.002$). (J GASTROINTEST SURG 2005;9:1191–1206)

KEY WORDS: Pancreaticoduodenectomy, periampullary adenocarcinoma, pancreatic adenocarcinoma, ampullary adenocarcinoma, resection

Periampullary adenocarcinoma (adenocarcinoma of the head of the pancreas, distal bile duct, ampulla of Vater, and duodenum) occurs in over 30,000 patients annually in the United States. Pancreatic cancer remains the fourth or fifth leading cause of cancer death in men and women and has a death-to-incidence

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ratio approaching 1. Pancreaticoduodenectomy (PD), or the Whipple procedure, remains the only chance for cure in these patients. Many factors have been shown to influence survival in this disease, including primary tumor characteristics (tissue of origin, size, degree of differentiation, resection margin status, and nodal status),[1–7] postresection CA 19-9 levels,[8] DNA content (ploidy),[9] molecular genetics,[10] and adjuvant chemotherapy and/or radiation.[7,11–14]

In the early 1970s, Fortner[15] proposed a more radical resection as a means for improving both resectability and survival for this disease. Their "regional resection of the pancreas" was extensive, involving total PD, subtotal gastrectomy, resection of the transpancreatic portion of the portal vein, and occasionally further vascular resection and reconstruction.[16–18] Currently, most studies evaluating "radical" resection define the procedure as wide en bloc pancreaticoduodenal resection with wide soft tissue margins, combined with harvesting of specific lymph node stations and a retroperitoneal lymphadenectomy. Several nonrandomized studies have supported the use of radical resection,[19–21] whereas others found no survival advantage.[22] A randomized multicenter report by Pedrazzoli and colleagues[23] suggested a survival advantage following radical lymphadenectomy for patients with node-positive tumors. A multicenter randomized trial in Japan by Nimura and colleagues[24] randomized 101 patients between two groups (regional resection versus regional resection plus extended lymphadenectomy) and found survival to be similar between the two groups.

At the Johns Hopkins Hospital, a prospective randomized trial was performed comparing pylorus-preserving PD resection for periampullary adenocarcinoma to radical resection including distal gastrectomy and retroperitoneal lymphadenectomy. An interim report of the first 114 patients[25] and a report after closing the trial with 294 patients[26] demonstrated similar operative mortality between the two groups. However, patients undergoing radical PD had a significantly higher overall complication rate, with a higher incidence of delayed gastric emptying, pancreatic fistula, and wound infection. This correlated with longer postoperative lengths of hospital stay in the radical group. A quality-of-life analysis of patients involved in the trial showed no differences between the two groups.[27]

The previous reports[25–27] did not demonstrate a statistically significant survival difference between the standard and radical groups for the entire cohort of periampullary cancer patients or for any subgroup of patients evaluated. The median live patient follow-up in the 2002 report[26] was only 31.5 months, precluding any conclusions regarding long-term survival (5-year survival). In those patients with pancreatic adenocarcinoma, there were similar median survivals when comparing the standard and radical groups. Using Kaplan-Meier actuarial survival statistics, the 5-year survival rate in patients undergoing standard PD for pancreatic adenocarcinoma was 10%, compared with 25% for those patients undergoing radical PD. This difference was not statistically significant but was intriguing and begged further long-term follow-up.

METHODS

Our previous reports detail the recruitment of patients into the study, the surgical technique, postoperative management, data collection, pathologic review, and statistical analyses.[25–27] As most patients did not have biopsy-proved cancer at the time of surgery, patients were recruited into the study preoperatively if they were to undergo PD for what was believed clinically to be a periampullary adenocarcinoma (tumor of the head, neck, or uncinate process of the pancreas, ampulla of Vater, distal bile duct, or peri-Vaterian duodenum). The study was approved by the Joint Committee on Clinical Investigation (JCCI) of the Johns Hopkins University School of Medicine. Informed consent was obtained preoperatively on all participating patients. Exclusion criteria included the absence of informed consent, preoperative chemotherapy or radiation, final pathology revealing disease other than adenocarcinoma primary to the periampullary region, or positive resection margins (by frozen section) at the time of pancreaticoduodenal resection. The trial was open for accrual from April 1996 through June 2001. Pancreaticoduodenectomy was performed in 983 patients during that time period, with 672 resections being for periampullary adenocarcinoma. Of these, 299 patients were randomized into the study.

After completion of a standard, margin-negative pylorus-preserving pancreaticoduodenal resection, a computer-generated random number pattern was used to randomize all eligible, consented patients to either standard or radical resection. For the standard resection group, pylorus-preservation was preferred, and the lymph node groups resected en bloc included the anterior pancreaticoduodenal lymph nodes (lymph node station 17 in the Japanese system), the posterior pancreaticoduodenal lymph nodes (station 13), nodes in the lower hepatoduodenal ligament (stations 12b and 12c), and nodes along the right lateral aspect of the superior mesenteric artery and vein (some station 14b and 14v). The

standard resection involved division of the duodenum 2–3 cm distal to the pylorus, with resection of all duodenum distal to the transection site, removal of the gallbladder and common bile duct (from the level of the cystic duct junction with the common hepatic duct distally), removal of 10–20 cm of the proximal jejunum beyond the ligament of Treitz, resection of the head, neck, and uncinate process of the pancreas (with division of the neck of the pancreas anterior to the portal vein–superior mesenteric vein axis), and extirpation of the periampullary tumor. Distal gastrectomy was performed only if the proximal duodenal margin was inadequate or the duodenal cuff appeared ischemic.

The radical resection (Fig. 1) added a 30–40% distal gastrectomy (including lymph node stations 5, 6, and some stations 3 and 4) and a retroperitoneal lymphadenectomy extending from the right renal hilum to the left lateral border of the aorta in the horizontal axis and from the portal vein to below the third portion of the duodenum in the vertical axis. The retroperitoneal dissection harvested nodes from stations 16a1 and 16b1 and sampled a celiac node (station 9).

The uncinate process was removed from underneath the superior mesenteric vein, flush with the superior mesenteric artery in both the standard and radical resections. Partial pancreatectomy was performed in the majority of cases. Reconstruction was typically performed to a single retrocolic jejunal limb with a proximal pancreaticojejunostomy, downstream hepaticojejunostomy, and further downstream duodeno- or gastrojejunostomy. Vein resection, vagotomy, tube gastrostomy, and feeding jejunostomy were not routinely used. All patients were managed postoperatively using a standard postoperative critical pathway.

Pathology specimens were reviewed to determine the type of carcinoma, site of the primary tumor, the margin status, the lymph node status, and the overall pathologic TNM stage. The retroperitoneal lymph node specimens were submitted in their entirety for histologic examination.

The primary endpoints of the study included perioperative complications, length of hospital stay, and survival of the two groups. The first report was an interim report[25] focusing on intraoperative and postoperative data. The second report was published at the close of the trial.[26] It also addressed survival, but the survival data at that point in time were immature. Quality of life was addressed in a separate report.[27]

All data were collected prospectively. This included the details of the operative procedure, a surgeon questionnaire detailing the operative findings, and other relevant clinical information. Follow-up information was complete on 289 of 294 patients, with only 5 patients lost to follow-up. The information was

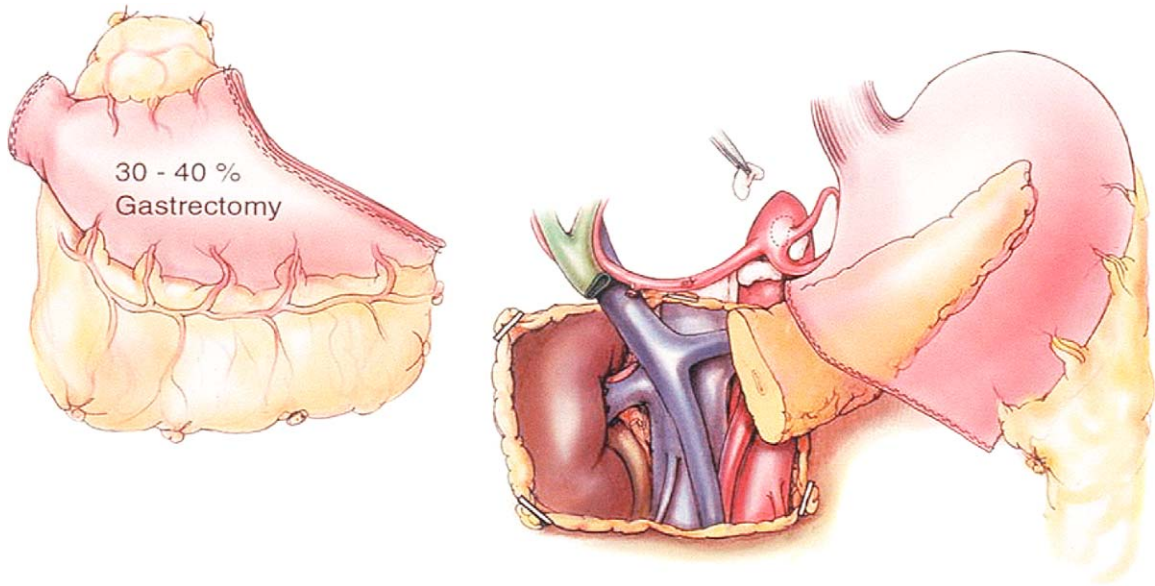

Fig. 1. Components of the radical procedure. At the left is the 30–40% distal gastrectomy specimen, which includes the pylorus and 2–3 cm of the duodenum. At the right is the retained stomach, the pancreatic body and tail, and an overview of the retroperitoneal dissection. Titanium clips have been placed to mark the extent of the retroperitoneal dissection. A celiac node is removed for histologic analysis. (From Yeo CJ, Cameron JL, Sohn TA, et al. Pancreaticoduodenectomy with or without extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma: Comparison of morbidity and mortality and short-term outcome. Ann Surg 1999;299:613–624, with permission.)

obtained through a Social Security Administration database, public records, office records, or telephone contact and was complete through December 2004.

At the time of initial study planning (1995), the number of patients determined to be necessary for statistical validity (one-sided) to improve overall 5-year survival from 20% to 35% (α set at .05; β set at .2; power = 80%) was 121 patients per arm (242 total patients). An interim statistical analysis in 1999 modified the study design, adjusting the overall 5-year survival rates from 30% to 40%, and increasing the number of patients per arm to 300. A survival analysis in 2001 showed no difference in survival between the two groups, and after review by the JCCI, the trial was closed for new patient accrual in June 2001.

All results are reported as mean ± SEM. Comparability of the standard and radical groups was performed using the Student *t* test and χ^2 analysis. Differences in survival between subgroups of patients were compared using a log-rank test. A Cox proportional hazards model was used to perform a multivariate analysis of factors influencing survival in the entire cohort and the subgroup of patients with pancreatic cancer. Significance was accepted at the 5% level.

RESULTS

As reported previously, 299 patients with periampullary infiltrating adenocarcinoma were randomized to either standard or radical PD. Five patients were subsequently excluded because their final pathology failed to reveal invasive periampullary adenocarcinoma, leaving 294 patients for analysis. One hundred forty-six patients underwent standard resection, and 148 underwent radical resection; 57% (n = 167) of patients had pancreatic adenocarcinoma, 21% (n = 63) had ampullary adenocarcinoma, 17% (n = 51) had distal bile duct adenocarcinoma, 3% (n = 9) had duodenal adenocarcinoma, and 2% (n = 4) had a pancreatic intraductal papillary mucinous neoplasm (IPMN) with an invasive carcinoma.

Demographics and Intraoperative Factors

The demographic factors, type and extent of resection performed, and intraoperative data have already been presented in the 2002 report[26] at the close of the study. These are not reviewed in detail but are presented in Table 1. The two groups were similar with regard to age and gender, but there was a significantly higher proportion of whites in the radical group ($P = 0.02$). While other intraoperative factors were similar, the median operative time was significantly longer in the radical group (6.2 hours versus 5.5 hours, $P = 0.002$), as would be expected with the addition of a distal gastrectomy and retroperitoneal lymphadenectomy.

Pathology

The pathology results for the entire cohort of patients are presented in Table 2. The standard and

Table 1. Patient demographics and intraoperative factors

	Standard (n = 146)	Radical (n = 148)	*P* Value
Demographics			
Mean age (yr)	66.2 ± 0.9 years	65.2 ± 0.9 years	0.46
Gender (% male)	58	51	0.19
Race (% white)	89	95	0.02
Intraoperative factors			
Type of resection (n)			
Pylorus-preserving	125 (86%)	0 (0%)	<0.0001
Classic (distal gastrectomy)	21 (14%)	148 (100%)	
Extent of pancreatic resection (n)			
Partial	141 (97%)	145 (98%)	0.46
Total	5 (3%)	3 (2%)	
Type of pancreatic anastomosis (n)			
Pancreaticojejunostomy	140 (99%)	141 (97%)	0.42
Pancreaticogastrostomy	1 (1%)	4 (3%)	
None	5	3	
Vein resection (n)	4 (3%)	4 (3%)	0.98
Median intraoperative blood loss (ml)	600	700	0.30
Median transfusions (units PRBCs)	0	0	0.96
Median operative time (hr)	5.5	6.2	0.002

PRBC = packed red blood cells.
Adapted from Yeo et al. Ann Surg 2002;236:355–368, Table 2.

Table 2. Pathology

	Standard (n = 146)	Radical (n = 148)	*P* Value
Site of tumor origin (n)			
Pancreas	84 (58%)	83 (56%)	0.40
Ampulla of Vater	35 (24%)	28 (19%)	
Distal bile duct	23 (16%)	28 (19%)	
Duodenum	2 (1%)	7 (5%)	
IPMN of pancreas with carcinoma	2 (1%)	2 (1%)	
Poor tumor differentiation (%)	39	45	0.27
Mean tumor diameter (cm)	2.6 ± 0.1	2.5 ± 0.1	0.42
Resected lymph node status (% positive)	73	74	0.76
Resection margin status* (% positive)	12	7	0.11
Perineural invasion (% positive)	70	77	0.17
Vascular invasion (% positive)	44	45	0.89

IPMN = intraductal papillary mucinous neoplasm.
Adapted from Yeo et al. Ann Surg 2002;236:355–368, Table 3.
*All positive margins were microscopically positive (R_1) on permanent section. No patient was randomized with microscopically positive margins by frozen section or grossly positive margins (R_2).

radical groups were comparable with respect to the site of tumor origin, tumor diameter, tumor differentiation, lymph node status, and presence of vascular or perineural invasion. Eighteen patients (12%) in the standard group and 10 patients (7%) in the radical group had positive microscopic margins on permanent section analysis; however, this difference did not achieve statistical significance ($P = 0.11$). Margins were most often positive at the uncinate process of the pancreas, adjacent to the visceral vessels (superior mesenteric vein [SMV] portal vein [PV], or superior mesenteric artery [SMA]).

For those patients with pancreatic adenocarcinoma (n = 167), the two groups were comparable with respect to lymph node status, differentiation, and vascular invasion (Table 3). Patients in the radical group tended to have smaller tumors (2.8 ± 0.1 cm) than those in the standard group (3.0 ± 0.1 cm), however this difference was not statistically significant ($P = 0.26$). The incidence of perineural invasion was slightly higher in the radical group (93% radical versus 84% standard, $P = 0.09$), but this did not achieve statistical significance. Of note, patients in the standard PD group had a statistically higher incidence of microscopically positive surgical margins on permanent section as compared with those patients in the radical PD group (20% versus 5%, $P = 0.003$). In our experience, the margin most commonly negative on frozen section, but then positive when analyzed on permanent section was the uncinate or visceral vessel (SMV, PV, or SMA) margin. This margin was not readdressed or altered when the patient was randomized between standard and radical PD. Thus, the higher incidence of margin positivity in the standard PD group appears to be a chance observation but one that could impact long term survival. One hundred sixty-two patients were included in the pancreatic cancer survival analysis. This group of 162 patients excluded perioperative mortalities (n = 4) and patients lost to follow-up (n = 1), as we were evaluating long-term survival. Seventeen of 80 patients in the standard PD survival analysis had positive margins (21%) and only 4 of 82 patients in the radical PD survival analysis had positive margins (5%, $P = 0.002$), a difference that was highly significant.

Table 3. Pathology of patients with pancreatic cancer

	Standard (n = 84)	Radical (n = 83)	*P* Value
Mean tumor diameter (cm)	3.0 ± 0.1	2.8 ± 0.1	0.26
Tumor differentiation (% poor)	40	51	0.29
Resected lymph node status (% positive)	82	77	0.42
Resection margin status* (% positive)	20	5	0.003
Perineural invasion (%)	84	93	0.09
Vascular invasion (%)	54	55	0.88

*All positive margins were microscopically positive on permanent section (R_1). No patient was randomized with microscopically positive margins by frozen section or grossly positive margins (R_2).

For those patients with ampullary and distal bile duct cancer, there were no differences in the pathologic characteristics of the standard and radical groups. The duodenal cancer and IPMN with cancer groups were too small to analyze for differences.

Lymph Node Analysis

In the standard group, 73% of patients had positive lymph nodes in the resection specimen, with the mean number of lymph nodes resected being 17. In the radical group, 74% of patients had positive lymph nodes, and as expected, the total number of lymph nodes resected was higher at 28.5 ($P = 0.001$). Only one patient in the radical group had negative lymph nodes in the pancreaticoduodenal specimen and a positive lymph node in the extended lymphadenectomy specimen (a perigastric lymph

node). A detailed analysis of the lymph node harvest can be found in Table 4 of the 2002 report.[26]

Postoperative Complications and Hospital Course

The postoperative complications and hospital course are summarized in Table 4. As these were previously reported,[25,26] they will not be extensively reviewed and the remainder of the Results section focuses on the 5-year follow-up data. Definitions of specific complications can be found in the previous reports.[25,26]

Postoperative Chemoradiation

Information on postoperative adjuvant chemoradiation was available on 217 patients. One hundred sixty-four patients received adjuvant therapy, while 53 did not receive adjuvant therapy. Of the 164 patients who received therapy, 81 were in the standard group and 83 were in the radical group.

Survival Analyses

Of the 294 patients with periampullary adenocarcinoma enrolled in the study, 9 died in the immediate postoperative period, leaving 285 patients available for follow-up. Of these patients, 5 were lost to follow-up and were not included, yielding a total of 280 patients in the survival analysis. One hundred thirty-six patients were in the standard group and 144 were in the radical group. The mean follow-up for the entire cohort was 35.7 ± 1.6 months. There are 77 patients (27.5%) who remained alive at the time of this report. The mean live-patient follow-up was 68.6 ± 2.2 months, with a median of 64 months. This compares to a mean follow-up of 24 months and a mean live patient follow-up of 31.5 months in the previous report.[26]

The 1-, 3-, and 5-year survival data for the entire cohort and different subgroups are summarized in Table 5. The actuarial survival curves are depicted in Figures 2–5. For the 136 patients in the standard group, the overall 1-, 3-, and 5-year actuarial survival rates were 78%, 42%, and 25% (median = 25 months) compared with 76%, 44%, and 31% (median = 28 months, $P = 0.57$; Fig. 2, *A*) for the 144 patients in the radical group. In the subgroup of periampullary cancer patients with positive lymph nodes, there were no differences in survival between the two groups (Fig. 2, *B*). Likewise, there were no differences in survival between standard and radical resection in node-negative patients (Fig. 2, *C*).

In the 162 patients with pancreatic cancer, the 80 patients undergoing standard resection had 1-, 3-, and 5-year actuarial survival rates of 75%, 34%, and 13% (median = 20 months) compared with 73%, 38%, and 29% (median = 22 months) for the 82 patients undergoing radical resection ($P = 0.13$; Fig. 3, *A*). However, it should be noted that patients in the standard group had a significantly

Table 4. Postoperative complications and hospital course

	Standard (n = 146)	Radical (n = 148)	*P* Value
Perioperative mortality (n)	6 (4%)	3 (2%)	0.30
Reoperation (n)	6 (4%)	6 (4%)	0.98
Any complication (n)	42 (29%)	64 (43%)	0.01
Delayed gastric emptying (n)	9 (6%)	24 (16%)	0.006
Pancreatic fistula (n)	9 (6%)	19 (13%)	0.05
Wound infection (n)	7 (5%)	16 (11%)	0.06
Intra-abdominal abscess (n)	5 (3%)	6 (4%)	0.77
Bile leak (n)	3 (2%)	7 (5%)	0.21
Cholangitis (n)	2 (1%)	3 (2%)	0.66
Lymphocele (n)	1 (1%)	4 (3%)	0.57
Median postoperative length of stay (days)	9	10	0.003

Table 5. Actuarial survival rates after standard versus radical pancreaticoduodenectomy*

	1 Year (%)	3 Years (%)	5 Years (%)	Median (mo)	*P* Value
Entire cohort (all pathologic diagnoses)					
Standard (n = 136)	78	42	25	25	0.57
Radical (n = 144)	76	44	31	28	
Node-positive patients (all pathologic diagnoses)					
Standard (n = 99)	76	31	15	19	0.23
Radical (n = 108)	76	39	28	20	
Node-negative patients (all pathologic diagnoses)					
Standard (n = 37)	84	73	50	59	0.51
Radical (n = 36)	94	61	41	45	
Pancreatic cancer					
Standard (n = 80)	75	34	13	20	0.13
Radical (n = 82)	73	38	29	22	
Pancreatic cancer, node positive					
Standard (n = 66)	73	26	10	19	0.36
Radical (n = 64)	69	33	24	18	
Pancreatic cancer, node negative					
Standard (n = 14)	86	71	29	43	0.21
Radical (n = 18)	89	56	44	44	
Ampullary cancer					
Standard (n = 32)	81	59	46	48	0.67
Radical (n = 27)	81	52	43	40	
Distal bile duct cancer					
Standard (n = 21)	81	38	28	23	0.51
Radical (n = 27)	78	44	15	25	

*Excludes perioperative deaths and patients lost to follow-up. Mean follow-up for live patients was 68.6 months.

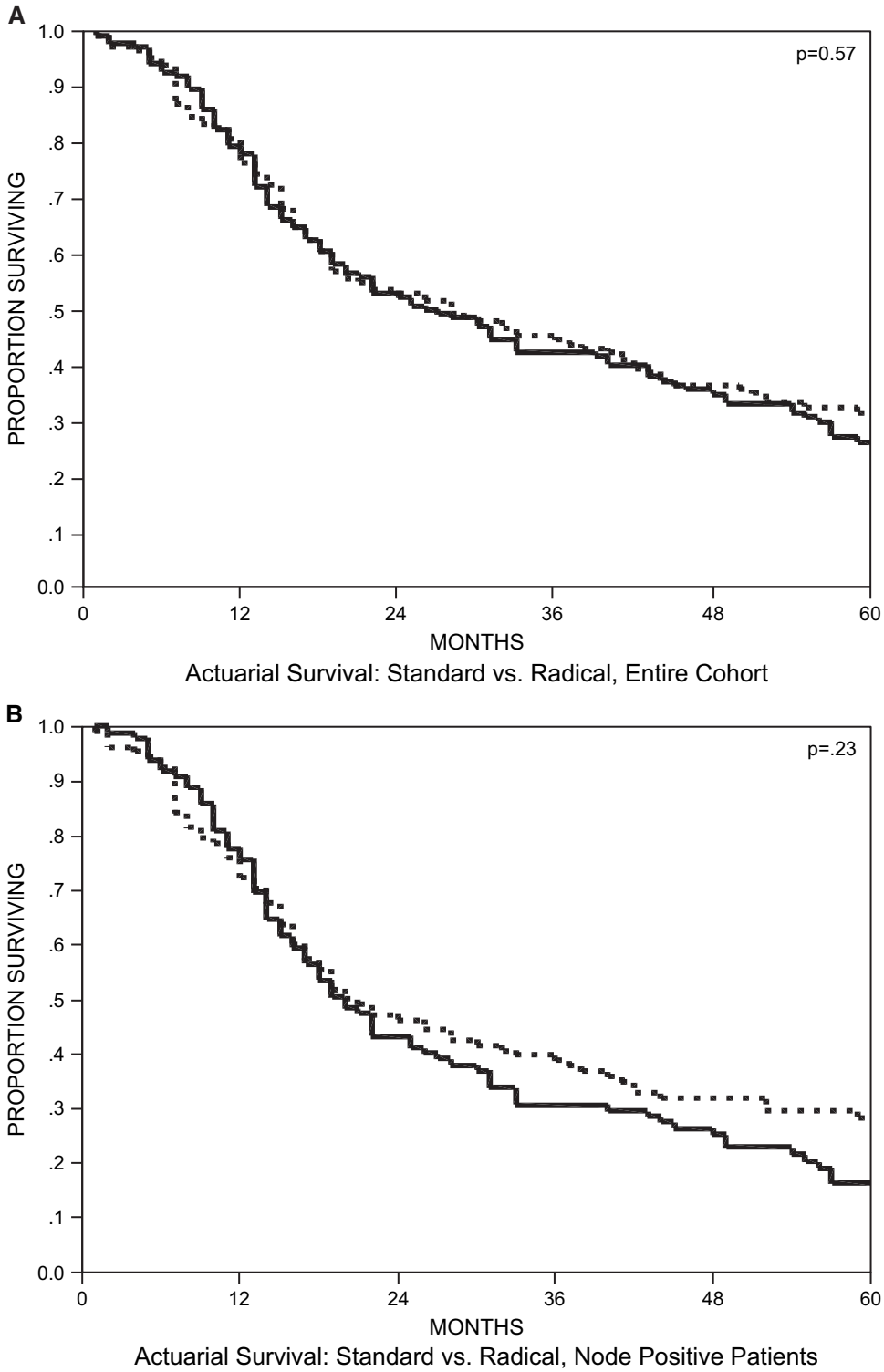

Fig. 2. (**A**) The actuarial survival curves for all patients (entire cohort) who survived the immediate postoperative period, comparing the standard resection group (n = 136, *solid line*) to the radical group (n = 144, *dashed line*). The 1-, 3-, and 5-year survival rates were 78%, 42%, and 25% for the standard group and 76%, 44%, and 31% for the radical group ($P = 0.57$), respectively. (**B**) The actuarial survival curves for patients (all periampullary adenocarcinomas) who survived the immediate postoperative period and had positive lymph nodes in the resection specimen, comparing the standard resection group (n = 99, *solid line*) to the radical group (n = 108, *dashed line*). The 1-, 3-, and 5-year survival rates were 76%, 31%, and 15% for the standard group and 76%, 39%, and 28% for the radical group ($P = 0.23$), respectively. (**C**) The actuarial survival curves for patients (all periampullary adenocarcinomas) who survived the immediate postoperative period and had negative lymph nodes in the resection specimen, comparing the standard resection group (n = 37, *solid line*) to the radical group (n = 36, *dashed line*). The 1-, 3-, and 5-year survival rates were 84%, 73%, and 50% for the standard group and 94%, 61%, and 41% for the radical group ($P = 0.51$), respectively.

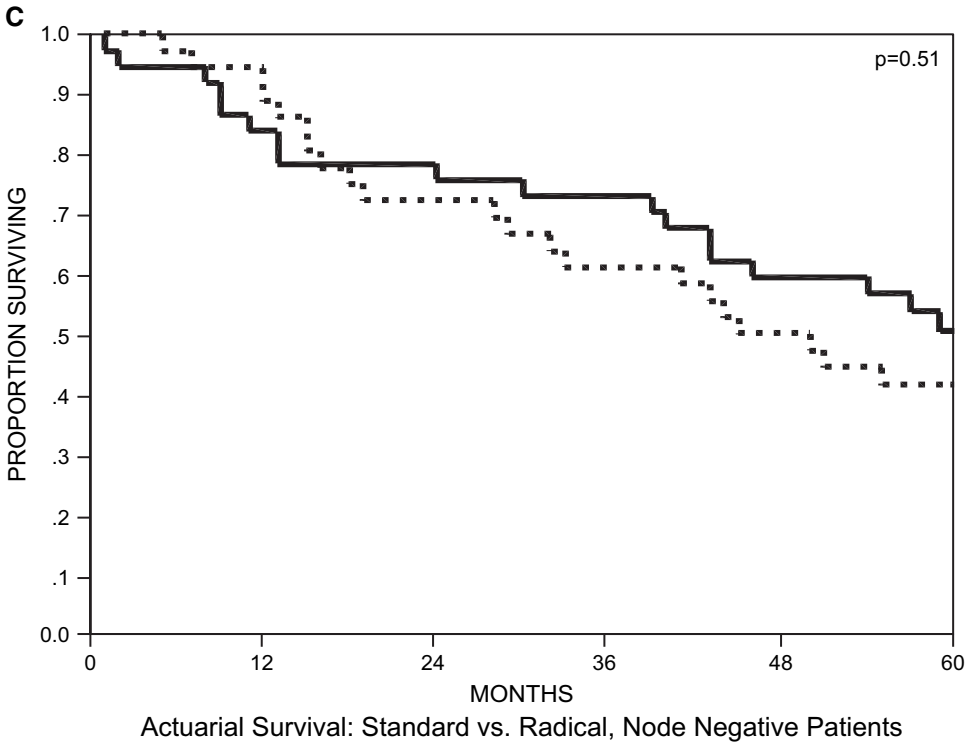

Fig. 2 (*continued*)

higher incidence of microscopically positive resection margins (21% versus 5% radical; $P = 0.002$).

For patients with node positive pancreatic cancer (n = 130), the 1-, 3-, and 5-year actuarial survival rates were 73%, 26%, and 10% (n = 66, median = 19 months) for those undergoing standard resection compared with 69%, 33% and 24% in the radical group (n = 64, median = 18 months, $P = 0.36$; Fig. 3, *B*). For the 32 node-negative patients, those undergoing standard resection had 1-, 3-, and 5-year survival rates of 86%, 71%, and 29% (n = 14, median = 43 months), while those undergoing radical resection had 1-, 3-, and 5-year survival rates of 89%, 56%, and 44%, respectively (n = 18, median = 44 months, $P = 0.21$; Fig. 3, *C*).

While there were not enough patients or deaths in the duodenal cancer group to perform a survival analysis, both the ampullary cancer and distal bile duct cancer subgroups had adequate numbers for analysis. The 1-, 3-, and 5-year actuarial survival rates were 81%, 59%, and 46% (n = 32, median = 48 months) for those patients undergoing standard resection for ampullary adenocarcinoma. Those undergoing radical resection had similar survival rates of 81%, 52%, and 43% (n = 27, median = 40 months, $P = 0.67$; Fig. 4).

For those patients with distal bile duct cancer, there were no differences in survival between standard and radical resection, with 1-, 3-, and 5-year actuarial survival rates of 81%, 38%, and 28% (n = 21, median = 23 months) for standard resection and 78%, 44%, and 15% (n = 27, median = 25 months, $P = 0.51$; Fig. 5) for radical resection.

For those patients undergoing radical resection, 74% had positive lymph nodes in the resection specimen and 15% had positive retroperitoneal lymph nodes. All patients with positive retroperitoneal nodes also had positive lymph nodes in the resection specimen. In patients with pancreatic cancer, 64 had positive lymph nodes in the resection specimen and 13 of those had positive retroperitoneal lymph nodes. When comparing survival of those pancreatic cancer patients with and without positive retroperitoneal nodes, those patients with positive retroperitoneal nodes (n = 13) had 1-, 3-, and 5-year survival rates of 46%, 15%, and 15%, while those patients with negative retroperitoneal nodes (n = 69) had 1-, 3-, and 5-year survival rates of 78%, 42%, and 32%. This difference approached significance, with a P value of 0.06.

Multivariate Models

Using a Cox proportional hazard model, a multivariate analysis of factors influencing survival for the entire cohort of patients (all pathologic diagnoses) and the cohort of patients with pancreatic cancer was performed (Table 6). Factors evaluated included (1)

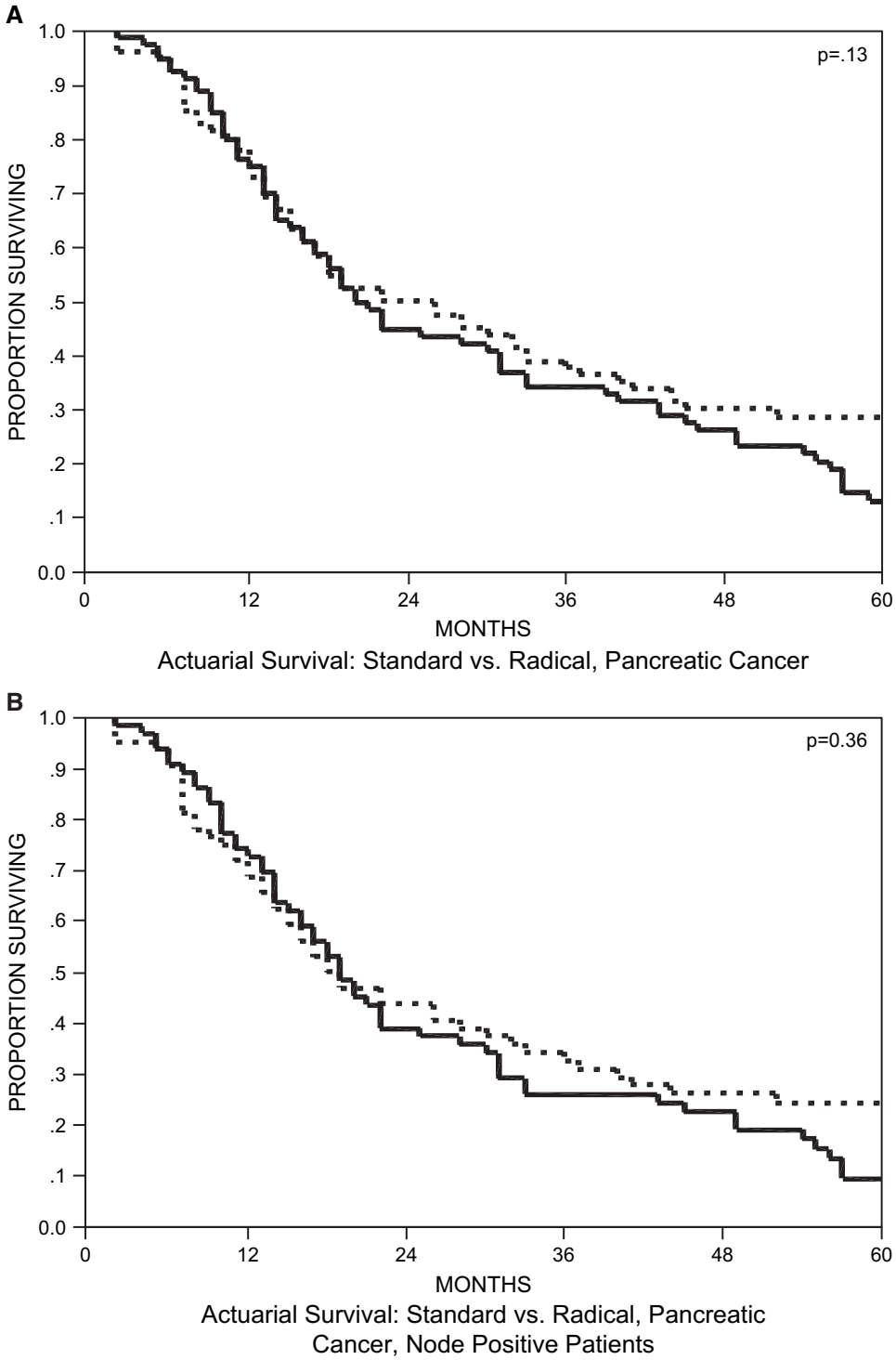

Fig. 3. (**A**) The actuarial survival curves for all patients with pancreatic adenocarcinoma who survived the immediate postoperative period, comparing the standard resection group (n = 80, *solid line*) to the radical group (n = 82, *dashed line*). The 1-, 3-, and 5-year survival rates were 75%, 34%, and 13% for the standard group and 73%, 38%, and 29% for the radical group ($P = 0.13$), respectively. (**B**) The actuarial survival curves for all patients with pancreatic adenocarcinoma who survived the immediate postoperative period and had positive lymph nodes in the resection specimen, comparing the standard resection group (n = 66, *solid line*) to the radical group (n = 64, *dashed line*). The 1-, 3-, and 5-year survival rates were 73%, 26%, and 10% for the standard group and 69%, 33%, and 24% for the radical group ($P = 0.36$), respectively. (**C**) The actuarial survival curves for all patients with pancreatic adenocarcinoma who survived the immediate postoperative period and had negative lymph nodes in the resection specimen, comparing the standard resection group (n = 14, *solid line*) to the radical group (n = 18, *dashed line*). The 1-, 3-, and 5-year survival rates were 86%, 71%, and 29% for the standard group and 89%, 56%, and 44% for the radical group ($P = 0.21$), respectively.

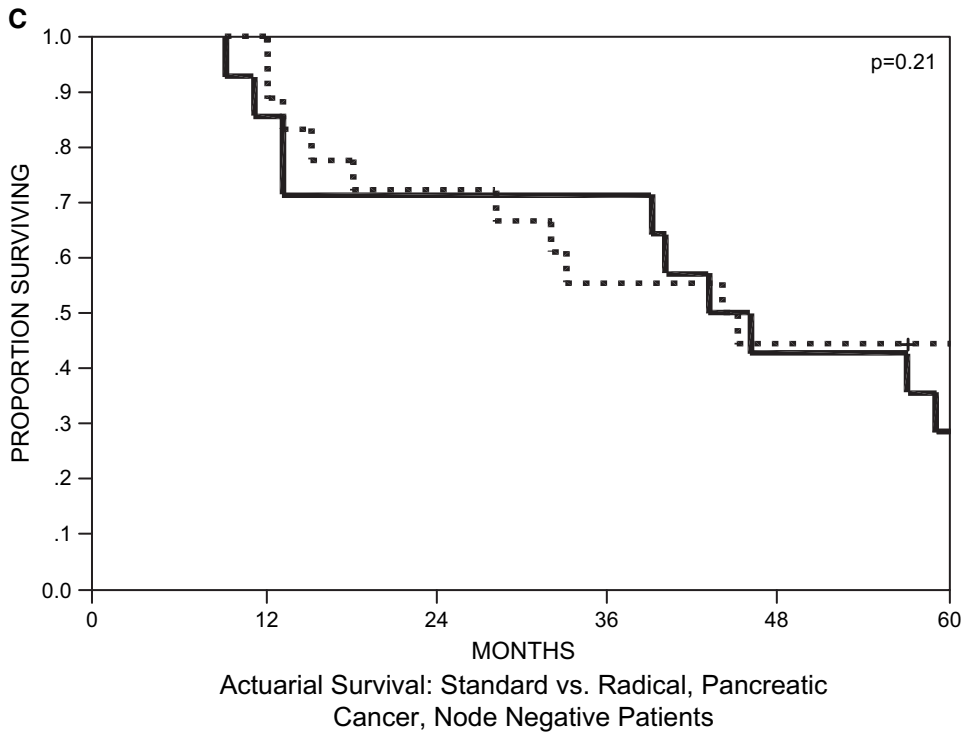

Fig. 3 (*continued*)

lymph node status, (2) diagnosis (pancreatic primary versus primary from duodenum, ampulla, or bile duct), (3) resection margin status, (4) tumor diameter (<3 cm versus $\geqslant 3$ cm), (5) adjuvant chemoradiation, and (6) standard versus radical resection. There were data for 203 patients for all of these factors. The hazard ratios, confidence intervals, and *P* values are shown in Table 6. For the entire cohort, only the presence of positive lymph nodes and the diagnosis of pancreatic adenocarcinoma had a significant negative influence on survival. Margin status also had a negative influence that approached, but did not achieve, statistical significance. Tumor diameter, adjuvant therapy, and radical resection were not significant predictors of survival in this multivariate model.

For those patients with pancreatic adenocarcinoma, the same factors (with the exclusion of diagnosis) were evaluated in another multivariate model. Of 163 patients, 126 had all factors known and were included in the model (see Table 6). In this analysis, only resected lymph node status was a significant predictor of survival. Again, radical resection did not confer a survival advantage.

DISCUSSION

The use of extended or regional lymphadenectomy has been proposed as a means to improve survival for many cancers. Extended lymphadenectomy has been most thoroughly studied in gastric cancer, where several randomized, controlled trials have failed to demonstrate a consistent survival advantage.[28–31] There are now multiple, nonrandomized and randomized, controlled trials evaluating the role of extended lymphadenectomy in pancreatic and periampullary cancer.[19–27]

The current report is a survival update of a previously reported randomized, controlled trial performed at the Johns Hopkins Hospital.[25–27] This trial compared standard pylorus-preserving PD to PD with distal gastrectomy and extended retroperitoneal lymphadenectomy (extending from the right renal hilum to the left lateral border of the aorta in the horizontal axis and from the portal vein to below the third portion of the duodenum in the vertical axis). This study included all periampullary adenocarcinomas, including pancreatic, ampullary, distal bile duct, and duodenal primaries.

The previous reports based on this randomized, controlled trial evaluated the endpoints of postoperative complications, intraoperative outcomes, quality of life, and short-term survival.[25–27] Patients undergoing radical resection had a significantly higher overall complication rate, with a higher incidence of delayed gastric emptying and pancreatic fistula formation. In addition, the patients in the radical group had a longer operative time and a longer

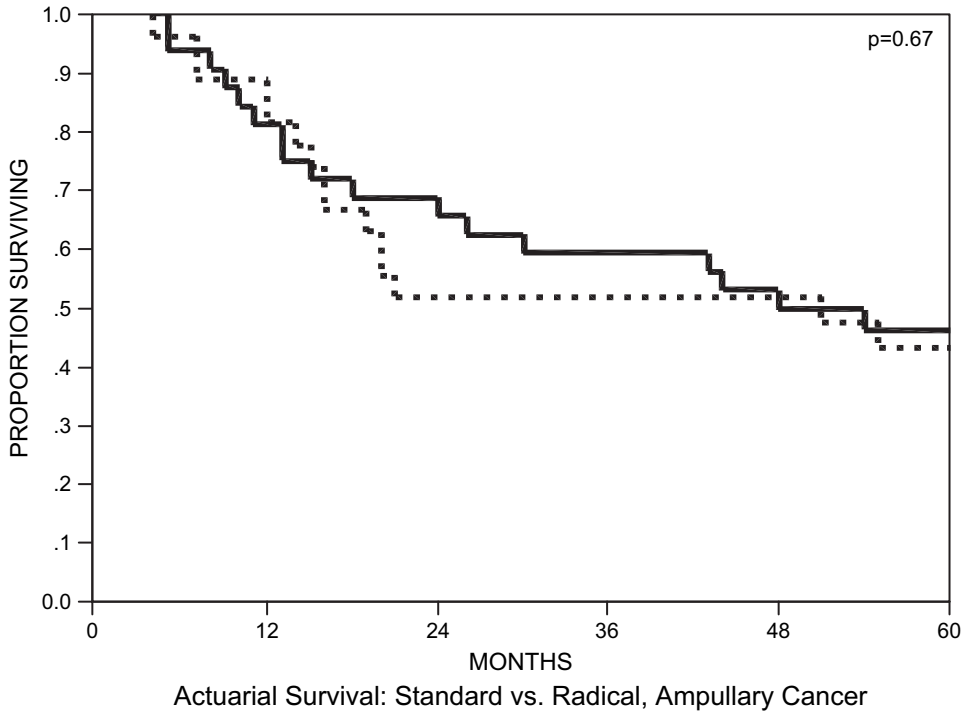

Fig. 4. The actuarial survival curves for all patients with ampullary adenocarcinoma who survived the immediate postoperative period, comparing the standard resection group (n = 32, *solid line*) to the radical group (n = 27, *dashed line*). The 1-, 3-, and 5-year survival rates were 81%, 59%, and 46% for the standard group and 81%, 52%, and 43% for the radical group (p = 0.67), respectively.

postoperative length of stay. After completion of the study in June 2001, a survival analysis for the entire cohort showed no difference in survival between the standard and radical groups.[26] Moreover, when comparing subgroups of patients with pancreatic cancer, ampullary cancer, and distal bile duct cancer, there also were no differences between the standard and radical groups. Interestingly, when studying only patients with pancreatic cancer, the standard and radical groups had similar median survival rates, but those patients in the radical group had a 5-year survival rate of 25%, compared with 10% in the standard group. While this difference was not statistically significant, it was intriguing to speculate that radical resection might benefit a small group of patients. However, the immaturity of the data suggested that long-term follow-up was warranted. In fact, the mean live patient follow-up in the 2002 report[26] was only 31.5 months. The current study focuses on survival with mature survival data, and the mean live patient follow-up is now 68.6 months.

For comparison, Henne-Bruns and colleagues[22] from Germany reported the results of a non-randomized trial evaluating patients with pancreatic cancer undergoing different extents of regional lymphadenectomy. One group of 26 patients underwent a lymphadenectomy including removal of nodes in the hepatoduodenal ligament, proximal celiac trunk, right side of the SMA, and ventral surface of the inferior vena cava. The other group of 46 patients underwent a more radical resection with removal of additional nodes including all lymphatic tissue along the left side of the SMA and the aorta. This analysis failed to demonstrate a survival benefit for the more extensive procedure.

Pedrazzoli and colleagues[23] randomized 81 patients with pancreatic cancer to either standard resection or resection with retroperitoneal lymphadenectomy, to include circumferential clearance of the SMA and celiac axis as well as removal of lymph nodes from the hepatic hilum, along the aorta from the diaphragmatic hiatus to the IMA, and laterally to both renal hila. In this study, the overall survival was not different between the two groups However, in the subgroup of patients with positive lymph nodes, they observed a significantly better survival rate.

Nimura and colleagues[24] of Japan recently reported the results of a multicenter, randomized, controlled trial comparing regional resection to regional resection with extended lymphadenectomy, which included the regional and para-aortic lymph nodes. This study included 101 patients, with no difference in

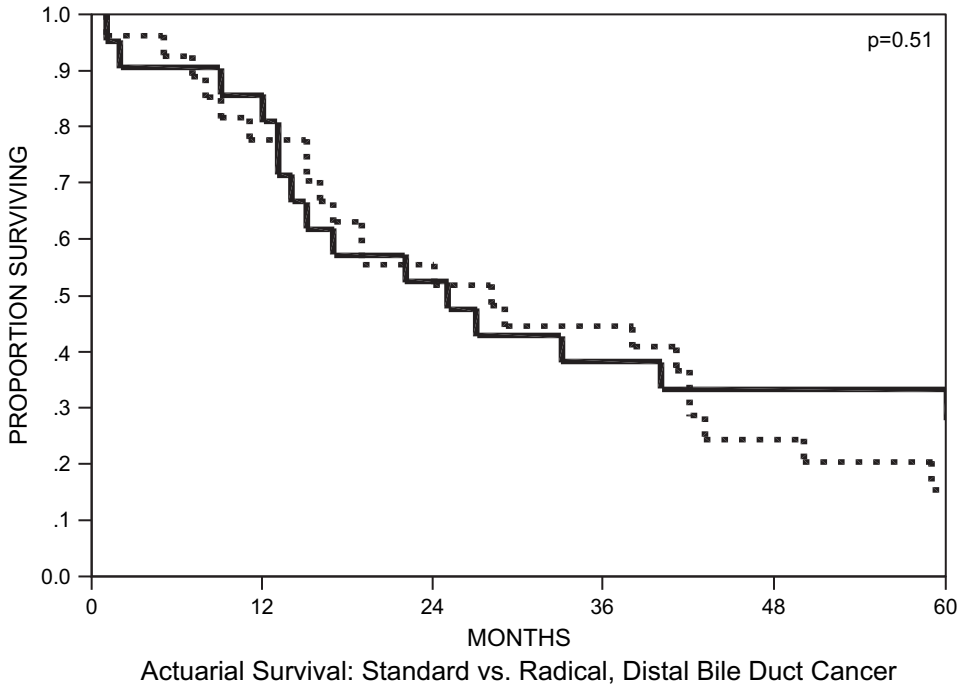

Fig. 5. The actuarial survival curves for all patients with distal bile duct adenocarcinoma who survived the immediate postoperative period, comparing the standard resection group (n = 21, *solid line*) to the radical group (n = 27, *dashed line*). The 1-, 3-, and 5-year survival rates were 81%, 38%, and 28% for the standard group and 78%, 44%, and 15% for the radical group ($P = 0.67$), respectively.

demographics between the two groups. As in our Johns Hopkins study, the mean operative times were longer in the extended group. They also noted a higher incidence of severe diarrhea in the extended group, which was statistically significant. They evaluated survival out to 3 years and found no differences between patients undergoing regional versus extended resection.

The effect of pylorus-preservation versus classic resection (including distal gastrectomy) has been debated. There have been two randomized trials evaluating this issue, neither of which included an extended lymphadenectomy.[32,33] There were no differences in survival between the classic and pylorus-preserving groups in these two trials. It is important to point this out, as distal gastrectomy was routinely included in the radical resection in our study, and the majority of patients undergoing standard resection had pylorus-preserving procedures.

With complete follow-up on 280 of 285 patients surviving the immediate postoperative period, we did not demonstrate a survival difference for the overall cohort of all periampullary adenocarcinoma patients, consistent with the previously reported data.[25–27] This lack of survival benefit is maintained when the entire cohort was stratified by nodal status, as neither node-negative nor node-positive patients had a survival benefit from the more radical resection.

When evaluating the subgroups of patients with ampullary and distal bile duct cancer, patients again

Table 6. Multivariate survival analysis

Factor	Hazard Ratio	Confidence Interval	*P* Value
Entire cohort (n = 203)			
Negative lymph nodes	0.49	0.31–0.76	0.002
Diagnosis other than pancreatic cancer (ampullary, bile duct, duodenum, IPMN with cancer)	0.61	0.42–0.88	0.009
Negative resection margins	0.66	0.41–1.10	0.09
Tumor diameter <3 cm	0.88	0.13–1.25	0.47
Adjuvant chemoradiation	0.98	0.85–1.12	0.75
Radical resection	1.01	0.72–1.42	0.94
Pancreatic cancer (n = 126)			
Negative lymph nodes	0.50	0.29–0.86	0.01
Negative resection margins	0.72	0.41–1.24	0.24
Tumor diameter <3 cm	0.76	0.51–1.15	0.20
Adjuvant chemoradiation	0.94	0.78–1.13	0.53
Radical resection	0.92	0.61–1.39	0.69

IPMN = intraductal papillary mucinous neoplasm.

derived no survival benefit from the more extended resection. For the 162 patients with pancreatic cancer, the median survival was 20 months in the standard group and 22 months in the radical group, with 5-year survival rates of 13% and 29%, respectively. This difference was not statistically significant, with a P value of 0.13. Rather than the apparent 5-year survival difference being a result of the radical resection, it is most likely that the patients with pancreatic cancer in the standard group had poorer survival because they had a significantly higher incidence of positive final microscopic margins (21% standard versus 5% radical, $P = 0.002$). Because the randomization did not affect the management of the uncinate/visceral vessel margin, it is likely that the higher incidence of positive margins in the standard group is a chance finding, which may unfairly impact 5-year survival and unfairly seem to favor radical resection. This is supported by the multivariate analysis of the entire cohort of patients, where radical resection was not a significant factor influencing survival, with a hazard ratio of 1.01. Similarly, in the multivariate analysis of only the pancreatic cancer patients, radical resection was not a predictor of survival, with a hazard ratio of 0.92.

From the mature data in this prospective randomized study and the data from other randomized and nonrandomized trials, it appears that the use of extended resections to include retroperitoneal lymphadenectomy will not improve long-term survival for patients with pancreatic or other periampullary adenocarcinoma. The longer operative times, longer lengths of stay, higher complication rates, and lack of survival benefit following radical resection led us to conclude that pylorus-preserving pancreaticoduodenectomy should remain the procedure of choice for most patients with resectable pancreatic and periampullary adenocarcinoma.

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Discussion

Dr. Andrew Warshaw (Boston, MA): Dr. Riall, that is a beautiful presentation of this randomized trial that compares standard pylorus-preserving pancreaticoduodenectomy versus operation to which was added, in a second phase, separate antrectomy and retroperitoneal lymph node dissection. Your prior analyses did not show any survival benefit for the extended operation, and follow-up in this analysis of the same patients has been extended from a median of 31.5 to 64 months. Survival at 1 and 5 years, as you have shown, is now 78% and 25% versus 76% and 31%, almost the same as the findings 2 years ago.

The "intriguing trend," as you have stated in your manuscript, to better survival, which must be discounted as not meaningful because it is not statistically significant, may be attributable to a statistically significant ($P = 0.002$) difference in R0 resection margins. Although you attribute this to chance, it is statistically very unlikely to have occurred by chance at that level of difference. Could there be another explanation despite the fact that the resection margins of the primary specimen were not further affected by the subsequent dissection?

My second issue is that separate harvesting of the retroperitoneal nodes perhaps violates en bloc oncologic principles. Since pancreatic cancer is highly implantable, might the technique itself, that is, the separate dissection, have obviated achieving further benefit from the lymph node clearance?

Patients were included for randomization only after negative frozen section of margins, yet a sizable number were found to be positive on permanent section. What was the problem? Could it have been spread along the perineural channels around the SMA, which have been reported to be positive in up to 40% of grossly normal margins? Do you think that the SMA margin failure may be the most significant fact in local-regional failure in this series, rather than any question related to the retroperitoneal nodes?

Finally, 164 of your patients received adjuvant therapy, about half in each group, but they were not allocated by randomization. Does any subgroup analysis show a survival difference between standard and extended dissection groups based on whether or not they received adjuvant therapy?

Once again, congratulations for the significant contribution of a carefully conducted and thoroughly analyzed randomized prospective trial on an important question, which should be an example to all of us.

Dr. Riall: Thank you for your questions, Dr. Warshaw. You asked about the difference in our margin status between the standard and radical groups and why this occurred. The way the resection was done and the way the study was designed, as you point out, one would not expect a difference in the margin status. We first performed the pancreaticoduodenal resection, confirmed negative resection margins, and then proceeded with randomization and retroperitoneal dissection when indicated. So the extent of resection should not have affected our margin status. This, in part, addresses your last question as well. I think it is a chance observation, and I think the reason that we had so many positive margins after our initial margins were negative is related to the thoroughness of our pathologists.

When we send it for frozen section, they ink the uncinate/retroperitoneal, bile duct, pancreatic neck, and duodenal margins. They take a look at a representative sample, but they don't look at the entire sample. Our pathologists are extremely good at finding these positive margins, and when they go back and do the final section I think they are just picking up very subtle positive margins that weren't seen on frozen section. When we document perineural invasion, it is not just perineural invasion at the SMA margin but perineural invasion throughout the tumor. I think that, by chance, more patients in the standard group had microscopically positive margins.

I appreciate your point about the separate harvesting of the tumor and the retroperitoneal lymph nodes. We felt, in doing so, that it was going to be the cleanest way to perform the randomization and make the groups comparable. Had we randomized at a point where we thought patients were resectable, we thought our positive margin rate would be higher.

We did look at patients who had adjuvant therapy, and if you look at the entire cohort, the data are similar to previous studies. Patients receiving adjuvant therapy do marginally better, and that holds true in both groups. However, it doesn't have a greater effect in either the standard or the radical group.

Dr. Martin Schilling (Homburg/Saar, Germany): I think the Hopkins group has to be congratulated for providing us with the best study so far on that subject. I have two questions. First, when we do radical lymphadenectomy, we do see a much better local control with only 20% of local recurrence. The patients die of their distant metastases and have a much better quality of life throughout their living time. Have you looked at local control in your patients?

And the second one, in your initial description of your lymphadenectomy, you did not remove lymph node 12-P. That means you only cleared the anterior aspect of the hepatoduodenal ligament. Why did you do that, and why did you not include lymph nodes 16-B and C?

Dr. Riall: In answer to your first question, we have survival data on these patients; however it is not disease-specific survival. We don't necessarily know the specific patterns of recurrence in these patients. In our experience, the large majority of patients recur distantly with liver metastases or peritoneal metastases. In many of these patients there is local recurrence as well, but by and large, these people are dying of widely metastatic disease.

As the study was designed, it did not include 12-P lymph nodes. It was uniform in the two groups and that was how we chose to do it.

Dr. Fabrizio Michelassi (New York, NY): First of all, congratulations for another excellent presentation. Yours is one of four prospective randomized studies now available analyzing whether an extended lymphadenectomy associated with a pancreatoduodenectomy confers a survival advantage to patients with pancreatic and periampullary adenocarcinomas. The other three studies include the one where I participated with Professor Pedrazzoli, the one by Nimura, and the one by Farnell, which was recently presented at the Central Surgical Association and will be published soon in Surgery. All of them offer data demonstrating that an extended lymphadenectomy does not confer an improved survival, but some of them suggest that the extended lymphadenectomy may actually worsen patients' quality of life. So my question focuses on the incidence of diarrhea after this extended procedure. Nimura has provided data on the incidence of diarrhea at 3, 6, and 12 months. In his experience, severe diarrhea occurred in 24% of patients at 6 months and decreased to 9% at 12 months. Have you had any experience with severe diarrhea in your patients?

Dr. Riall: As you point out, there is certainly a different extent of resection as the operation is described in the different papers; however, we feel our study is probably comparable. The average number of lymph nodes harvested in our radical group was 28 lymph nodes compared to 20 in your study and 24 in the Henne-Bruns, Germany, study. So we are certainly harvesting a similar number of lymph nodes, and I feel that the resections are probably similarly radical in their extent of resection, although they are difficult to compare.

In terms of diarrhea, we did do a study that we published separate from this which looked at quality of life. Because we don't circumferentially dissect around the celiac axis and the SMA/SMV like the Japanese do, we really don't see an incidence of diarrhea. Farnell and his group at Mayo described it early in their quality of life study, but they said, too, in their study that over time this improved. We haven't really had a significant problem with diarrhea.

Dr. Keith Lillemoe (Indianapolis, IN): As immediate past president, I am going to take the unusual prerogative of commenting on a paper on which I am a co-author. I would like to dispel a myth and make a statement.

For those of you who have seen the Hopkins group present over the last 10 years and are aware of the number of publications on the topic of pancreatic cancer and other periampullary tumors, I would like to dispel the myth and state that Taylor Riall is not a full professor at Hopkins. If you look at her CV

and the number of publications she has, you might think that. Taylor, in fact, will be finishing her super chief year at Hopkins next month.

The comment I would like to make is that although a lot of us have left Hopkins over the last few years, including many of us in the room, the loss of Taylor and her contributions to everything that the Hopkins pancreatic group has done, including her analysis of retrospective and prospective databases and all of her contributions to the clinical trials, will be a major loss to that institution. I congratulate Dr. Nealon on recruiting her to Galveston.

Dr. Riall: Thank you, Dr. Lillemoe.

Hepatic Resection of Hepatocellular Carcinoma in Patients With Cirrhosis: Model of End-Stage Liver Disease (MELD) Score Predicts Perioperative Mortality

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Hepatic resection for hepatocellular carcinoma (HCC) in patients with cirrhosis is generally recommended for patients with Child-Turcotte-Pugh (CTP) Class A liver disease and early tumor stage. The Model for End-Stage Liver Disease (MELD) has been shown to accurately predict survival in patients with cirrhosis, but whether MELD is useful for selection of patients with cirrhosis for hepatic resection is unknown. We examined whether MELD was predictive of perioperative mortality and correlated MELD with other potential clinicopathologic factors to overall survival in patients with cirrhosis undergoing hepatic resection for HCC. A retrospective chart review was undertaken of patients with HCC and cirrhosis undergoing hepatic resection between 1993 and 2003. Eighty-two patients (62 men, 20 women; mean age, 62 years) were identified. Forty-five patients had MELD score ≥ 9 (range, 9−15) and CTP score ranged from 5 to 9 points. Fifty-nine patients underwent minor (<3 segments) hepatic resections (MELD ≤ 8, n = 29; MELD ≥ 9, n = 30) and 23 underwent major (≥ 3 segments) hepatic resections (MELD ≤ 8, n = 8; MELD ≥ 9, n = 15). Perioperative mortality rate was 16%. MELD score ≤ 8 was associated with no perioperative mortality versus 29% for patients with an MELD score ≥ 9 ($P < 0.01$). Multivariate analysis demonstrated that MELD score ≥ 9 ($P < 0.01$), clinical tumor symptoms ($P < 0.01$), and ASA score ($P = 0.046$) are independent predictors of perioperative mortality. Multivariate analysis showed MELD ≥ 9 ($P < 0.01$), tumor size >5 cm ($P < 0.01$), high tumor grade ($P = 0.03$), and absence of tumor capsule ($P < 0.01$) as independent predictors of decreased long-term survival. MELD score was a strong predictor of both perioperative mortality and long-term survival in patients with cirrhosis undergoing hepatic resection for HCC. In patients with cirrhosis, hepatic resection (minor or major) for HCC is recommended if the MELD score is ≤ 8. In patients with MELD score ≥ 9, other treatment modalities should be considered. (J GASTROINTEST SURG 2005;9:1207–1215)

KEY WORDS: Hepatocellular carcinoma, MELD, cirrhosis

Cirrhosis is a well-established predisposition for hepatocarcinogenesis. Regardless of etiology, the risk of hepatocellular carcinoma (HCC) is significantly greater in patients with cirrhosis.[1] Currently, HCC is among the most common abdominal malignancies worldwide and has an incidence of nearly 500,000 cases per year. Moreover, the incidence has increased 80% in the United States over the past two decades[2] and is projected to continue to increase because of the increased incidence of hepatitis C.

Partial hepatic resection remains the primary curative treatment option for most patients with HCC.[3–5] Although even extended hepatic resections are well tolerated in patients without underlying liver disease, perioperative risk of partial hepatic resection in patients with cirrhosis is strongly influenced by the degree of hepatocellular compromise and hepatic reserve.[6,7] Consequently, candidacy for hepatic resection of HCC in patients with cirrhosis has been limited. To assess the fitness of patients with cirrhosis

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and HCC for fitness for hepatic resection, numerous clinical, pathologic, and biochemical indices of the patient, the HCC, and the underlying liver disease have been correlated with perioperative outcome and used for selection of patients for operation. The Child-Turcotte-Pugh (CTP) classification specifically was developed and modified to stratify perioperative risk for patients with cirrhosis undergoing various types of operations.[8,9] However, difficulties in accurately quantitating several components of the CTP class have partially rendered this index subjective and of variable reliability.[10–12] In 2001, the Model for End-Stage Liver Disease (MELD) was developed to predict mortality in patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunt (TIPS) based on three reliably reproducible serum measurements.[13] Subsequently, this model has provided a more objective measure of short-term liver disease−related mortality and has been validated repeatedly as a prognostic index for a broad spectrum of clinical conditions in patients with cirrhosis.[14–16] However, the value of MELD has been evaluated in few patients with cirrhosis undergoing hepatic resection for HCC.[17] The primary aim of this study was to determine whether MELD score is a predictor of perioperative mortality in patients with cirrhosis undergoing hepatic resection for HCC. Our secondary aim was to correlate MELD score and other known clinical and pathologic factors to long-term survival.

METHODS

After approval from the Mayo Foundation Institutional Review Board, all patients 18 years of age or older with cirrhosis who underwent hepatic resection for HCC between January 1993 and December 2003 at the Mayo Clinic, Rochester, Minnesota, were identified. Clinical, laboratory, and pathologic data were abstracted from clinical records and tabulated. American Society of Anesthesia score was obtained from operative anesthesia records. HCC was confirmed pathologically in all patients. The simplified AJCC staging system was used for pathologic tumor stage.[18] Three preoperative laboratory tests were used to calculate MELD score: international normalized ratio (INR), serum total bilirubin, and serum creatinine. MELD score was calculated using the following formula: MELD = $9.57 \times \log_e$ (Cr mg/dL) + $3.78 \times \log_e$ (bili mg/dL) + $11.20 \times \log_e$ (INR) + 6.43 (13). Patients who were anticoagulated and those with chronic renal insufficiency requiring hemodialysis were excluded from the study. The validity for grouping patients with MELD score ≤ 8 and MELD score ≥ 9 has been used by others.[19,20] CTP score was calculated based on preoperative prothrombin time, albumin, bilirubin, and clinical findings of ascites or encephalopathy.[9] CTP score was stratified as class A (5−6), B (7−9), or C (10−15). Perioperative mortality was defined as any death occurring during hospitalization for the resection of HCC or any death occurring within 30 days of operation after hospital discharge.

The Pearson χ^2 test was used to compare binary outcome variables. Survival was determined using the Kaplan-Meier method,[21] and the comparisons were performed with log-rank statistics. Multivariate logistic regression and the Cox proportional hazard model were used to identify predictive variables for perioperative mortality and survival, respectively.[22] Any P value <0.05 was considered statistically significant. The c-statistic was used to discriminate the prognostic validities of variables. Concordance (range, 0.0−1.0) is equivalent to the area under the receiver operating characteristic curve. The 30-day operative mortality c-statistics of MELD were calculated.

RESULTS

From 1993 through 2003, 82 patients with cirrhosis underwent partial hepatic resection for HCC at the Mayo Clinic, Rochester, Minnesota. There were 62 men and 20 women with a mean age of 62 years. Mean follow-up was 3.2 years (range, 0.5−9.8 years) and was complete in all patients to death or July 2004. All patients had clinical evidence of cirrhosis and high-grade hepatic fibrosis on biopsy (fibrosis score, 5−6).[6] Etiology of cirrhosis included viral hepatitis in 39 (hepatitus B virus-7; hepatitus C virus-32), alcohol (n = 16), cryptogenic (n = 14), primary biliary cirrhosis (n = 5), autoimmune hepatitis (n = 3), hemochromatosis (n = 3), and primarily sclerosing cholangitis (n = 2). Thirty (37%) patients had symptoms deemed related to the tumor at presentation.

The mean size of HCC was 5.2 cm (range, 1.4−14 cm). Serum α-fetoprotein level was elevated in 56% of patients with a mean level of 2417 (1−53,000) ng/ml among those patients. Simplified AJCC stage was I in 26 patients, II in 21 patients, and III in 35 patients. Overall individual MELD score ranged from 6 to 17. MELD score was ≤ 8 in 37 patients and ≥ 9 in 45 patients. The CTP classification was class A in 80 patients and class B in two patients (Fig. 1). Among the 80 CTP class A patients, MELD score was ≤ 8 in 37 patients and ≥ 9 in 43 patients. Overall minor hepatic resections (<3 segments) were performed in 59 patients, and

major resections (≥3 segments), in 23 patients. Of the 37 patients with MELD ≤8, hepatic resections were major in 8 patients and minor in 29 patients. Of the 45 patients with MELD ≥9, hepatic resections were major in 15 patients and minor in 30 patients. One patient with MELD ≤8 had a major hepatic resection with radiofrequency ablation (RFA) of a contralateral HCC. Two patients with MELD ≤8 and three patients with MELD ≥9 had minor resections with RFA for multicentric HCC. Of the 80 CTP class A patients, hepatic resections were major in 23 patients and minor in 57 patients. Mean operating time was 3 hours (range, 1.1−7.1 hours). Patients in the two MELD groups had similar clinical and pathologic characteristics except that patients with MELD score ≥9 had significantly longer operative times (4.1 versus 3 hours, $P < 0.01$). Only 11 patients underwent ischemic preconditioning by vascular inflow occlusion during resection.

Perioperative Mortality

The overall perioperative mortality rate was 16% (13 patients). Death was secondary to hepatic failure (n = 9), sepsis (n = 2), coagulopathy (n = 1), and myocardial infarction (n = 1). Univariate analysis identified MELD score ≥9 ($P < 0.01$), presence of clinical tumor symptoms ($P < 0.01$), and ASA score ($P < 0.05$) as significantly associated with perioperative mortality. The perioperative mortality for patients with MELD score ≥9 (29%) was significantly greater than that for patients with MELD score ≤8 (0%) ($P < 0.01$). Other patient demographics and pathological factors were not associated with perioperative mortality (Table 1). MELD score did not correlate with CTP class ($P = 0.12$). Multivariate logistic regression identified MELD score ≥9 ($P < 0.01$), the presence of clinical tumor symptoms ($P < 0.01$), and ASA score ($P = 0.046$) as independent predictors of increased perioperative mortality. MELD score ≤8 versus MELD ≥9 did not have significant correlation with other clinicopathologic factors. The c-statistic for prediction of perioperative mortality by MELD score was 0.83 (95% confidence interval, 0.71−0.96).

Survival

The overall 5-year survival rate was 40%, and median survival was 33 months (Fig. 2). MELD score ≥9 ($P < 0.01$), presence of clinic tumor symptoms ($P < 0.01$), absence of tumor capsule ($P < 0.01$), tumor size >5 cm ($P < 0.01$), and HCC grade 3−4 ($P < 0.01$) were univariate predictors of decreased survival (Table 2). Cox regression analysis identified MELD score ≥9 ($P < 0.01$), presence of clinical tumor symptoms ($P < 0.01$), tumor size >5 cm ($P < 0.01$), and high HCC grade ($P = 0.03$) as independent predictors of decreased survival. Survival rate of patients by MELD score (≤8 versus ≥9), tumor size (≤5 cm versus >5 cm), and tumor grade (1−2 versus 3−4) is shown in Figure 3.

To further stratify long-term survival on the basis of preoperatively identifiable noninvasive criteria,

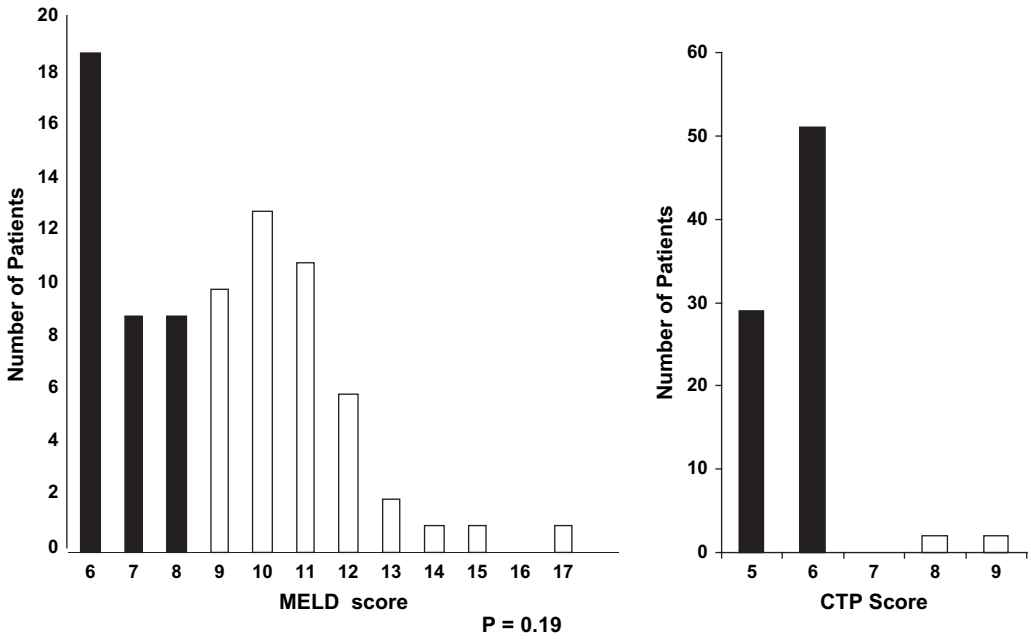

Fig. 1. Distribution of MELD in patients with cirrhosis. MELD = model for end-stage liver disease; CTP = Child-Turcotte-Pugh.

Table 1. Univariate analysis of perioperative mortality in patients with cirrhosis with hepatocellular carcinoma

Variable	No. of patients	Perioperative mortality, n (%)	*P* value
Age (yr)			0.7
≤65	35	5 (14)	
>65	47	8 (17)	
Gender			0.9
Male	62	10 (16)	
Female	20	3 (15)	
Symptoms			<0.01
Present	30	9 (30)	
Absent	52	4 (8)	
Portal hypertension			0.5
Present	11	1 (9)	
Absent	71	12 (17)	
Ascites			0.6
Present	1	13 (16)	
Absent	81	0 (0)	
Etiology of cirrhosis			0.06
Viral	39	6 (15)	
Alcoholic	16	5 (31)	
Cryptogenic	14	2 (14)	
PSC/PBC	7	0 (0)	
Other	6	0 (0)	
CTP class			0.5
A	80	13 (16)	
B	2	0 (0)	
MELD score			<0.01
≤8	37	0 (0)	
≥9	45	13 (29)	
Tumor size (cm)			0.06
≤5	45	4 (9)	
>5	37	9 (24)	
Grade			0.6
1	6	0 (0)	
2	50	9 (18)	
3	24	4 (17)	
4	2	0 (0)	
Stage			0.3
1	26	3 (12)	
2	21	2 (10)	
3	35	8 (23)	
Extent of resection			0.1
Minor	59	7 (12)	
Major	23	6 (26)	
Surgical margin			0.3
Positive	9	2 (22)	
Negative	73	11 (15)	
ASA class			0.046
1	0	0 (0)	
2	14	0 (0)	
3	62	11 (18)	
4	6	2 (33)	
AFP (ng/ml)			0.9
<5	24	4 (17)	
6–500	37	7 (19)	
>501	21	2 (10)	
Albumin (g/dl)			0.3
≤3.5	16	4 (25)	
>3.5	66	9 (14)	
BUN (mg/dl)			0.2
≤40	80	12 (15)	
>40	2	1 (50)	
AST (u/L)			0.2
≤40	18	1 (6)	
>40	64	12 (19)	
Platelet count ($\times 10^9$)			0.1
≤150	34	3 (9)	
>150	48	10 (21)	

CTP = Child-Turcotte-Pugh; MELD = model for end-stage liver disease; ASA = American Society of Anesthesiologists; AFP = alpha-fetoprotein; BUN = blood urea nitrogen; AST = aspartate aminotransferase.

subset analyses combining the significant predictors of MELD score and tumor size were performed (Fig. 4). Subset analysis of the 20 patients with MELD ≤8 and HCC size ≤5 cm showed a 5-year survival rate of 74% versus 29% for the 17 patients with MELD ≤8 and HCC size >5 cm ($P = 0.02$) (Fig. 4, *a*). Subset analysis of the 25 patients with MELD ≥9 and HCC size ≤5 cm showed a 5-year survival rate of 32% versus 14% for the 20 patients with MELD ≥9 and HCC size >5 cm ($P = 0.01$) (Fig. 4, *b*). Subset analysis of patients with MELD ≤8 and low-grade HCC showed a 5-year survival rate of 62% versus 27% for patients with MELD ≤8 and high-grade HCC ($P = 0.02$). Subset analysis of the 31 patients with MELD ≥9 and low-grade HCC showed a 5-year survival of 36% versus 0% for the 14 patients with MELD ≥9 and high-grade HCC ($P = 0.05$).

DISCUSSION

Our findings show that MELD score ≥9 is strongly predictive of increased perioperative mortality in patients with cirrhosis undergoing hepatic resection for HCC. No other clinical and pathologic factors, except for clinical tumor symptoms and ASA score, were predictive of perioperative mortality. We did not find that CTP score, a standard method for rating clinical liver decompensation, either stratified patients into risk categories or correlated with perioperative mortality. MELD score also significantly

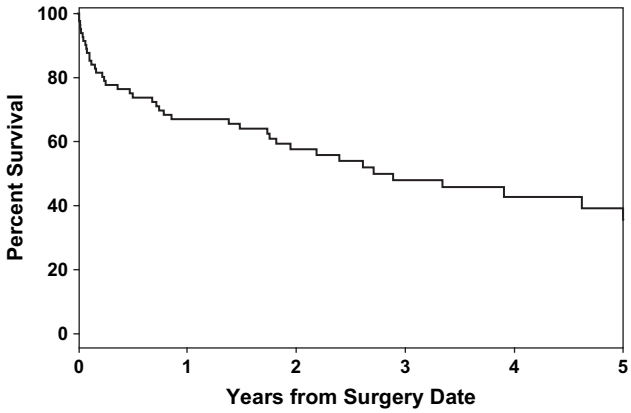

Fig. 2. Overall survival rate of patients with cirrhosis undergoing resection of HCC. HCC = hepatocellular carcinoma.

correlated with long-term survival, as did tumor size and grade. Moreover, subset analysis of survival by MELD score ⩽8 and tumor size ⩽5 cm identified patients with recognizable preoperative characteristics who would potentially derive significant benefit from resection despite the presence of cirrhosis.

The degree of hepatocellular compromise or conversely hepatocellular reserve has been correlated to perioperative mortality in patients with cirrhosis.[7,23–25] Operative risks clearly increase with decreased hepatic functional reserve for patients with cirrhosis undergoing a variety of either intra-abdominal or extra-abdominal operations.[20,26] Indeed, the risk of perioperative mortality increases proportionally to the degree of hepatic functional impairment once chronic liver disease is established. Similar increases in perioperative mortality have been recognized for patients with cirrhosis undergoing hepatic resection.[27] The major dilemma in recommending hepatic resection for HCC in patients with cirrhosis has been balancing the appropriate oncologic resection with tumor-free margins while retaining an adequate functional hepatic remnant. Major resections have acceptable perioperative risks in patients with cirrhosis only if markers of hepatic function are nearly normal. Consequently, minor resections are used most frequently because of impaired hepatic reserve. Ablative approaches have evolved to further reduce procedural risk yet permit potentially curative treatment of HCC. Although numerous methods have been used to evaluate hepatic functional reserve preoperatively, accurate assessment of perioperative mortality risk for patients with cirrhosis undergoing hepatic resection of HCC has been difficult.

The CTP classification is perhaps the most widely used index of disease severity for chronic liver disease. This noninvasive, preoperative risk stratification system was designed to assess perioperative mortality after portosystemic shunts in patients with cirrhosis.[8] Despite attempts to quantitate several of the components of this index to more accurately stratify CTP class, the CTP score has limited discriminatory utility.[10–12] CTP score was not useful in stratifying our patients undergoing resection for HCC. Although not all factors influencing selection of patients for resection herein were retrievable due to the retrospective study design, low CTP class coupled with early clinical stage of HCC was a major factor influencing operative intervention. Unexpectedly, despite the predominance of CTP class A chronic liver disease herein, all perioperative deaths occurred in that category and most were caused by liver failure. This finding suggests that CTP score was not a reliable indicator of hepatic reserve and further confirms that CTP score has limited utility in assessing perioperative mortality risk for patients with cirrhosis undergoing resection for HCC. Although others with larger patient populations have shown that CTP class has correlated with perioperative mortality, the stratification of operative risk based on CTP class remains imprecise. In fact, the range of preoperative mortality reported for patients with CTP class A liver disease undergoing resection of HCC attests to its discriminatory limits.[3–7]

The primary focus of this study was to determine whether MELD score could predict the risk of perioperative mortality in patients with cirrhosis undergoing resection for HCC. MELD was developed as an objective index to noninvasively assess the severity of end-stage liver disease and predict mortality in patients with cirrhosis.[13,14] The predictive value of MELD has been validated repeatedly and has been used increasingly in a variety of clinical settings.[15] We showed that MELD score ⩾9 was an independent predictor of perioperative mortality. Moreover, MELD score ⩽8 identified patients at low risk for perioperative mortality. Patient stratification by MELD score clearly discriminated perioperative risk. This MELD level has been recently used by others in patients with cirrhosis to predict risk for other intra-abdominal operations.[19,20] In fact, the range of MELD scores herein indicated a much broader severity of liver disease than that demonstrated by the CTP system. Our data were inadequate to assess whether MELD scoring of patients with CTP class B liver disease would identify a subgroup of patients at low risk of perioperative mortality.

We assessed other clinical and pathologic factors as predictors of perioperative mortality, but only clinical tumor symptoms and ASA class were significant. Interestingly, the National Surgical Quality Improvement Program criteria did not stratify

Table 2. Univariate analysis of clinicopathologic factors associated with survival after hepatic resection of hepatocellular carcinoma in patients with cirrhosis

Variable	1-Year survival (%)	3-Year survival (%)	5-Year survival (%)	*P* value
Age (yr)				0.3
⩽65	62	53	47	
>65	68	43	24	
Gender				0.6
Male	66	46	32	
Female	64	58	58	
Symptoms				< 0.01
Present	43	17	7	
Absent	80	68	58	
Type of cirrhosis				0.6
Viral	59	52	33	
Alcoholic	55	39	39	
Cryptogenic	79	50	50	
PBC/PSC	86	51	0	
Other	83	42	42	
Child class				0.6
A	66	47	38	
B	50	50	0	
MELD score				< 0.01
⩽8	89	63	51	
⩾9	46	34	23	
Grade				< 0.01
1	100	100	100	
2	72	60	41	
3	51	30	20	
4	0	0	0	
Tumor size (cm)				< 0.01
⩽5	79	68	38	
>5	43	29	23	
Stage				0.6
1	72	66	60	
2	84	36	18	
3	50	39	29	
Extent of resection				0.06
Minor	73	53	37	
Major	45	30	30	
Surgical margin				0.8
Positive	43	43	43	
Negative	66	52	35	
Extent of disease				0.5
Unilobar	65	47	33	
Multilobar	65	35	35	
Focus of disease				0.5
Unifocal	60	51	41	
Multifocal	75	29	14	
Vascular invasion				0.09
Yes	57	25	17	
No	68	52	40	
Capsule				<0.01
Yes	73	53	43	
No	37	14	0	
AFP level (ng/ml)				0.6
<5	66	41	27	
6–500	67	45	20	
>501	64	57	50	

PBC = primary biliary cirrhosis; PSC = primary sclerosing cholangitis; MELD = model for end-stage liver disease; AFP = alpha-fetoprotein.

operative risk for patients with cirrhosis undergoing resection of HCC.[28] This lack of correlation was likely related to the fact that all patients underwent an elective operation and thrombocytopenia from secondary hypersplenism was common in these patients. Moreover, our perioperative mortality was likely due, in part, to referral of patients with cirrhosis and other comorbidity to a tertiary care center.

The clinical staging of HCC has become increasingly important to assess prognosis and guide treatment intervention. Both pathologic tumor stage and hepatic functional reserve have been recognized as key factors that affect outcome for patients with HCC. Long-term survival of patients in this study was associated with an MELD score ⩽8, HCC size ⩽5 cm, and low HCC histologic grade. The association of HCC size ⩽5 cm with prolonged survival is consistent with the revised and simplified AJCC staging system for HCC and prognostic histologic indicators.[18] Similar predictors of patient survival have been demonstrated by others in both patients undergoing partial hepatic resection and transplantation for HCC.[3–5,29–32] Other components of the current AJCC staging system and overall TNM stage did not correlate with survival and, in part, may be due to the fact that the fibrosis score of all patients herein was 5 or 6. Interestingly, MELD score ⩽8 was also significantly associated with long-term survival. Recently, another study identified MELD score <10 as an independent predictor of survival and showed that MELD was a more reliable predictor of survival than CTP class but only 12 patients underwent resection.[17] Three current staging systems for HCC, Barcelona Clinic Liver Cancer (BCLC) staging system,[33] Cancer of the Liver Italian Program (CLIP),[34] and Japan Integrated Staging (JIS),[35] use CTP class as a primary component for determining prognosis. Although each staging system is predictive of survival for patients with HCC,

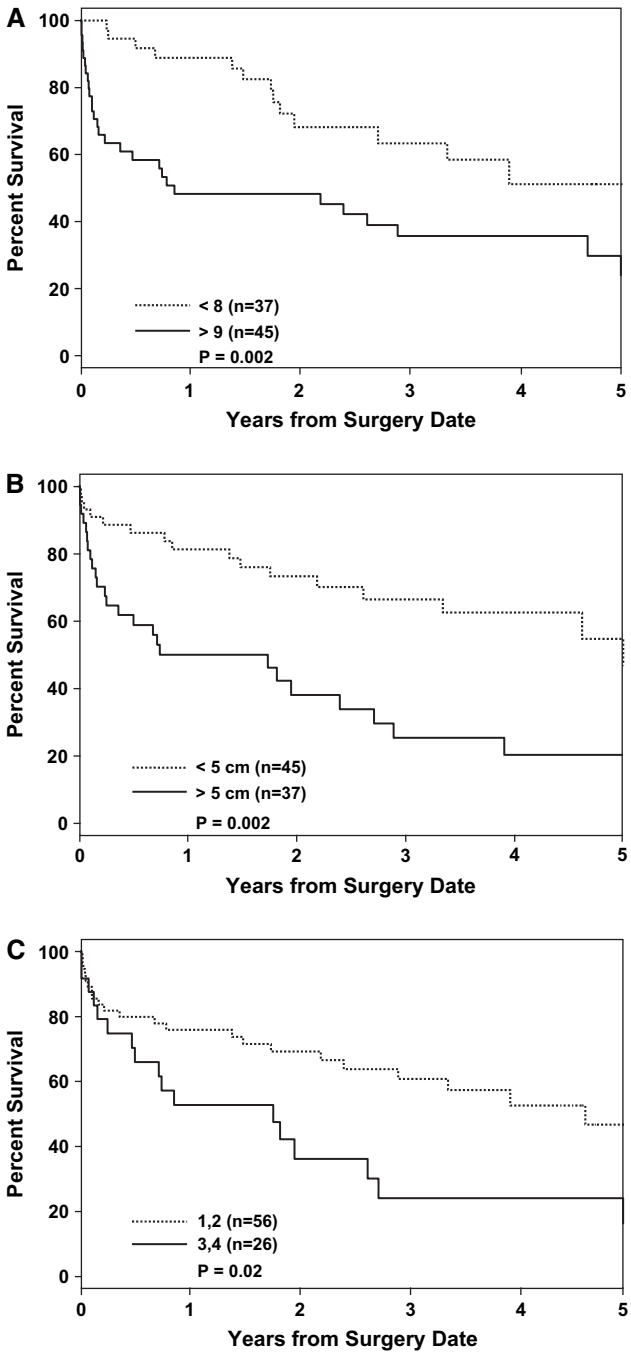

Fig. 3. Survival rate of patients with cirrhosis undergoing resection of HCC by independent predictors of survival. (**A**) MELD ⩽8 versus MELD ⩾9. (**B**) Size ⩽5 cm versus size >5 cm. (**C**) Low-grade HCC (grade 1−2) versus high-grade HCC (grade 3−4). HCC = hepatocellular carcinoma; MELD = model for end-stage liver disease.

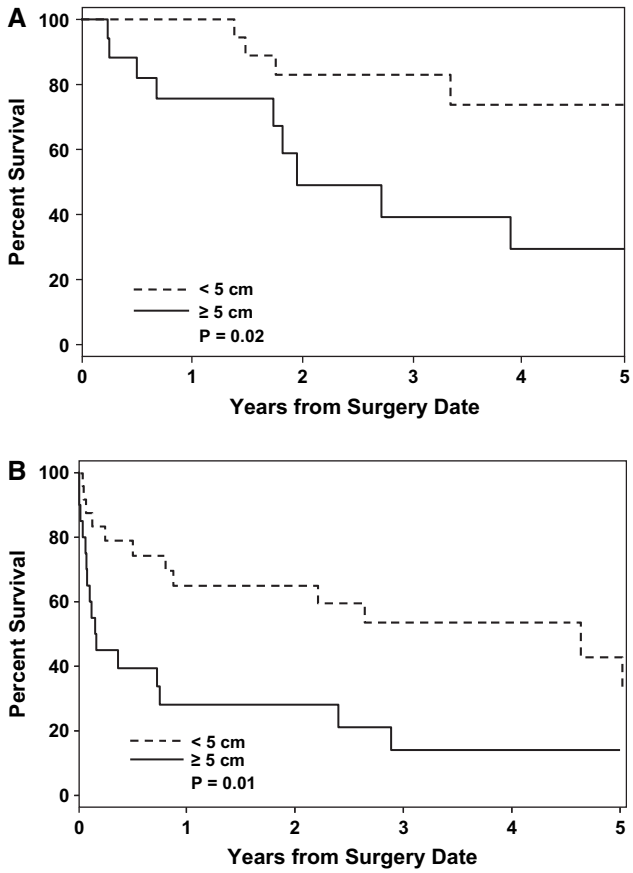

Fig. 4. Survival rate of patients with cirrhosis undergoing resection of HCC by combined MELD and tumor size. (**A**) MELD ⩽8 and HCC size ⩽5 cm versus HCC size >5 cm. (**B**) MELD ⩾9 and HCC size ⩽5 cm versus HCC size >5 cm. HCC = hepatocellular carcinoma; MELD = model for end-stage liver disease.

only CLIP has been externally validated.[36] There is no consensus on which staging system is best.[37] Because MELD correlated with both perioperative mortality and long-term survival, the use of MELD score, instead of CTP score, to increase the discriminatory ability of these staging systems is an attractive area for further study.

Finally, our study showed that patients with an MELD score ⩽8 and HCC ⩽5 cm in size had a prolonged survival. Despite the small size of this subset, these findings support the observation of others[3,5,31,38,39] that there are subgroups of patients with cirrhosis and HCC in whom partial hepatic resection can result in excellent long-term survival. The fact that identifiable subgroups of patients with cirrhosis benefit from resection and may be comparable to outcome reported after liver transplantation further supports multi-institutional study to better define selection criteria. Our findings require further confirmation that MELD score can be used preoperatively as a selection criterion for partial hepatic resection. The rising incidence of HCC warrants continued investigation of selection criteria for partial hepatic resection for HCC in patients with

cirrhosis either as a definitive treatment or as a bridge to liver transplantation.

In conclusion, our study showed that MELD score was predictive of both perioperative mortality and long-term survival for patients with cirrhosis undergoing resection of HCC. These findings suggest that MELD score $\leqslant 8$ should be a selection criterion for hepatic resection of HCC in patients with cirrhosis and, conversely, MELD score $\geqslant 9$ should prompt consideration of other treatment options for patients with cirrhosis and HCC. Our results should be further evaluated in larger, independent study populations to assess the discriminatory value of MELD score and other patient, tumor, and interventional factors on early and late outcomes.

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Discussion

Dr. Kevin Behrns (Chapel Hill, NC): Congratulations, Dr. Teh and colleagues, for a superb and timely study examining the utility of the model of end-stage liver disease or MELD scoring system in cirrhotic patients undergoing hepatic resection for HCC. You retrospectively showed that the MELD was not only prognostic for perioperative mortality, but it also predicted survival. In addition, in these 82 patients, MELD provided finer discrimination of mortality than the Child-Pugh score.

In this study you chose to dichotomize the MELD score in the patients with a score of less than or equal to 8, these patients had a mortality of 0%, versus patients with a MELD score of 9 or greater with a mortality of 29%. However, your MELD scores ranged from 6 to 17. In addition, with this analysis you showed that the C-statistic was quite good at 0.83. Did you think about or analyze the data in three separate MELD score classifications, for instance, less than or equal to 8, 9 to 11 or 12, and then greater than 12? This may be quite important because what we want to do is avoid the danger of denying a patient a hepatic resection who might have a slightly higher but acceptable mortality risk.

Finally, this study provides further evidence that hepatic function is a primary determinant of survival from HCC. A recent study published in Hepatology found that the Barcelona Clinic Liver Cancer system provided the best prediction of survival, but this system has been criticized for its lack of discrimination of patients with intermediate-type HCC. Given your data, how would you recommend that these findings be integrated into the Barcelona system or to the other six complex systems that are used for staging HCC?

Dr. Teh: Thank you, Dr. Behrns. The first question is, can we offer surgery to more patients with an acceptable surgical risk? We did look at our data and analyzed them in many ways. Subset analysis of patients with MELD score greater than 9 was performed. They are further divided into n group with MELD score of 9, 10, and 11. The other group has a MELD score of 12. The perioperative mortality is about 23% and 33%, respectively. This trend is observed, but the *P* value is not significant. This is likely due to the sample size. There are many things we are trying to tweak, but we still cannot avoid a pretty high mortality rate after you pass the MELD score beyond 9. This is the first paper to try and correlate the MELD score with the patient who had hepatic resection for HCC. We hope that this paper will stimulate the liver community to do prospective or a larger retrospective study to see what is the exact cutoff point that is best for the patient.

The second question regards how accurate and reliable are the currently available prognostic systems for HCC. There are at least seven systems so far, and in some way this is telling us that none of them is perfect. Every patient with cirrhosis is different, every tumor is different, and putting them together, the complexity increases. I think we are unlikely to get a perfect scoring or prognosis system. An ideal system should have several vital qualities—reflective, quantitative, reproducible, and the result of a prospective multicenter study.

For the reflectiveness, a system needs to take the following criteria into consideration—the tumor factors, host factors, and treatment factors. Tumor factors and treatment factors have been studied extensively, more recently, by Dr. Vauthey et al., where simplified staging systems for HCC were established. As for the host factors, we believe that the hepatocellular reserve for a particular patient with cirrhosis is very important, and we believe MELD is reflective of the hepatocellular reserve. Therefore, a MELD score–based system might be superior to a non-MELD system. A future multicenter prospective study is needed to derive a MELD score–based prognostic system.

Molecular Predictors of Lymph Node Metastasis in Colon Cancer: Increased Risk With Decreased Thymidylate Synthase Expression

Avo Artinyan, M.D., Rahila Essani, M.D., Jeffrey Lake, M.D., Andreas M. Kaiser, M.D., Peter Vukasin, M.D., Peter Danenberg, Ph.D., Kathleen Danenberg, Ph.D., Robert Haile, M.D., Robert W. Beart, Jr., M.D.

TNM staging in colon cancer has several limitations. Prognostic molecular markers are now being developed to address these limitations. The aim of this study was to identify a combination of genes and markers whose expression is predictive of nodal status and outcome in colon cancer. The expression of 12 genetic markers were examined in 66 node-positive and 65 node-negative T3 colon cancers. Gene expression was quantified using real-time polymerase chain reaction. Microsatellite instability status was available through the registry. Association with lymph node status was examined using univariate and multivariate logistic regression. Thymidylate synthase expression was statistically significantly associated with lymph node status (odds ratio 0.36; 95% confidence interval: 0.16–0.81). Microsatellite instability and the other genes were not associated with nodal status. Multiple logistic regression did not identify a significant multivariate predictive model. Decreased expression of thymidylate synthase is associated with a higher risk of lymph node metastasis in patients with T3 colon cancers. Microsatellite instability and the expression of other genes are not predictive of nodal status in this population. Thymidylate synthase gene expression may help identify patients at greater risk for progression of disease. (J GASTROINTEST SURG 2005;9:1216–1221)

KEY WORDS: Molecular markers, colon cancer, lymph node metastasis, thymidylate synthase

Prognostic information in colon cancer is largely derived from postoperative data detailing the anatomic extent of disease. Such pathologic factors form the basis of the current TNM staging system, which has been in universal use since 1987.[1] There are several problems associated with the current staging system for colon cancer. Although there is general correlation between TNM stage and prognosis, there is often significant variability in tumor behavior and individual patient outcome that is unaccounted for by pathologic factors alone.[2,3] In addition, TNM staging in colon cancer provides an estimate of prognosis only after definitive operation is complete and, as a result, greatly limits the ability of the physician to tailor the extent and sequence of treatment to the specific pattern of disease.

Over the past 10 to 15 years, there has been a growing trend toward the identification of predictive markers at the molecular and genetic level. The use of such molecular markers has several advantages. Molecular markers offer the potential for a significant improvement in prognostic accuracy. Furthermore, the small amount of tissue required for genetic analysis could make it possible to assess disease prognosis preoperatively from a colonoscopic biopsy specimen.

Several molecular markers have already been suggested to be predictive of outcome in colon cancer. These include chromosomal deletions,[4,5]

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microsatellite instability (MSI),[6] several gene mutations, and other measurable genetic alterations. However, most of these markers have been studied in a univariate manner, using nonuniform methodology, and without statistical adjustment for other potentially significant prognostic markers.

The aim of our study was to examine several of these well-studied molecular markers in a multivariate manner with the goal of identifying a combination of genes/markers whose pattern of expression would be predictive of outcome measures in colon cancer. In this initial study, we chose lymph node metastasis as the outcome measure with the intent of incorporating survival data once they became available at a later date.

MATERIALS AND METHODS

Tissue Samples and Study Population

Formalin-fixed paraffin-embedded archived specimens of primary colon cancer were acquired from the Colon Cancer Family Registry (CCFR), which collects tissue from hospitals throughout Southern California. The study population consisted of 131 patients who had undergone resection of T3 colon cancers divided into two cohorts: (1) 66 patients with node-positive disease (T3N+) and (2) 65 patients with node-negative disease (T3N0). Lymph nodes were uniformly examined using hematoxylin-eosin staining based on protocols in place at the individual hospitals participating in the CCFR. Lymph node status (positive or negative) was determined by reviewing the pathology reports maintained by the registry, which contained specific data regarding lymph node examination. Informed consent for participation in this and other studies was obtained at the time of recruitment into the CCFR.

Gene/Marker Selection

The literature was critically reviewed to identify molecular genetic markers that had been shown to be associated with colon cancer outcome. Measures of outcome included lymph node and distant metastases, as well as overall survival. A total of 21 genes and markers were found to have significant and repeatedly demonstrated associations with colon cancer progression. The markers were ranked on the basis of strength of association, giving priority to those that had been studied in a multivariate manner with larger sample sizes. Twelve genes and markers were selected for study on the basis of rank and the availability of well-designed primers and probes for real-time polymerase chain reaction within our laboratory. Table 1 lists the 12 genes and markers selected for study.

Table 1. List of genes selected for study with selected references

Gene/Marker
Bcl-2[20,21,22]
Cox-2[23]
Cyclin E[24]
DPD[25]
EGF-R[26]
MDR-1[27,28]
MMP-9[29,30]
P27[31,32]
Thymidylate synthase (TS)[12–17]
VEGF[33,34]
Thymidine phosphorylase (TP/PD-ECGF)[35]
Microsatellite instability (MSI)[3,6,36]

VEGF = vascular endothelial growth factor.

Measurement of Gene Expression

Microdissection. Microdissection was performed on all samples to limit the heterogeneity of cell types in a given specimen. A hematoxylin-eosin–stained section was initially used to identify the boundaries of a given tumor. Sample sections were then stained with nuclear fast red. Cancer cells were excised using scalpel manual microdissection and laser-capture microdissection, when necessary, to attain 90% or greater homogeneity. The excised specimens were submitted for mRNA extraction.

mRNA extraction and quantitation. The extraction of RNA from formalin-fixed paraffin-embedded tissue was performed using a proprietary method of the University of Southern California and Response Genetics Incorporated (U.S. patents 6,248,535 and 6,613, 518). mRNA was isolated using the QuickPrep micro-mRNA isolation kit (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) according to the manufacturer's instructions and converted to cDNA as previously described.[7]

cDNA of the target genes and an internal reference gene (β-actin) were then quantitated using a fluorescence based real-time detection method (ABI PRISM 7700 Sequence Detection System [TaqMan], Perkin Elmer Applied Biosystems, Foster City, CA). The mRNA expression values of the target genes were calculated relative to the β-actin.

Data Gathering and Statistical Analysis

Demographic and pathologic data were acquired from the clinical database maintained by the CCFR. Variables recorded included age (at time of surgery), race (white, African-American, Hispanic, Asian, other), gender, tumor location, MSI, and lymph node status. Information regarding MSI status had

been determined in accordance with definitions of the National Cancer Institute and was made available through the registry.

By using pilot data, we estimated greater than 80% power to detect significant associations between lymph node status and gene expression values (expressed as categoric variables) at a 5% significance level. For analysis of continuous measures of gene expression, we estimated 80% power to detect a 1.7-fold increase or decrease in odds of nodal involvement for a change of 1 standard deviation in gene expression.

The association of lymph node status with gene expression and MSI status was examined using univariate and multivariate logistic regression. Gene expression levels were categorized into "high" and "low" groups by dichotomizing at the sample median values. Univariate and multivariate relationships were adjusted for age (expressed as a continuous variable) and gender. Effect modification by race and tumor location was evaluated by incorporating appropriate interaction terms. Results are reported as adjusted odds ratios with corresponding 95% confidence intervals.

The statistical analysis was performed using STATA (version 8.0; Stata Corporation, College Station, TX).

Table 2. Demographic and descriptive characteristics of study population

Variable	No. of patients (%) (n = 131)
Age (yr) (mean ± SD)	57.0 ± 12.6
Gender	
Male	63 (48.1)
Female	66 (50.4)
Unknown	2 (1.5)
Race	
White	87 (66)
African-American	10 (7.6)
Hispanic	16 (12.2)
Asian	3 (2.3)
Other	8 (6.1)
Unknown	7 (5.3)
Tumor location	
Cecum	20 (15.3)
Ascending	33 (25.2)
Tranverse	11 (8.4)
Descending	7 (5.3)
Sigmoid	27 (20.6)
Rectum	21 (16.0)
Unknown	12 (9.2)

SD = standard deviation.

RESULTS

Demographic and descriptive data for the study population are summarized in Table 2. Of note, the mean age of the population was 57.0 ± 12.6 years. Men comprised 49% of the sample. The majority of patients were of white/Caucasian descent (66%). Tumors were nearly equally divided between right-sided lesions (cecum and ascending colon: 41%) and left-sided lesions (descending, sigmoid, and rectum: 42%) with a significantly smaller proportion of transverse colonic tumors (5%). Data regarding tumor location were missing for 12 patients (9%).

Association of Lymph Node Status With Gene Expression

The results of univariate logistic regression are shown in Table 3. A statistically significant association was noted between lymph node status and the expression of thymidylate synthase (TS) (odds ratio 0.36, 95% confidence interval 0.16–0.81). The relationships between lymph node status and the other 10 genes did not reach statistical significance. Similarly, no significant association was noted between nodal status and MSI status (Table 3). The results did not vary by race or tumor location (data not shown). On the basis of the above result, individuals with low TS expression are approximately threefold more likely to have lymph node metastases than those with high expression values.

Multivariate logistic regression demonstrated no additional significant associations in a model combining TS with each of the other 10 genes and MSI (all P values $> .10$). Predictive multivariate model building techniques using stepwise, forward, and backward approaches did not identify any significant predictive models beyond a univariate model containing TS (data not shown).

DISCUSSION

The current TNM staging system for colorectal cancer has been useful in predicting outcome after definitive resection, but the shortcomings of postoperative pathologic staging have become evident. There is a great deal of heterogeneity among individual tumors that is unaccounted for by pathologic stage. Attempts have been made to better predict tumor behavior by identifying novel biologic prognostic factors. Histologic markers that have been shown to have potential value in this regard include tumor grade,[8] blood and lymphatic vessel invasion,[8,9] perineural invasion,[10] and peritumoral lymphocytic

Table 3. Results of univariate logistic regression with lymph node status versus gene expression

	TNM stage		
Gene	**T3N+**	**T3N0**	**OR (95% CI)**
Bc1-2			
High	23 (49%)	24 (51%)	1.11
Low	23 (46%)	27 (54%)	(0.49, 2.53)
Cox-2			
High	22 (39%)	35 (61%)	0.61
Low	27 (52%)	25 (48%)	(0.28, 1.33)
Cyclin E			
High	28 (47%)	31 (53%)	1.05
Low	28 (47%)	31 (53%)	(0.50, 2.19)
DPD			
High	28 (52%)	26 (48%)	1.56
Low	24 (41%)	35 (59%)	(0.73, 3.33)
EGF-R			
High	33 (55%)	27 (45%)	1.64
Low	25 (42%)	34 (58%)	(0.79, 4.42)
MDR-1			
High	26 (48%)	28 (52%)	0.97
Low	27 (49%)	28 (51%)	(0.45, 2.07)
MMP-9			
High	17 (46%)	20 (54%)	1.43
Low	13 (37%)	22 (63%)	(0.54, 3.77)
P27			
High	17 (47%)	19 (53%)	1.17
Low	15 (41%)	22 (59%)	(0.63, 4.64)
Thymidylate synthase			
High	22 (39%)	35 (61%)	0.36
Low	34 (59%)	24 (41%)	(0.16, 0.81)
VEGF			
High	29 (48%)	31 (52%)	0.99
Low	29 (48%)	31 (52%)	(0.48, 2.05)
Thymidine phosphorylase			
High	27 (48%)	29 (52%)	0.91
Low	29 (51%)	28 (49%)	(0.43, 1.93)
MSI status			
MSS	44 (52%)	40 (48%)	1.0 (Reference)
MSI-L	12 (55%)	10 (45%)	2.21 (0.66, 7.37)
MSI-H	10 (40%)	15 (60%)	1.93 (0.75, 5.00)

OR = odds ratio; CI = confidence interval; VEGF = vascular endothelial growth factor; MSI = microsatellite instability.
Gene expression values are dichotomized at the sample median. Odds ratios are age and gender-adjusted and are presented with corresponding 95% confidence intervals.

infiltrate.[11] However, none of these variables have been consistently shown to improve on the prognostic ability of TNM staging.

Molecular markers at the genetic level offer the potential for significant improvement in prognostic accuracy, and many such markers have already been identified. However, most previous studies have examined only one or a small number of genes together. The current study is the only one we are aware of, to date, that has examined the association of a large number of genes in combination with outcome measures in colon cancer.

The present study identified TS expression as a potential predictor of lymph node status in colon cancer, with lower expression values predicting a threefold increase in the probability of lymph node metastasis. TS catalyzes the conversion of dUMP to dTMP, a rate-limiting step in DNA synthesis.[12] The enzyme serves as a target of the fluoropyrimidines commonly used for the systemic treatment of colon cancer, 5-fluorouracil and 5'-fluoro-2'-deoxyuridine.[13]

Previous studies examining the prognostic role of TS expression have reached somewhat conflicting results. There is some suggestion that cancers with higher levels of TS expression are associated with poorer survival and a poorer response to adjuvant chemotherapy.[14–17] There are, however, a number of studies that have shown improved prognosis with higher TS expression, in agreement with our data.[18,19] The reasons for these conflicting findings are unclear but may reflect fundamental differences in tumor behavior and biochemistry within different subsets of patients.

The lack of significant associations with other markers also conflicts with the literature. There are several potential explanations for this discrepancy. Previous studies for the most part have not used microdissection in their analysis of expression. Therefore it is conceivable that some of the significant findings reported in the past may have been related in part to characteristics of peritumoral cells rather than the cancer cells themselves. Most previous studies have also used immunohistochemistry for the quantitation of gene expression. This technique is more qualitative than measurement of RNA levels and measures gene expression at the protein level. As a result, previous findings may suffer from subjectivity or may reflect features not identifiable at the mRNA level.

Our initial goal was to identify a predictive model incorporating several genes, with the hope of demonstrating significant association with overall survival in a future study. A model of this nature would facilitate preoperative staging using a small amount of tissue acquired from a biopsy specimen and would help the physician individualize the extent of treatment based on the preoperatively predicted aggressiveness of an individual tumor. Given the lack of significant associations beyond TS, we were unable to identify a multivariate predictive model as we had initially hoped. The analysis of additional genes, including the deleted in colorectal cancer and tissue factor genes, is currently under way.

The identification of significant associations among this new set of genes may still allow the description of such a model within this study population.

CONCLUSION

Our study demonstrates a significant association between TS expression and lymph node metastasis, with lower gene expression levels predicting a threefold higher probability of nodal disease. Additional studies including those with survival data are warranted and currently in progress to validate these results.

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Discussion

Dr. James Fleshman (St. Louis, MO): My congratulations to the authors on a well thought out study and an excellent presentations. I believe, as they do, that we are at a critical point in understanding colon cancer behavior. It is important to focus on molecular markers of prognosis because of the inaccuracies of the pathologic staging process as it currently exists, the possibility of resecting an aggressive tumor early in its course, which yields a favorable pathologic stage but after already metastasizing on a cellular level, and the possibility for incisionless surgery with no need for tissue extraction as minimally invasive techniques improve.

I have four questions for the authors. Were these patients with familial cancer from your registry and can these data be extrapolated to sporadic cancer?

Are you able to classify the thymidylate synthase levels by the number of gene repeats? We know that there are zero, one, or two repeats and that this may influence the response to 5FU and may also select for prognosis based on this alone.

Number three, how long were your samples stored and can that affect your outcomes? Would fresh tissue extraction of RNA or DNA be better?

And number four, how many of the markers that you tested reflect cancer development and how many reflect the potential for metastasis, because this may more greatly influence lymphatic spread and survival?

I want to thank the authors again for allowing me to review their article before today and to the Society for the opportunity to comment. Thank you.

Dr. Artinyan: As far as the registry itself, there are a significant number of familial cancers, and it is possible that there is a different mechanism. It has been shown that familial cancers have a higher degree of microsatellite instability, they are more likely to have mutations in some of the familial cancer genes, the nonpolyposis genes, and their mechanism may be slightly different. Can we extrapolate it to spontaneous cancers? As far as gene expression, probably, but I can't give you a definite answer.

As far as the thymidylate synthase gene itself, we did not study it at the DNA level, which is where such repeats occur. We only looked at the quantity of transcribed mRNA. The actual mRNA product should be, qualitatively, the same regardless of how many nontranscribed repeats appear in the DNA.

Storage is always an issue, and this has been one of the difficulties with formalin-fixed specimens, which were used in this case. It has been difficult in the past to extract mRNA in a quantitative manner from formalin-fixed tissue. For this reason, most previous studies examining expression at the mRNA level have been performed with frozen tissue from blocks of tumor. However, a lab within our area, the Response Genetics Lab, has come up with and patented a method of quantitatively extracting mRNA from formalin specimens, and the technique has been validated using a number of trials. We believe and have evidence that the technique is quantitative and reproducible. But, yes, the method of extraction can affect the quantitative nature of the experiment.

Several genes have been shown to be involved in the initiation of cancer and others in the progression of cancer. When we reviewed the literature, we looked specifically for genes predictive of outcome measures once the cancer had already been diagnosed. In this respect, we didn't address the issue of initiation. As far as outcome measures, a number of these genes have been shown to be associated with increased metastatic potential to lymph nodes, distant sites, or both and other genes with end points such as increased or decreased overall and/or disease-free survival. The outcome measure in this case was lymph node metastasis. It is clear that lymph node metastasis is associated with survival, but to what degree we can make the jump from a marker's association with lymph node status to its potential association with survival is not clear.

Endoscopic Ultrasonography and Magnetic Resonance in Preoperative Staging of Rectal Cancer: Comparison With Histologic Findings

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The development of new surgical techniques and use of neoadjuvant therapy have increased the need for accurate preoperative staging of rectal cancer. We compared the ability of endoscopic ultrasonography (EUS) and two magnetic resonance imaging (MRI) coils to locally stage rectal carcinoma before surgery. Forty-nine patients with histologically proven rectal carcinoma were T and N staged by EUS and either body coil MRI or phased-array coil MRI. After radical surgery, the preoperative findings were compared with histologic findings on the surgical specimen. For T stage, accuracies were 70% for EUS, 43% for body coil MRI, and 71% for phased-array coil MRI. For N stage, accuracies were 63% for EUS, 64% for body coil MRI, and 76% for phased-array coil MRI. For T stage, EUS had the best sensitivity (80%) and the same specificity (67%) as phased-array coil MRI. For N stage, phased-array coil MRI had the best sensitivity (63%) and the same specificity (80%) as the other methods. EUS and phased-array coil MRI provided similar results for assessing T stage. No method provided satisfactory assessments of local N stage, although phased-array coil MRI was marginally better in assessing this important parameter. Although none of the results differed significantly, phased-array coil MRI seems to be the best single method for the preoperative staging of rectal cancer. (J GASTROINTEST SURG 2005;9:1222–1228)

KEY WORDS: Rectal cancer, preoperative staging, endoscopic ultrasonography, magnetic resonance imaging

Therapeutic approaches and clinical outcomes in rectal cancer are strongly influenced by preoperative staging.[1] In recent years there has been increased use of sphincter-saving procedures for low rectal cancers such as low anterior resection with coloanal anastomosis and local excision for lesions confined to the rectal wall,[2,3] and these procedures are progressively replacing abdominoperineal resection in patients with midrectal and low rectal cancer.[4–6] Small cancers confined to the mucosa or submucosa can be excised locally by transanal endoscopic microsurgery and conventional surgery.[7,8] In addition, short preoperative irradiation and prolonged preoperative chemoradiotherapy have been tried in selected patients, and neoadjuvant chemoradiotherapy can now be recommended for advanced rectal cancer to downstage the lesion.[9–11] The importance of the circumferential resection margins after total mesorectal excision has been increasingly acknowledged.[12,13]

Assessment of the invasion depth (T stage) and lymph node involvement (N stage) are vital components of preoperative staging. The three main techniques currently used are computed tomography (CT), endoscopic ultrasonography (EUS), and

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magnetic resonance imaging (MRI) using various coils. CT was the first technique to be introduced; it is useful for revealing advanced disease and distant metastases, but not as good at assessing the extent of wall invasion or identifying microscopic involvement of perirectal fat and metastatic lymph nodes.[14–17] EUS can assess the extent of wall invasion, provide good estimates of longitudinal and circumferential extent, and visualize perirectal lymph nodes (to direct fine-needle biopsy); however, liver metastases and distant nodal involvement are not revealed because they are outside the range of the probe. Because of its high soft tissue contrast, MRI can assess wall penetration and identify perirectal nodal involvement and distant metastases.

The utility of EUS and MRI for staging rectal cancer has been amply demonstrated.[18–28] Two reviews, one with a meta-analysis, have compared the two techniques.[29,30] The choice of procedure is also influenced by the cost and availability of radiologists and endoscopists experienced in assessing rectal malignancies.[31]

The present study comparatively assesses the ability of EUS, body coil MRI (BC-MRI), and phased-array coil MRI (PA-MRI) to preoperatively stage rectal carcinoma, using the histologic findings on the surgical specimen as the gold standard.

PATIENTS AND METHODS

Forty-nine consecutive patients (34 men and 15 women), with a mean age of 64 years (range 30–85 years), who had resectable rectal carcinoma were enrolled from January 1999 to January 2004. Carcinoma was demonstrated by histologic analysis of endoscopic biopsy samples, and the rectal site was considered as extending from the anal verge to the rectosigmoid junction. Patients undergoing emergency surgery or who underwent previous chemotherapy or radiotherapy were excluded. Twenty cancers (40.8%) were in the upper third of the rectum, 17 cancers (34.7%) were in the middle third of the rectum, and 12 cancers (24.5%) were in the lower third of the rectum. Forty-three patients (87.8%) underwent a low anterior resection, and six patients (12.2%) underwent an abdominoperineal resection. The surgical technique included high vascular ligation of the inferior mesenteric vessels, radical lymphadenectomy, and total mesorectal excision.[12] Laparoscopy was used to perform anterior resection in 28 (57.1% of total) cases. For abdominoperineal resection, the perineal cavity was closed and a drainage tube was left in the perineal space for the first few postoperative days. The surgical specimen was subjected to full pathologic examination and staged (TNM) according to the guidelines of the American Joint Committee on Cancer.[32]

Endoscopic Ultrasonography

EUS examinations were performed using a Pentax FG36UX ultrasound scanner (Pentax Precision Instruments, New York, NY), with a flexible convex probe containing a 7.5 MHz transducer. Examinations were conducted with the patient in a left lateral decubitus position. The tip of the transducer was covered with a latex balloon filled with degassed water. The probe was introduced past the level of the tumor and then withdrawn slowly. The extent of wall invasion was assessed according to the criteria for dividing the rectal wall described by Hildebrandt and Feifel:[33] Stage uT1 tumors were confined to the mucosa and submucosa; uT2 tumors were confined to the rectal wall; uT3 tumors penetrated the rectal wall and invaded perirectal fat; and uT4 tumors invaded surrounding organs.

The sonographic criteria for identifying involved lymph nodes were as follows: size greater than 5 mm, hypoechoic, sharply demarcated borders, and spherical (rather than ovoid or flat) volume. All endoscopic examinations were performed by a single endoscopist (A.M.).

Magnetic Resonance Imaging

MRI of the pelvis was performed with a 1.0 T unit (Philips Gyroscan, General Electric Medical Systems, Milwaukee, WI). The first 28 patients, enrolled up to September 2002, were studied using a linear body coil supplied with the MRI machine. The next 21 patients were examined with a multichannel PA system of four coils (two anterior and two posterior). The principal difference between a linear body coil and PA body coil system is that the latter consists of several small coils each producing separate images that are combined by dedicated software to produce the high-resolution images. The advantage of a PA system is that it achieves the sensitivity of a small local surface coil, but over a larger field of view. All patients gave informed consent for the examination. An enema was given the day before the examination. Images were acquired with the patient prone, after intravenous injection of 1 mL Buscopan (Boehringer-Ingelheim, Milan, Italy) and introduction of a small Foley catheter into the rectum that was inflated with 300 mL of room air. A single experienced radiologist (G.P.), blinded to the EUS results, acquired and assessed the images. Sagittal T2 turbo spin-echo scans were first obtained to locate the tumor. T2 turbo spin-echo scans, some

with fat suppression, and T1 spin-echo scans paratransverse to the tumor were then obtained. After contrast injection, the examination was completed with sagittal and paratransverse T1 scans. Coronal scans were only obtained to examine the ischiorectal fossa and perianal lesions. T1 scans, especially after contrast, were used to assess the perirectal fat, lymph nodes, and external rectal contour. T2 scans were more useful for evaluating the rectal wall layers and revealing involvement of other pelvic structures. Lesions with smooth margins confined to the rectal wall were considered localized to the rectum (T1–T2). Extension of low signal soft tissue into high-signal perirectal fat and disruption of the outer margins of the rectal wall were staged as T3. Adjacent organ involvement was classified as T4. Lymph nodes greater than 0.5 cm were considered pathologic.

Statistical Methods

The sensitivity, specificity, positive predictive value, and negative predictive value of each staging technique were calculated with reference to the histologic findings. The 95% confidence interval (CI) of the accuracy of the estimates of the T and N stages were calculated by the Wilson Score Method.[34] The data were analyzed using the Stata 8.0 statistical package (StataCorp. LP, College Station, TX).

RESULTS

A total of 931 lymph nodes were examined pathologically, an average of 19 per patient (range 1–53), 89 (9.5%) of which were positive. The mean time from preoperative staging to surgery was 7.5 days. The analysis was performed on patients with T1, T2, and T3 tumors. Patients with T4 tumors were excluded because they received neoadjuvant therapy. Accuracies of T staging were 70% for EUS, 43% for BC-MRI, and 71% for PA-MRI (Table 1). In all cases the 95% CIs overlapped, indicating no significant difference between the techniques in terms of accuracy (Table 1). The accuracies of N staging were 63% for EUS, 64% for BC-MRI, and 76% for PA-MRI (Table 1). Again, there were no significant differences between these figures. The sensitivities and specificities of the three methods for T and N staging are shown in Table 2, and the positive and negative predictive values are presented in Table 3. Overstaging and understaging in assessment of T and N are shown in Table 4.

Table 1. Accuracies of endoscopic ultrasonography, body coil magnetic resonance imaging, and phased-array coil magnetic resonance imaging in estimating T and N stage

	Accuracy T	95% CI	Accuracy N	95% CI
EUS	0.70	(0.65–0.90)	0.63	(0.50–0.80)
BC-MRI	0.43	(0.39–0.75)	0.64	(0.47–0.82)
PA-MRI	0.71	(0.52–0.91)	0.76	(0.58–0.94)

CI = confidence interval; EUS = endoscopic ultrasonography; BC-MRI = body coil magnetic resonance imaging; PA-MRI = phased-array magnetic resonance imaging.

Table 2. Comparison of sensitivities and specificities of endoscopic ultrasonography, body coil magnetic resonance imaging and phased-array coil magnetic resonance imaging in estimating T stage and N stage

	T stage		N stage	
	Sensitivity	Specificity	Sensitivity	Specificity
EUS	0.80	0.67	0.47	0.80
BC-MRI	0.55	0.63	0.62	0.80
PA-MRI	0.75	0.67	0.63	0.80

EUS = endoscopic ultrasonography; BC-MRI = body coil magnetic resonance imaging; PA-MRI = phased-array magnetic resonance imaging.

DISCUSSION

The studies published by Kwok et al.[29] in 2000 and Bipat et al.[30] in 2004 systematically reviewed published data on rectal cancer staging with CT, EUS, and MRI using different coils. Kwok et al.[29]

Table 3. Comparison of positive and negative predictive values obtained by endoscopic ultrasonography, body coil magnetic resonance imaging, and phased-array coil magnetic resonance imaging in estimating T stage and N stage

	T stage		N stage	
	Positive predictive value	Negative predictive value	Positive predictive value	Negative predictive value
EUS	0.85	0.64	0.67	0.64
BC-MRI	0.79	0.36	0.73	0.71
PA-MRI	0.79	0.57	0.75	0.77

EUS = endoscopic ultrasonography; BC-MRI = body coil magnetic resonance imaging; PA-MRI = phased-array magnetic resonance imaging.

Table 4. Comparison of proportions of cases overstaged and understaged by endoscopic ultrasonography, body coil magnetic resonance imaging, and phased-array coil magnetic resonance imaging

	T staged		N staged	
	Overstaged	Understaged	Overstaged	Understaged
EUS	0.17	0.12	0.10	0.27
BC-MRI	0.25	0.32	0.14	0.21
PA-MRI	0.14	0.14	0.09	0.14

EUS = endoscopic ultrasonography; BC-MRI = body coil magnetic resonance imaging; PA-MRI = phased-array magnetic resonance imaging.

concluded that EUS was the most accurate technique for assessing wall penetration. However, in studies that compared MRI with an endorectal coil with EUS, the former was found to be as effective as EUS for assessing T stage and was the more effective in assessing nodal involvement. Kwok et al.[29] concluded that MRI with an endorectal coil was the single investigation that most accurately predicted pathologic stage in rectal cancer. The meta-analysis of Bipat at al.[30] also found that EUS was the best technique for assessing local invasion, but stressed its limitations: operator dependency, no assessment of stenotic tumor, inability to visualize with a rigid probe, tumors located in the upper rectum, inability to detect lymph nodes outside the range of the transducer, and inability to visualize the mesorectal fascia. The authors emphasized that none of the techniques were able to identify involved lymph nodes with satisfactory accuracy.

The present single-center study reports our experience, first with BC-MRI and then with PA-MRI, always complemented by EUS. PA coils were not available with the first release of the MRI machine, but they were introduced with an upgrade. From September 2002 to the present, all examinations have been performed with the PA coil because the slices are thinner and images are more detailed than those obtained with a BC. We found that EUS and PA-MRI had the same accuracy for assessing T stage (70% and 71%, respectively). The sensitivity of EUS was 80%, inferior to the 93% reported by Kwok et al.[29] and inferior to the 85% to 94% sensitivity for detecting perirectal tissue invasion reported by Bipat et al.[30] The main problem with EUS in our experience is that it overestimated T stage in 17% of cases (Table 4). Even highly experienced operators tend to overstage because perineoplastic inflammation can be mistaken for tumor, and a prior endoscopic biopsy that can induce necrotic and hemorrhagic microfoci within the rectal wall can be mistaken for tumor.[21,22,25,26]

In contrast, the T-staging sensitivity of 75% we obtained for PA-MRI is closer to the 79% of Bipat et al.,[30] not too far from the 86% of Kwok et al.[29] We found considerable differences between BC-MRI and PA-MRI in terms of the accuracy (43% vs. 71%) and sensitivity (55% vs. 75%) of T-stage estimates, and BC-MRI was the least accurate method for T stage (Table 1), although none of these differences were significant. We note that the 95% CI for EUS accuracy was narrower than for the MRI techniques. It is our clinical impression that the PA-MRI modality is the most promising, and we speculate that if more patients had been studied with it, the 95% CI would have narrowed, possibly rendering it significantly more accurate than EUS.

In the assessment of T stage, EUS had higher sensitivity (80%), positive predictive value (85%), and negative predictive value (64%) than the two MRI coils, whereas specificities were similar: 67% for EUS, 63% for BC-MRI, and 67% for PA-MRI. However, for the identification of lymph node involvement, the overall accuracy of PA-MRI was, at 76%, higher than that of EUS (65%). The sensitivity (63%) and specificity (80%) of PA-MRI in assessing lymph node involvement were similar to those reported by Kwok et al.[29] (sensitivity 65% and specificity 80%) and Bipat et al.[30] (sensitivity 66% and specificity 76%). The differences we found between BC-MRI and PA-MRI in assessing N stage were small. The accuracy (64%) of BC-MRI was lower than that of PA-MRI (76%), but the sensitivity and specificity were similar. We found that the sensitivity (47%) of EUS in assessing N stage was much lower than the overall figures quoted by Kwok et al.[29] (71%) and Bipat et al.[30] (67%).

To conclude, our study suggests that EUS and PA-MRI provide similar results in assessing the T stage of rectal cancer. In addition, PA-MRI permits good assessment of tumor penetration, permits good visualization of rectal wall layers, is less operator dependent than EUS, and is not influenced by tumor size or location. Lymph node evaluation remains problematic, but our results, in accord with published data, indicate that both MRI techniques have slightly better accuracy and sensitivity than EUS. MRI can also detect lymph nodes distant from the perirectal zone, whereas new lymph node-specific contrast agents may further increase the use of MRI in the near future.[35,36] MRI can also probe the mesorectal fascia, although its ability to do this

is still being investigated.[14] EUS cannot assess this structure. PA-MRI seems to be the single preoperative staging examination that most accurately predicts the pathologic stage of rectal cancer.

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Discussion

Dr. Fabrizio Michelassi (New York, NY): I thank the Society for the privilege of discussing this article and the authors for sending the article well in advance of the meeting.

Accurate preoperative staging of rectal cancer is essential to select the appropriate therapeutic options and maximize chances of cure and maintenance of transanal defecation. Preoperative chemoradiation treatment of locally advanced cancer has been shown to downstage tumors, potentially increasing long-term survival and feasibility of sphincter-saving procedures. Also, some early tumors located in the lower third of the rectum can be excised transanally.

The feasibility and validity of these choices are predicated on the accuracy of preoperative staging, as it has become clear that preoperative chemotherapy is not without morbidity and local exicision of rectal cancer can be associated with a high local recurrence rate in T3 tumors and possibly also in T2 tumors, thus, the importance of this article comparing local preoperative staging obtained with two different modalities, endoscopic ultrasound (EUS) and magnetic resonance imaging (MRI). The findings of the study suggest that both modalities offer similar rates of accuracy and predictive value with EUS easier to perform, probably less costly, and more operator-dependent, and MRI better for detecting liver metastases and for higher lesions and lesions that have already undergone a biopsy.

I have three questions. Can the accuracy of staging of endoscopic ultrasound be increased by the addition of targeted lymph node biopsies?

You calculate the accuracy and predictive value of the two modalities on the concordance of the preoperative staging with the pathologic staging for 931 lymph nodes. As we treat patients rather than lymph nodes, which modality is more reliable in avoiding over- and understaging of patients?

And last, in your last slide you state that MRI is the best single modality; in your practice, have you switched to using only MRI for the staging of patients with rectal cancer?

Congratulations for the study, and thank you very much for the opportunity to discuss its findings.

Dr. Bianchi: This is a good point on biopsies with EUS. We do not usually perform biopsies of lymph nodes, so I cannot have an answer to your question, because usually our endoscopist does not perform these biopsies. In the literature it is reported that it is not difficult to biopsy the lymph nodes, and in an article this staging was demonstrated to reduce tumor recurrence risk.

Which is the best way to evaluate the N stage preoperatively is difficult to say. EUS has a field of view very small, so it is difficult to evaluate the lymph nodes with this method, but there is no concordance. The volume is the only criteria to evaluate lymph nodes with MRI. Maybe the use of some contrast agents that are lymph node-specific may answer your question, but this is still under investigation.

In regard to the final question about the MRI as a single examination, in this study we found there is no significance in the different methods. We do not know the best method. EUS alone is not sufficient to obtain accurate preoperative staging, particularly for the bulky tumor, the tumor with stenosis, and the tumor located in the upper third of the rectum. It is necessary to have another method to evaluate, a CT or an MRI. MRI can evaluate the mesorectal fascia. This is a crucial point. Mesorectal fascia has an important role in the preoperative evaluation because it can show the distance of the tumor from the mesorectal fascia. For this reason we assume that MRI could be the only preoperative staging method to best evaluate rectal cancer.

Dr. Richard Hodin (Boston, MA): I don't think you told us the actual stage of the patients or, for example, how many patients turned out to be positive in terms of lymph nodes. The reason I am asking is because you included only patients who did not receive preoperative XRT and chemotherapy, so I think we can assume that this was a subset of fairly early lesions. Is that true? And then the question is, of course, whether the data apply for more advanced lesions.

Dr. Bianchi: Good question. The reason we excluded patients with preoperative radiotherapy or chemoradiotherapy was that MRI can really be altered by radiotherapy, and so the results may be different. We have no T4 stages because T4 stages were treated with preoperative radiotherapy. In this study we used the histologic findings on the surgical specimen as the gold standard, and I think restaging after radiotherapy can alter the true accuracy of the method. On this basis we decided to exclude patients with preoperative radiotherapy.

Colonoscopic Perforations: A Retrospective Review

Corey W. Iqbal, M.D., Yun Shin Chun, M.D., David R. Farley, M.D.

Colonic perforation is no longer a rare complication of colonoscopy. Our previous report identified 45 such iatrogenic injuries from 1980 through 1994 (3082 colonoscopies per year). This follow-up of the ensuing 7 years examines changing trends of endoscopic usage in addition to management and prognosis of patients with colonoscopic perforations. Retrospective analysis of 78,702 colonoscopies (1994 through 2000, 11,243 colonoscopies per year) allowed assessment of medical records in all patients treated at our institution for colonic perforation. Sixty-six patients from our institution (perforation rate, 0.084%; 1 per 1192 procedures) and six patients from outside institutions were treated for colonic perforation following colonoscopy (41 women, 31 men; ages, 30–92 years; median, 73 years). Sixty-two patients underwent laparotomy, while 10 were managed nonoperatively. All 10 patients managed nonoperatively were void of peritoneal irritation by physical examination; eight patients did well (median hospital stay, 5.5 days; range, 0–12), but one death (family declined operative intervention) and one pelvic abscess requiring percutaneous drainage were noted. Peritoneal irritation by physical examination was evident in 57 of 62 patients undergoing laparotomy. Perforations occurred throughout the colon: right, 22 (31%); transverse, 5 (7%); left, 44 (61%); and unknown, 1 (1%). Thirty-eight patients (61%) underwent primary repair or resection with anastomosis. Fecal diversion was used in 100% of patients with extensive peritoneal contamination (n = 12) and 40% of patients with moderate contamination (12 of 30). Perioperative morbidity (39%) and mortality (8%) were significant. Factors predicting a poor outcome included delayed diagnosis, extensive peritoneal contamination, and patients using anticoagulants ($P < .05$). Compared with our prior study, the present review highlights a higher prevalence of injury based on more frequent use of colonoscopy. Perforation rates remain around 0.08%. While nonoperative management is viable in patients void of peritonitis, expedient surgical intervention seems to facilitate patient recovery. (J GASTROINTEST SURG 2005;9:1229–1236)

KEY WORDS: Colon, colonoscopy, endoscopy, colonoscopic perforation, colon injury, colon trauma

The incidence of colonoscopic perforation in recent, large-volume series (>10,000 colonoscopies) is reportedly 0.03%–0.19%.[1,2] Despite a low incidence of perforation, the rising use of colonoscopy has led to a higher prevalence of colonoscopic injuries. In our previous series we reported on 12,581 colonoscopies being performed at our institution over an 8-year period (1980–1987).[3] In the year 2000 alone, we exceeded the previous entire collective experience with 15,393 colonoscopies performed.

A review of the literature reveals that 50%–100% of patients with colonoscopic perforations undergo emergent laparotomy, with postoperative morbidity and mortality rates of up to 34% and 25%, respectively.[3–6] In this study we reexamine our institutional practice of colonoscopy and specifically focus on perforation trends: etiology, recognition, and optimum treatment.

MATERIAL AND METHODS

Study Subjects

An institutional computer-based search of records of colonoscopic trauma between 1994 and 2000 disclosed 72 patients with iatrogenic perforation, excluding patients reported in our previous study.[3] Sixty-six patients underwent colonoscopy at our institution, while six were treated after colonoscopy performed elsewhere. Data was obtained regarding

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indications for colonoscopy, degree of difficulty of the colonoscopic procedure, quality of bowel preparation, presenting symptoms, medical and operative management, length of hospitalization, and morbidity and mortality.

Statistical Analysis

Rates of morbidity and mortality were estimated for various risk factors including anticoagulation, extensive contamination, and prior hospitalization. In addition, 95% exact binomial confidence intervals were calculated. Fisher's exact tests were used to assess associations between each risk factor and 30-day postoperative morbidity. Similarly, 30-day postoperative mortality related to operative procedure and mechanism of colon injury was assessed. An α level of $<.05$ was considered to be statistically significant.

RESULTS

Demographics

The study group consisted of 41 women and 31 men, ranging in age from 30 to 92 years (mean 71.5, median 73). Fifty-eight patients (81%) underwent colonoscopy as outpatients, while 14 (19%) were already hospitalized, including 6 patients in the intensive care unit. Fifty patients (69%) had a prior abdominal, groin, or pelvic operation. Significant comorbid conditions included corticosteroid use in 13 patients (18%), anticoagulation in 9 (13%), current malignancy in 6 (8%), inflammatory bowel disease in 3 (4%), and organ transplantation in 2 (3%).

Endoscopy

Colonoscopy was performed or supervised by either staff gastroenterologists or staff colorectal surgeons. Analgesics (i.e., meperidine hydrochloride) and sedatives (i.e., midazolam) were used as needed to ensure patient comfort in all but one patient, who refused medication.

The indications for colonoscopy are shown in Table 1. Of the 66 colonoscopies resulting in perforation performed at our institution, the endoscopist noted difficulty performing the procedure and/or visualized patient discomfort during 42 examinations (64%). Bowel prep was suboptimal in 18 patients (27%). The endoscopist saw or suspected a perforation during 19 examinations (29%). Forty-one patients (62%) had diverticuli noted during colonoscopy, with 8 (12%) patients having dense or wide-mouthed diverticuli, which rendered the examination more difficult. Other colonic abnormalities included angiodysplasia in 9 (13%) patients, colon cancer in 3 (4%), and inflammatory bowel disease in 3 (4%). Thirty-one patients (43%) underwent therapeutic colonoscopy, while 41 patients (57%) had a diagnostic examination, with or without biopsy (Table 2).

Table 1. Indications for colonoscopy

Indication	No. of Patients (%)
Known or history of polyp	26 (36)
Gastrointestinal bleeding	16 (22)
Family history of colon cancer	10 (14)
Change in bowel habits (rule out cancer or colitis)	7 (10)
Iron-deficiency anemia	4 (6)
Stricture associated with Crohn's colitis	2 (3)
Ogilvie syndrome	2 (3)
Abnormal barium enema	2 (3)
Surveillance for ulcerative colitis	1 (1)
Carcinoma of unknown primary	1 (1)
Known colon cancer, rule out synchronous tumor	1 (1)
Total	72 (100)

Manifestations

After colonoscopy, seventy (97%) patients developed abdominal pain and distention. Two patients were asymptomatic: one had perforation visualized during colonoscopy and underwent emergent laparotomy, and the other had free intraperitoneal air on abdominal radiography but was treated conservatively without sequelae.

Sixty-two patients underwent operative management. Fifty-seven (92%) of these 62 patients had peritoneal irritation on physical examination. Of the five patients without peritoneal irritation, four underwent surgery within 1 hour of colonoscopy because the endoscopist saw the perforation, and the other had massive ascites obscuring the physical exam. Of the 10 patients managed conservatively, none had peritoneal irritation on physical examination.

Table 2. Colonoscopic procedure

Procedure	No. of Patients (%)
Examination only	30 (42)
Polypectomy	22 (30)
Examination with biopsies	11 (15)
Coagulation of bleeding	5 (7)
Stricture dilatation	2 (3)
Colonic decompression	2 (3)

Fifty-seven patients managed operatively underwent abdominal roentgenograms; 48 (84%) had free intraperitoneal air, and 9 (16%) did not. Among the eight patients managed conservatively who underwent abdominal radiography, four patients had free air, and four did not. As shown in Table 3, the positive predictive value of pneumoperitoneum on radiography for patients requiring laparotomy was 92%, while the negative predictive value of the absence of pneumoperitoneum for patients undergoing nonoperative management was 31%.

Eighteen patients underwent computed tomography (CT) scanning of the abdomen and pelvis: 12 after equivocal or unremarkable abdominal radiographs and 6 without a prior radiograph. In patients with equivocal or unremarkable radiographs, CT disclosed free air in six patients, abscess in three, and microperforation in two. One patient had massive ascites, which obscured the CT examination.

Water-soluble contrast enema done in two patients showed no perforation. Of these two patients, one was managed conservatively, and the other required laparotomy for peritoneal irritation.

Seventy-one patients underwent urgent complete blood cell count: among the 62 patients undergoing operative management, 29 (47%) had preoperative leukocytosis, and 11 (18%) had fever ($>38^{\circ}C$). Among the 10 patients undergoing conservative management, 5 (50%) developed leukocytosis, and 4 (40%) had fever.

Delayed Diagnosis

Nineteen patients were diagnosed at the time of colonoscopy by the endoscopist, and an additional 39 patients were diagnosed within 24 hours of colonoscopy. Nine patients (13%) were diagnosed between 24 and 48 hours after colonoscopy. Five patients (7%) had a delayed presentation or diagnosis at greater than 48 hours.

Among 14 patients with a delay in diagnosis of greater than 24 hours, morbidity and mortality rates were 57% and 14%, respectively (morbidity was 28% and mortality 5% for patients diagnosed within 24 hours, $P = 0.1$). Among these 14 patients, 4 were taking corticosteroids, 2 underwent colonoscopic decompression for Ogilvie syndrome, 1 presented late secondary to severe dementia, 1 was taking narcotic analgesics, and 1 had massive ascites, which confounded the physical examination and radiographic studies.

Table 3. Results of abdominal radiography

	+ Pneumoperitoneum	– Pneumoperitoneum	Total
Laparotomy	48	9	57
Nonoperative management	4	4	8
Total	52	13	65

Nonoperative Management

Ten patients were managed nonoperatively (one complication and one death). Length of hospitalization ranged from 0 to 14 days (mean 6.7, median 6). Eight patients recovered completely, with resolution of abdominal pain, normal temperature, and normal leukocyte count in 1–7 days (mean 3.7, median 4). All eight patients successfully managed nonoperatively were diagnosed within 24 hours of colonoscopy. All but one were admitted to the hospital for intravenous antibiotics, hydration, withholding of oral intake, and serial abdominal examinations. One patient was treated as an outpatient with liquid diet for 2 days and oral antibiotics.

Nonoperative management resulted in one complication in a patient with Crohn's disease undergoing colonoscopic dilatation of a stricture. She presented 3 days postcolonoscopy with abdominal pain, distention, and fever but did not have peritonitis on physical examination. CT showed microperforation, which was treated nonoperatively. Sixteen days postcolonoscopy, she was readmitted with a pelvic abscess requiring percutaneous drainage. This was complicated by a colovesical fistula, repaired surgically 4 months later.

The fatal outcome occurred in an 88-year-old male patient in the intensive care unit with multiple comorbid conditions including left hip fracture status post open reduction and internal fixation prompting his hospitalization, coronary artery disease, chronic obstructive pulmonary disease, and dementia. Emergent evaluation for hematochezia led to a descending colon endoscopy-related injury causing a tension pneumoperitoneum. The patient's family declined further intervention, and the patient died less than 24 hours post-colonoscopy.

Operative Management

Sixty-two patients underwent operative management. The median duration of hospital stay was 10 days (mean 12.5, range 6–49 days). The operative procedure was colonic resection with anastomosis in 22 (35%) patients, colonic resection with stoma in 21 (34%), simple suture repair in 16 (26%), and simple suture repair with a diverting ileostomy or colostomy in 3 (5%) patients (Table 4). Thirty-three percent (6 of 18) of patients with right-sided colonic

Table 4. Techniques of operative repair in study group

Operative Procedure	No. of Patients (%)
Colonic resection with primary anastomosis	22 (35)
Colonic resection, anastomosis, and ostomy	21 (34)
Simple closure of defect	16 (26)
Simple closure with diverting ileostomy or colostomy	3 (5)
Total	62 (100)

perforations required fecal diversion, compared with 41% (16 of 39) of left-sided and 40% (2 of 5) of transverse colon perforations. Intestinal continuity was eventually restored in 10 of the 24 (42%) patients with a stoma.

The amount of intraperitoneal contamination ranged from minimal in 20 (33%) patients, to local soiling in 30 (48%), and diffuse fecal peritonitis in 12 (19%). Fecal diversion was performed in all patients (12 of 12) with diffuse feculent peritonitis and in 12 of 30 patients with moderate contamination. No patients with minimal contamination (n = 20) required a stoma. Intraperitoneal irrigation (5 L or more of isotonic saline) was used in all operative cases.

Simple surgical closure was performed in 7 of 15 patients diagnosed with perforation immediately by the endoscopist and in none of the 13 patients with delayed diagnosis of greater than 24 hours (Table 5). Fecal diversion was necessary in 62% of patients diagnosed greater than 24 hours after colonoscopy. The degree of contamination related to time to diagnosis as is shown in Table 6. Among patients diagnosed greater than 24 hours postcolonoscopy, 54% had extensive peritoneal contamination (P = NS).

Postoperative Morbidity

Thirty-four complications occurred in 24 patients, including 5 deaths, resulting in postoperative morbidity and mortality rates of 39% and 8%, respectively (Table 7). Major morbidity, excluding wound infection or superficial (skin) dehiscence, was 29% (18 of 62). Postoperative complication rates for patient risk factors are summarized in Table 8. Major morbidity and mortality related to the operative procedure are summarized in Table 9.

Table 5. Operative procedure related to time to diagnosis

Time of Diagnosis	Simple Closure	Resection With Anastomosis	Fecal Diversion
Immediate	7/15 (47%)	2/5 (13%)	6/15 (40%)
<24 Hours	9/34 (27%)	15/34 (44%)	10/34 (29%)
>24 Hours	0	5/13 (38%)	8/13 (62%)

Table 6. Degree of contamination related to time to diagnosis

Diagnosis	Minimal	Moderate	Extensive
Immediate	41%	59%	0
<24 Hours	39%	45%	16%
>24 Hours	8%	38%	54%

Anticoagulated Patients

Among risk factors for postoperative morbidity, anticoagulation was statistically significant. Reasons for anticoagulation were atrial fibrillation in five patients, cardiac thrombus in one, coronary stent in one, and liver failure in one. Among the eight patients, indications for colonoscopy were hematochezia in four patients, polyp in one, iron deficiency anemia in one, abnormal colon radiograph in one, and change in bowel habits in one. The four colonoscopies for hematochezia were performed emergently, and endoscopic visualization was obscured by blood in the colon in all four patients.

Table 7. Postoperative morbidity

Complication	No. of Patients
Wound infection or superficial dehiscence	7
Death	5
Adult respiratory distress syndrome	3
Fascial dehiscence	2
Cardiac arrhythmia	2
Septic shock	2
Pulmonary embolism	2
Aspiration pneumonitis	1
Devascularization of preexisting ileal conduit	1
Pancreatitis	1
Acalculous cholecystitis	1
Myocardial infarction	1
Incarcerated incisional hernia	1
Parastomal hernia	1
Bleeding ileostomy	1
Pelvic abscess	1
Anaphylactic reaction	1
Renal failure	1

Table 8. Morbidity related to patient risk factors

Risk Factor	Morbidity	*P* Value
Anticoagulation	75% (6/8)	.02
Extensive contamination	67% (8/12)	.07
Prior hospitalization	58% (7/12)	.12
Active malignancy	67% (4/6)	.14
Delayed diagnosis	57% (8/14)	.11
Steroid use	58% (7/12)	.12

Postoperative Mortality

Five postoperative deaths occurred in four men and one woman. Deaths resulted from underlying cardiopulmonary disease in two patients and aspiration pneumonitis in a third patient. A fourth death occurred after colonoscopic polypectomy in a 61-year-old man with α_1-antitrypsin deficiency and massive ascites, awaiting liver transplantation. A perforation at the rectosigmoid junction was found unexpectedly 7 days postcolonoscopy during laparotomy for planned liver transplant. He died 14 days later of sepsis and multisystem organ failure.

The fifth death occurred in a 54-year-old man with steroid-dependent bullous emphysema, admitted with bilateral pneumothoraces and pneumomediastinum. He underwent emergent colonoscopic decompression for Ogilvie syndrome. Seven days postcolonoscopy, he underwent laparotomy for presumed small bowel obstruction and was found to have a transverse colon perforation sealed by omentum and a loop of bowel. He died 12 days postoperatively from respiratory failure and withdrawal of support.

Among these five patients, two were diagnosed with perforation immediately by the endoscopist, one was diagnosed within 24 hours, and two patients had a delayed diagnosis of 7 and 12 days postcolonoscopy.

Mechanism of Injury

Inspection of the colonic injury by the endoscopist, surgeon, and pathologist provided subjective evidence of three mechanisms of injury—direct colonoscopic trauma from mechanical manipulation (perforation without evidence of thermal injury); polypectomy (perforation in region of removed tissue); and electrocautery (burn after coagulation of bleeding or after biopsy). The mechanism of injury was mechanical/torque in 39 patients (54%), polypectomy in 17 (24%), and electrocautery in 16 (22%).

Twenty-four percent (4 of 17) of patients with polypectomy injuries were managed conservatively compared with 10% (4 of 39) and 13% (2 of 16) of patients with mechanical and electrocautery injuries, respectively (P = NS).

Table 9. Morbidity related to operative procedure

Operative Procedure	Morbidity (n)	Mortality (n)
Primary closure	1/16 (6%)	1/16 (6%)
Resection with anastomosis	10/22(45%)	3/22 (14%)
Resection with end colostomy/ileostomy	5/21 (24%)	0
Repair, loop ostomy	2/3 (66%)	1/3 (33%)

Site and Size of Perforation

Most perforations occurred at the rectosigmoid junction or sigmoid colon (Fig. 1.). Perforation size ranged from 0.1 to 6.0 cm (mean, 1.7 cm). The largest perforations resulted from mechanical injury, while the smallest perforations were the result of electrocautery injury (Table 10). More patients required fecal diversion after mechanical injury (P = NS). Electrocautery injury resulted in the highest postoperative morbidity, while mortality was lowest after polypectomy injury (Table 11, P = NS).

DISCUSSION

The utility of colonoscopy continues to broaden as a diagnostic and therapeutic tool, and we have seen an increase in the number of patients undergoing this procedure. Consequently, the number of

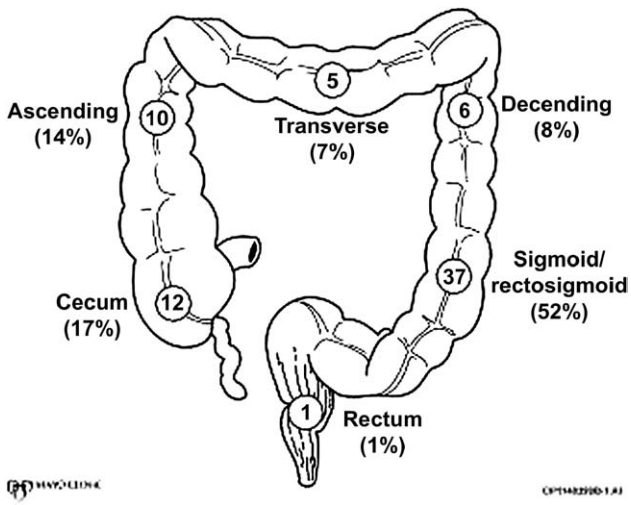

Fig. 1. Cecum, 12 (17%); ascending, 8 (11%); hepatic flexure, 2 (3%); transverse, 5 (7%); splenic flexure, 3 (4%); descending, 3 (4%); sigmoid, 26 (36%); rectosigmoid junction, 11 (16%); rectum, 1 (1%); unknown, 1 (1%).

Table 10. Mechanism of injury and size of perforation

Mechanism	Size of Perforation, mean (range)
Electrocautery	0.9 cm (0.1–2 cm)
Polypectomy	1.3 cm (0.1–4 cm)
Mechanical	1.9 cm (0.2–6 cm)

associated complications has also risen. Moreover, colonoscopies are being performed on older and less fit patients who are more susceptible to iatrogenic, colonoscopic injury.

In the past two decades (1980–2000), our institution performed 135,730 colonoscopies, and 111 patients sustained an iatrogenic perforation (an incidence of 0.082%, or 1 in 1220 procedures). Over the two study periods, the incidence of perforation has not changed. Certain patient variables also remained remarkably similar in our two study periods in terms of site and mechanism of injury, extent of intra-abdominal contamination, and type of operative intervention. However, we did observe a marked change in other variables including an increase in median patient age, an increase in the number of patients managed conservatively, and an increase in postoperative morbidity and mortality rates (Table 12). While this retrospective study with low numbers makes demonstrating any statistical significance difficult, we feel that these observed trends represent the evolving use of colonoscopy in a more diverse patient population.

Due to such a large volume of colonoscopies, even a low complication rate of 0.084% at an institution that averages more than 12,000 colonoscopies per year will yield at least one iatrogenic perforation per month; this is a complication that gastroenterologists and gastrointestinal surgeons need to be able to recognize and manage.

Perforations occur via one of three different mechanisms: mechanical, removal of tissue, and thermal. Mechanical injuries, often due to torque in the rectosigmoid region, are associated with the largest perforations, whereas thermal injuries are associated with the smallest perforations. In our current study, the rate of perforation due to one of the specific mechanisms of injury correlated with the indication for examination. For example, the most common mode of injury was mechanical and the most common indication for colonoscopy was examination only. Other factors can make colonoscopy difficult and likely contribute to colonoscopic perforations. These include endoscopist experience, suboptimal bowel prep or active bleeding, and the presence of dense or wide-mouthed diverticuli. When the perforation is not recognized by the endoscopist at the time of the procedure, abdominal pain and distention are the most frequent presenting complaints and should raise the suspicion for colonoscopic perforation. Patients may also exhibit fever, chills, leukocytosis, hypotension, tachycardia, nausea, emesis, and obstipation.[3–9]

Table 11. Fecal diversion/postoperative morbidity and mortality related to mechanism of injury

Mechanism	Fecal Diversion	Morbidity	Mortality
Mechanical	43% (15/35)	34% (12/35)	11% (4/35)
Electrocautery	36% (5/14)	50% (7/14)	7% (1/14)
Polypectomy	31% (4/13)	31% (5/13)	0

Table 12. Changing practice

	1980–1995	1994–2000
Incidence of colonoscopic perforation	0.075% (1 in 1333 procedures)	0.084% (1 in 1192 procedures)
Median age (yr)	69	73
Conservative management	6.7%	14%
Mechanism of injury		
Electrocautery	20%	22%
Polypectomy	32%	24%
Mechanical trauma	48%	54%
Intra-abdominal contamination		
Minimal	31%	33%
Local soiling	48%	48%
Diffuse peritonitis	21%	19%
Postoperative morbidity	29%	39%
Overall mortality	0%	8%

Abdominal roentgenogram is a quick, cost-effective, and useful means for detecting the presence of pneumoperitoneum with a positive predictive value of 92%. However, a negative study may not be satisfactory to rule out perforation (negative predictive value of 31%). In this setting, computed tomography, in a clinically stable patient devoid of peritonitis, can aid in the diagnosis by detecting free air, microperforations, and/or abscess.

The presence of pneumoperitoneum alone is not an indication for operative management. Highly selected patients who are clinically stable and who do not exhibit signs of peritoneal irritation or abdominal sepsis have good outcomes with observation coupled with intravenous antibiotic therapy, bowel rest, and serial abdominal examinations.[1,7,9–14] Nonoperative management in the appropriate patient results in a shorter length of hospitalization and

lower morbidity. The only death observed among patients not undergoing operative intervention involved a patient in the intensive care unit whose family refused such intervention. This patient did not meet the criteria we use to determine a patient's potential for nonoperative management.

Immediate operative intervention is not necessarily mandatory. Patients diagnosed more than 24 hours following colonoscopy seem to have a higher rate of extensive fecal contamination. However, there was no difference in this current study in extent of contamination between those patients who underwent immediate celiotomy compared to those who underwent celiotomy within 24 hours of perforation. Therefore, if further resuscitation, reversal of coagulopathy, or correction of other medical issues is indicated, it may be corrected preoperatively, if in a timely manner.

Operative management was determined by the intraoperative findings. In cases of minimal to moderate intra-abdominal contamination coupled with healthy tissues and limited comorbidities, primary closure or resection with primary anastomosis is favored. With extensive contamination, poor tissue quality and/or significant comorbidities resection with stoma or primary repair with fecal diversion is favored. Only two preoperative factors were identified that suggest which type of procedure would be required. First, those patients diagnosed within 24 hours of perforation were more likely to undergo a primary closure of the perforated site compared with those diagnosed beyond 24 hours due to the higher rate of extensive fecal contamination in the latter group. Second, mechanical injury was associated with larger perforations (mean, 1.9 cm), and these patients may be more likely to require resection with fecal diversion. However, the mechanism of injury cannot always be determined preoperatively. No other preoperative factors reliably predicted which operation would be indicated.

The morbidity and mortality rates increased from our previous study. We think this can be attributed to an older patient population with more comorbidities. The only significant predictor of postoperative morbidity that we identified was coagulation status. Those patients on anticoagulation or with preexisting coagulopathies had a higher incidence of morbidity. In addition, those patients with extensive fecal contamination trended toward a higher morbidity rate ($P = .07$).

CONCLUSION

Colonoscopic perforation remains a rare complication of colonoscopy, however as its usage continues to grow, the prevalence of colonic perforation has risen. Colonoscopic perforation in itself is quite morbid because the majority of patients will require an exploratory celiotomy for fecal peritonitis and abdominal sepsis. Patients undergoing colonoscopy will continue to age and have comorbidities, and the postoperative morbidity and mortality rates will likely remain high. The role of nonoperative management should be undertaken in that highly selected group of patients who do not exhibit signs of peritoneal contamination or abdominal sepsis. However, delaying operative intervention beyond 24 hours will result in more extensive intra-abdominal contamination, limiting the intraoperative options and possibly contributing to even higher morbidity and mortality rates.

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Discussion

Dr. Robert Martin (Louisville, KY): I congratulate the authors on an impressive study of the largest review of colonoscopic procedures complicated by perforation. With the increasing age in the population, the continued efforts of increasing colon cancer screening, and, more importantly, the inaccuracies of CT colonoscopy, the need for colonoscopic evaluation will continue to grow. Dr. Farley's group presents a retrospective study of over 78,000 colonoscopies in which 66 patients sustained a perforation. There are two primary issues in regard to colonoscopy and the risk for perforation. One, who is at risk for perforation; two, what is the optimal management and diagnosis of these patients? For these issues I have three questions for the authors.

From the wealth of data presented, the one data point that seems to be missing is the endoscopic experience of the endoscopist. Since the career numbers of the endoscopist would not be available, it would be interesting to see how many have been done on an annual basis. It would obviously be concerning that an endoscopist who is doing only five or six procedures a year and has two of these perforations may be a marker for you. In the era of volume specialization as well as the continued efforts to make endoscopy suites closed to surgeons, especially general surgeons, who continue to take a great deal of funding from these, I think this type of risk factor would be important.

Since the majority of perforations were in the rectosigmoid area, which may be affected by prior surgery, were you able to capture how many of these patients had prior operations? Such as women who had prior hysterectomies, or how many of these patients had prior diverticular disease?

Last, in regard to the operative management, you reported 69% of patients with perforations who underwent an operation in less than 24 hours. Yet you then report that only 18 of these had a suboptimal prep but nearly 30 of them had moderate to extensive contamination. That seems to be counterintuitive if a lot of these patients had optimal colon prep? I would assume that this extensive contamination is probably from the number of inpatients who were having emergent colonoscopies for GI bleeding or Ogilvie's syndrome. If so is it possible that these patients could have already had microperforations that were exacerbated by the colonoscopy performed?

These are limitations of a retrospective study, but I do think it should be mentioned in the manuscript just to help us gain further criteria of risk. I believe this will be an often-quoted study, especially by general surgeons, who are continuing to do this procedure.

I thank the opportunity of the Society and the authors for allowing me to review their manuscript.

Dr. Iqbal: Thank you for your comments, Dr. Martin. In response to experience of the endoscopist, we did not look at perforations in terms of whose complication it was or their experience. We considered looking at this in terms of the time of year that perforations happen with regard to resident or fellow training to see if there was a trend toward earlier in the academic year.

In response to your second question, 50 patients did have a previous abdominal or pelvic operation, which was 69% of the total patients with perforation. We did not analyze that as a risk factor.

With regard to bowel prep, 18 patients were noted to have a suboptimal prep. In addition six patients were scoped in an ICU/emergent setting and those examinations can be obscured by not just suboptimal bowel prep but bleeding as well. In fact, all four of the patients on anticoagulation who underwent endoscopy for hematochezia had their examinations obscured by bleeding. In these patients, the quality of the bowel prep is difficult or impossible to determine. Additionally, it is possible but unlikely that some of these patients did have a preexisting perforation.

Cost-Saving Effect of Treatment Algorithm for Chronic Anal Fissure: A Prospective Analysis

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Evidence-based medicine suggests that in the management of chronic anal fissure (CAF), lateral internal sphincterotomy (LIS) is far more effective than medical treatment in lowering the anal sphincter tone and curing the fissure. In the current study, we developed a treatment algorithm from topical nitroglycerin (NTG) to botulinum toxin type A (Botox [BTX]) to LIS and analyzed its cost benefit by calculating the effective and potential costs based on the treatment success and the rate of avoided surgeries. Patients presenting between November 2003 and December 2004 with CAF and symptoms for greater than 3 months were prospectively treated according to a treatment algorithm which started with (1) topical NTG, in case of failure (2) injection of BTX, thus limiting (3) surgery to those who failed both nonsurgical options or at any point chose the surgical approach. Based on the primary end points of fissure healing or surgery, we calculated the true cost (algorithm) and the potential incremental cost (BTX plus surgery or surgery in all patients, respectively). Sixty-seven patients with CAF (25 men and 42 women; median duration of symptoms, 16 weeks) were treated according to the algorithm. NTG alone was successful in fissure healing in 31 of 67 patients (46.2%). Two developed a recurrent fissure and then received BTX as part of the protocol. Of the 36 patients who failed NTG trial, 3 requested surgery; the others were treated with BTX, which was successful in 84.8%. Five patients (15.2%) failed BTX and subsequently required surgery. The overall surgery rate in the whole study group was 11.9%, whereas CAF healed in 88.1% of our patients with medical treatment alone. Cost for NTG is $10; for 100 units BTX, $528; and for outpatient surgery, $1119. The total cost for these 67 patients therefore was $33,252 ($290 for NTG, $20,580 for NTG plus BTX, $3,357 for NTG plus LIS, and $9,025 for NTG plus BTX plus LIS). If all patients had received BTX with a 15% failure rate, the total cost would have been $56,688 (70.3% cost increase). If all patients had undergone surgery as initial/only treatment, the total cost would have been $74,973 (125% cost increase). Our treatment algorithm for CAF with stepwise escalation can avoid surgery in 88% of the patients. It is highly cost-efficient and resulted in savings of 41% (compared with BTX plus LIS) and up to 70% (compared with surgery in all patients), respectively. (J GASTROINTEST SURG 2005;9:1237–1244)

KEY WORDS: Fissure in ano, cost analysis, nitroglycerin, botulinum toxin type A, sphincterotomy

Anal fissures are a high-frequency anorectal pathology of uncertain etiology, resulting in significant cumulative health care cost. The pathology consists of a longitudinal tear commonly in the posterior, less frequently in the anterior midline of distal anal canal or at the anal verge, which causes characteristic symptoms of bright red rectal bleeding and a range of discomfort to severe pain during and after defecation.[1,2] While most acute fissures heal spontaneously within a few weeks, some persist and form a chronic lesion, often with reactive morphologic changes such as sentinel fibrous skin tags, hypertrophic anal papillae, exposure of the anal sphincter muscle fibers, and indurated wound edges.[2] Fissure persistence has been associated with sphincter hypertonia, that is, increased manometric resting pressures and a hypertrophic internal anal sphincter muscle. Even though the exact pathophysiologic mechanisms and the role of the local blood supply remain a matter of debate,[3] treatment success has correlated with the ability to

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lower the sphincter tone either by surgical or pharmacologic means.[1]

Lateral internal sphincterotomy (LIS) has long been considered the gold standard of treatment for chronic anal fissures (CAF) with cure rates of 94%–100%. However, concerns about the surgical morbidity of an irreversible sphincter defect with some degree of incontinence to stool or gas in up to 35% of patients in earlier series, less than 10% in more recent assessments,[4–6] have prompted a search for less invasive approaches.[7–10] Temporary pharmacologic "sphincterotomies" using topical nitroglycerin (NTG), calcium channel blockers (diltiazem, nifedipine), or botulinum toxin type A (BTX) have evolved as alternative nonsurgical strategies with reported healing rates of 67%–88%.[1,2,11–13] While a multiarm randomized trial directly comparing the various approaches would be desirable, it has never been performed and therefore, the technique of meta-analysis was used to assess the available evidence. Nelson's meta-analysis questioned the true success of these conservative treatments and suggested a chance of cure only marginally better than with placebo and far less effective than surgery.[14] However, inclusion of studies that lacked a distinction between acute and chronic fissures might have blurred the overall picture.[15]

In our current communication, we aimed at prospectively studying the effectiveness of a treatment algorithm for chronic anal fissures with stepwise treatment escalation from NTG to BTX to LIS. We hypothesized that the algorithm would (1) be effective from a therapeutic aspect (overall cure rate and tolerance), (2) keep the rate of necessary irreversible surgeries at a minimum, and hence (3) be cost-saving. Based on the numbers of clinical effectiveness, we performed a cost analysis by calculating the effective and hypothetical total and per capita cost of our algorithm as well as of two alternative treatment models.

PATIENTS AND METHODS

Eligible patients who presented between November 2003 and December 2004 with CAF were treated according to the treatment algorithm and were prospectively entered into a database. Included were patients with symptoms for longer than 3 months and characteristic clinical findings. Excluded were patients with cardiac disease, patients taking other smooth muscle relaxant medications, pregnancy, HIV-related ulcers, inflammatory bowel disease, current chemotherapy, or pelvic radiation therapy. Data collection included demographics, duration of symptoms, previous treatments, any side effects, and reasons for treatment failure; during every follow-up visit, treatment success and pain scores on a visual analog scale (VAS) from 1 to 10 were recorded. Primary end points of our study were the rate of fissure healing and the rate of necessary surgeries. Secondary end point was the cumulative direct treatment cost of the whole study group.

Treatment Algorithm

All patients were offered a treatment algorithm (Fig. 1) with stepwise escalation, starting with (1) topical NTG, (2) injection of BTX into the internal anal sphincter, and (3) lateral internal sphincterotomy (Fig. 1). Patients were followed at least every 4 weeks to assess the effectiveness of the treatment with regard to pain, bleeding, and healing of the fissure. Lack of either a partial or a complete resolution noted on follow-up or prohibitive side effects from the treatment were considered a failure of that level, and the next level of the algorithm was recommended to the patient. At any moment, patients had the option to shortcut the algorithm and advance to the next level or to proceed with surgery from the beginning. The study protocol was approved by the Institutional Review Board of the University of Southern California and was compliant with HIPAA regulations.

NTG was delivered as a 0.2% ointment and applied twice per day with a fingertip amount to the anus. BTX (Botox, 100 units, Allergan, Irvine, CA) was dissolved in normal saline and supplied in two syringes containing 20 units each; after local desinfection, it was injected bilaterally into the internal anal sphincter muscle.

Statistical Analysis

Results were reported in descriptive statistics using Microsoft Excel and expressed as mean ± standard deviation.

Cost Analysis

Costs analysis was based on the direct costs for NTG, BTX, and LIS as well as on the assumption that the number of follow-up office visits would be equal for all patients regardless of the treatment. Purchase costs were $10 for NTG and $528 for 100 units of BTX (currently the only vial size available). Procedural costs, based on Medicare reimbursement rates, were $148 for the BTX injection and $1119 for the LIS, which includes the surgeon's fee as well as the hospital/office cost for the procedure. Based on the primary end points of fissure

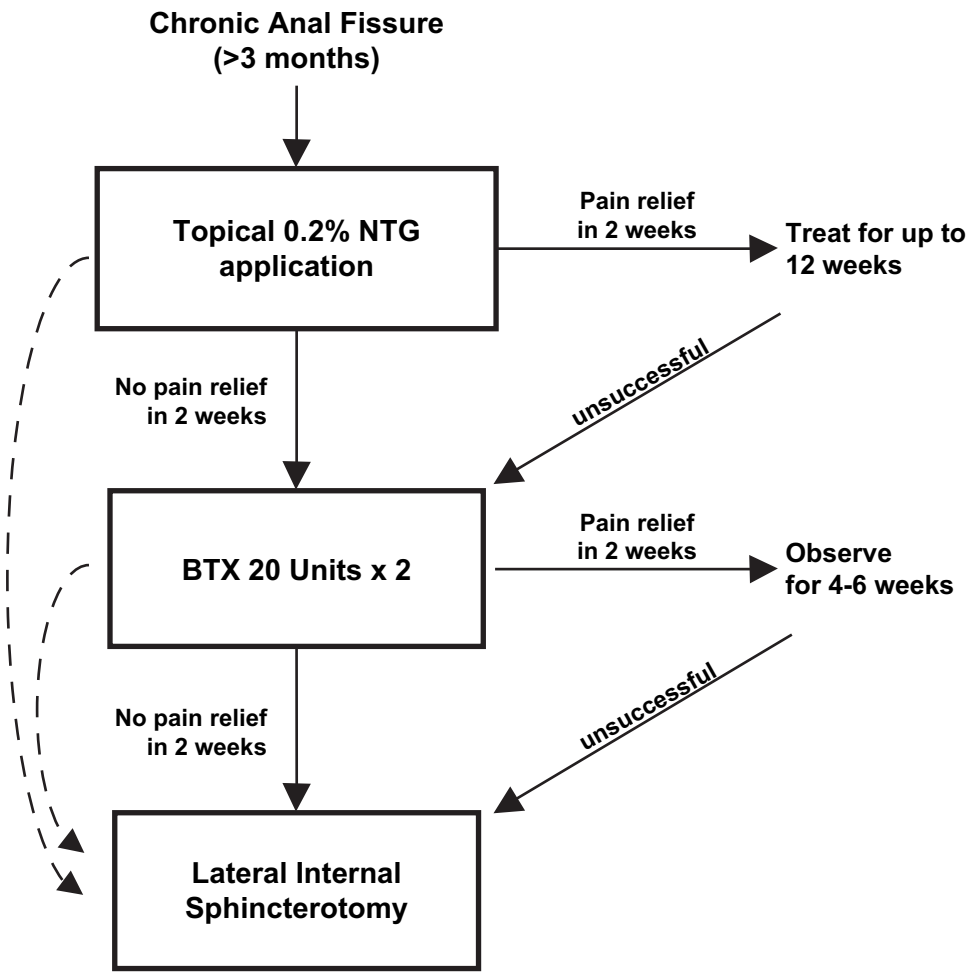

Fig. 1. Treatment algorithm (see text). NTG = nitroglycerin; BTX = botulinum toxin.

healing or surgery, we calculated the true cost of the algorithm, as well as the potential incremental cost if (A) "Brisinda approach": as NTG is less effective than BTX, all patients would be started with BTX and underwent surgery in case of failure[12] or (B) "Nelson approach": as medical management is less effective than surgery, all patients would undergo surgery as the only treatment.[14] Not included in the cost analysis were follow-up visits, indirect hospital costs, and potential costs from fecal incontinence. Because the overwhelming majority of patients complained of a local problem that did not affect their overall functionality and ability to work, we have not included the fissure healing and the time off work in our analysis.

RESULTS

Within the study period, 67 patients with CAF were eligible for the treatment algorithm. Mean age was 49 ± 14.4 years (range, 25–85 years), and there were 30 (44.8%) females and 37 (55.2) males. The median duration of the symptoms was 16 weeks (range, 12 weeks to 5 years). Median pain score on VAS at presentation was 8. Of the 67 patients, 3 patients have had their fissures previously treated; the rest had their first presentation with CAF. Mean follow-up at this point in the study was 6 months.

Treatment Success

NTG application alone was successful in healing the fissure in 31 of 67 patients (46.2%). Two patients later developed recurrences and were treated with an injection of BTX as part of the protocol. Of the 36 patients who failed NTG trial, 3 requested surgery; the others were treated with BTX injections, which was successful in 84.8% (28 of 33 patients). Five of 33 patients (15.2%) failed BTX treatment and subsequently required LIS. The overall surgery rate in the whole study group was 11.9%, whereas CAF healed in 88.1% of our patients with medical treatment

alone. No patient in the whole cohort complained of incontinence.

Cost Analysis

Total cost for these 67 patients was $33,282 ($497 per capita), which included $290 for 29 patients with NTG alone, $20,580 for NTG plus BTX, $3,387 for NTG plus surgery and $9,025 for NTG plus BTX plus surgery (Table 1).

Based on the above-mentioned rates of healing of the fissures with BTX (84.8%), we subsequently calculated the hypothetical costs for the following two scenarios:

Assumption A (Brisinda approach): NTG would not be used at all; the cohort of patients would primarily be treated with BTX,[12] leaving surgery for the BTX failures (15.2%). In this scenario, the total cost for these 67 patients would rise to $56,688 ($810 per capita), which combines the cost for 100 units of BTX and the injection in the office setting in all patients, as well as the cost of surgery ($1,119) in the 15.2% of patients failing BTX. The $56,688 would represent a 70.3% increase of the costs compared to the patients treated according to our treatment algorithm ($33,282).

Assumption B (Nelson approach): No role for conservative treatment; all patients of the cohort would primarily and only undergo surgery.[14] In this scenario, the total cost for the whole study group would rise to $74,973 ($1,119 per capita), which would represent a 125.3% cost increase compared with the total cost of patients in our treatment algorithm ($33,282).

BTX currently is available only in a 100-unit vial, and it is therefore a common hospital practice to charge a patient for the whole 100 units of BTX even if only a fraction of it is being used. Our calculations were therefore based on the vial price of $528. However, if the costs were calculated with the assumption that the price would truly be adjusted to the used fraction (e.g., 40 units), the total costs in the algorithm would be somewhat reduced ($22,194 instead of $33,282), whereas the surgery-only costs would remain unchanged. This adjusted calculation would therefore result in an even more dramatic cost-saving effect of our algorithm compared with the two above-mentioned hypothetical scenarios (Fig. 2).

Table 1. Distribution of Treatment Modalities and Cost Analysis

Treatment	No. of Patients	100 Units Botox	40 Units Botox
NTG alone	29	$290	$290
NTG + Botox	30	$20,580	$11,076
NTG + surgery	3	$3,357	$3,357
NTG + Botox + surgery	5	$9,025	$7,440
Total	67	$33,282	$22,194

NTG = nitroglycerin; Botox = botulinum toxin type A.

DISCUSSION

Successful treatment for chronic anal fissure has remained a continued hot topic throughout the past 50 years, and a general consensus remains out of reach. Nonetheless, it has become clear that the increased resting tone of the internal anal sphincter plays a fundamental role in the perpetuation of the nonhealing anal wound.[1] In that sense, the mainstay in the management of patients with chronic anal fissures is the lowering of the sphincter pressure.[1,2]

A rational approach to defining the role of the various surgical and nonsurgical means depends on disease-related factors (rate of and time frame for healing), patient-related factors (quality of life, absence of treatment side effects), and health care system–related factors (utilization costs, morbidity, recurrence rates, sick leaves). Increasing financial restraints within the health care system demands a search for effective and inexpensive approaches with minimal side effects.[16,17] Lateral internal sphincterotomy has evolved as the gold standard in the treatment of chronic anal fissures. It is predictably fast in promoting a lasting healing of the fissure; however, the irreversible weakening is not completely free of risks, and decreased sphincter control to stool or gas ranges from 3% to 16% and remains the biggest long-term fear.[4,6,18] In addition, the surgery accumulates several cost items that results in a high end price. The belief that the increased sphincter tone is not only the cause but also the result of the fissure supports the quest for a temporarily limited sphincter relaxation by pharmacologic means that would seem to be sufficient to just break the vicious circle and allow the fissure to heal but at the same time avoid the surgical short- and long-term morbidity.

In recognizing the role of nitric oxide in reducing the tone of the internal anal sphincter, topical NTG has been promoted as a chemical sphincterotomy.[19–23] Despite its appealing low cost and several encouraging trials with healing rates of up to 68%–86%, higher failure rates and recurrences have subsequently been reported due to side effects (mostly headaches, tachyarrhythmias) and tachyphylaxis.[24] Calcium channel blockers that might have some theoretical advantage appeared to be equal from

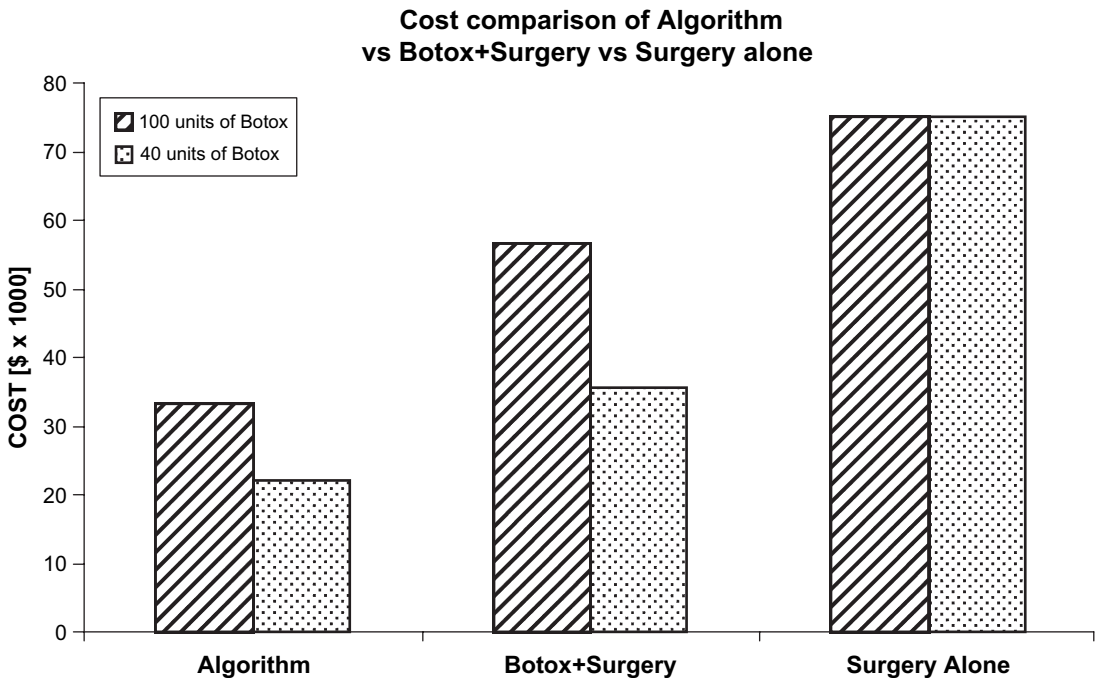

Fig. 2. Comparison of the cost-effectiveness between algorithm cohort and two hypothetical models. Each model was calculated for use of 100 units of BTX and of 40 units of BTX. BTX = botulinum toxin.

a practical standpoint.[25] Injection of the paralytic agent BTX has a more lasting pharmacodynamic effect of several weeks and was shown in randomized trials to be more effective than NTG.[12] It has therefore been recommended by some authors as the first-line treatment for chronic anal fissures.[12] However, it should be noted that the purchase price for the lyophilized BTX is roughly 50 times higher than a several-week course with NTG, and general use of BTX might therefore be a waste of resources.[16]

While one trend pointed toward a more conservative approach in the treatment of anal fissures to avert the risk of permanent injury to the internal anal sphincter, critical voices have questioned the value of nonoperative management and suggested a low risk and clear superiority of the surgical sphincterotomy.[21,24] A recent meta-analysis of 31 published trials comparing 21 different regimens concluded that medical therapy was far less effective than surgery.[14]

In our current prospective open design study, we intended to analyze the feasibility of a treatment algorithm for chronic anal fissure with stepwise escalation of the modalities from the least to the most invasive and effective method. As the patients had been suffering for several months to years of their symptoms, they concurred that there was commonly no need to rush a particular treatment but welcomed the opportunity to attempt the nonsurgical measures first, unless they took the liberty to choose surgery at any time prior. In contrast to published randomized trials, our approach did not directly compare success/failure of two or more different regimens but aimed at defining the overall success rate and cost effectiveness of a tailored conservative management, which reflects the considerations in a clinical practice setting. Our end points were, on one hand, the overall healing of the fissure and the rate of necessary surgeries and, on the other hand, a calculation of the direct costs based on the purchase costs and Medicare reimbursement schedules. For the latter purpose, we had to make a few simplifying assumptions, such as that the baseline management (e.g., stool management), the number of follow-up visits, and the time off work would be the same regardless of the treatment. The rational for this simplification was that the typical patient with a chronic anal fissure complained of a persisting local problem but only rarely was affected in his or her overall functionality and ability to work; if at all, we would speculate that there would be additional costs (perioperative/postoperative time off work) in the surgery group. We also left out several potential cost items that are likely to occur in the surgical group in addition to the direct surgical fees, such as anesthesia and other indirect hospital fees. And we did not include costs that might result from the surgical morbidity (e.g., bleeding, infection) and possible incontinence. Our data suggest that conservative management in an unselected cohort of patients with chronic anal fissures, as defined by a duration of longer than 3 months and morphologic features of

chronicity, is highly successful with a 88% healing rate and thus avoidance of a necessary surgical intervention. Our data are compatible with previously reported studies on both NTG and BTX; 46% of the fissures healed with the cheapest method of topical NTG, which is somewhat lower than in the previous randomized trials.[20] One might speculate that compliance in the group failing NTG treatment was less than perfect due to not acknowledged side effects. Even though some authors contributed an NTG success in part to a placebo effect,[15] the open design of our study neither depends on nor discredits such a beneficial impact. But the long duration of symptoms in some patients of up to 5 years prior to initiating our treatment suggests that the specific treatment beyond bulking agents was of significant relevance.

We subsequently compared the cumulative cost incurring to the whole cohort of patients with two alternative hypothetical scenarios. One scenario would follow the conclusion of Brisinda et al.[12] that NTG was less effective and therefore treat all patients primarily with BTX, leaving the surgery for our 15.2% BTX failures. The other scenario would follow Nelson's suggestion that surgery is far superior to nonsurgical management and thus not perform any conservative treatment.[14] Our calculations clearly demonstrate not only that there is a strong financial incentive to avoid the surgery-alone approach (54% cost saving of our algorithm) but also that the inexpensive success with NTG in almost half of the patients contributes to a high cost efficiency of our treatment algorithm compared with the Brisinda approach (41% cost saving of our algorithm). To rule out that the cost benefit was solely related to the high per-vial charge (full price) for the BTX instead of a per-administered dose–adjusted charge, we recalculated the costs based on the effective price per 40 units BTX. Both results were similar in their cost-saving effect between the algorithm and the BTX/LIS group (37% cost saving versus 41% cost saving). However, there was an even more dramatic cost-saving effect (70% cost saving versus 54%) of our algorithm compared with the surgery-only group scenario.

In summary, our study suggests that a stepwise approach to the treatment of chronic anal fissures is feasible and highly successful and that the escalation from the cheapest to the more expensive modalities results in a highly cost-saving effect. When given the option to decide, the majority of patients prefer the nonoperative treatment as a first-line approach.

CONCLUSION

Our treatment algorithm for CAF with stepwise escalation as needed can avoid surgery in 88% of the patients and thus saves direct costs of up to 70%. Neither surgery nor BTX should therefore be used as the initial treatment but should be reserved for when less expensive treatment modalities fail.

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Discussion

Dr. Susan Galandiuk (Louisville, KY): I commend Dr. Essani and her colleagues for their very thoughtfully designed study to look at the cost-saving effects of a treatment algorithm on chronic fissure in ano. I have a number of questions for the authors.

Chronic fissure in ano is frequently the result of constipation and/or straining, and as such, the addition of fiber bulking agents alone has always been a key part of treatment. In how many patients did constipation rather than diarrhea play a role in the etiology of this fissure, and were bulking agents added to the initial part of treatment in those patients who received nitroglycerin ointment? If that wasn't done, would that have improved treatment efficacy?

Second, the application of nitroglycerin is often limited by side effects such as headache or drops in blood pressure. Was this or patient compliance a limiting factor that might explain the fact that nitroglycerin worked in only 46% of patients in whom this was the initial treatment? I always tell patients to very carefully check their labeling of their product simply because in my practice, even with very careful handwriting, I am amazed at how often pharmacists will substitute 2% nitroglycerin for 0.2%, which, as you can imagine, can result in a lot of side effects.

Third, many insurance carriers, despite the presence of publications that cite the effectiveness of Botox in the treatment of chronic fissure in ano, refuse to pay for Botox in the treatment of fissure in ano. Was that a problem in any of your patients? As you cited, one of the problems with using Botox is that it only comes in a 100-unit vial, so that one has to discard the remaining unused portion. I have always been interested in patient requests to use the additional Botox for other body areas.

Last, in any cost analysis of a treatment, other factors contribute to actual cost, including the time to healing of the fissure and the effect of this on actual time taken off from work. Do you have any data regarding this? For example, more rapid healing and fewer days taken off from work, for example, with surgery, could theoretically offset the cost savings that you described.

Dr. Essani and Dr. Kaiser: Thank you for the questions. Regardless of the etiology (constipation or diarrhea), we routinely recommend all our patients as an adjunctive measure to improve stool regularity and consistency, e.g., by means of bulking agents. In our cohort, this probably did not have a sufficient impact as the patients all suffered from chronic fissures with a duration of symptoms of 3 months to 5 years, during which they had attempted these and other nonspecific approaches without success. Apparently, they did not do well with just the bulking agents and dietary compliance alone.

With regard to the second question, we made sure that the patients filled the prescription at our hospital pharmacy to ascertain that 0.2%, and not 2%, ointment was given. In our study, we did not have a built-in mechanism to verify the patients' compliance. Only two patients clearly complained of severe headaches; however, there might be a number of patients in the group failing nitroglycerin treatment who did not apply the ointment as directed, secondary to (underreported) side effects.

In our practice, we attempt to line up several patients on the same clinic day to minimize wasting of the Botox. However, if we are not able to do so, we have to discard the rest. Fortunately, we did not encounter major problems with authorizations, and the patients have not requested to get the rest of the vial.

As to the last question, the typical patient with a chronic anal fissure complains of some persisting local problem that only rarely affects the individual's functionality and ability to work. In that sense, we have not included the fissure healing and the time off work in our analysis; if at all, we would speculate that there would be additional costs (time off work) in the surgery group.

Dr. Henry Pitt (Indianapolis, IN): What percentage of all patients with anal fissure come to this point of having a chronic problem? Is this a majority of patients, or is this a rare problem?

Dr. Essani: The majority of patients in our practice have chronic fissures because we are a colorectal surgery group, and the patients may often be referred to us only when they fail the simple things.

Dr. Pitt: Well, sure, but do most of these people heal on their own and only 5% come to you, or do 80% of all the patients come to you?

Dr. Essani: Unfortunately, we do not have that denominator and it is unlikely that we will ever get that number. A number of patients with acute fissures might not even consult a physician; an even lower number will eventually end up with a specialist. This translates into describing our patient cohort as a group with rather refractory fissures.

Dr. Michael Schoenberg (Munich, Germany): What happened after the 6-month observation period? Are you still following these patients, and what were the results later on?

Dr. Essani: Yes. This is an ongoing study. At this point, they have been followed for a mean duration of 6 months.

Dr. Schoenberg: What are the results? How is it after 1 year, after 1.5 years?

Dr. Essani: We haven't completed our 1-year follow-up yet; it is still in progress.

Dr. Pitt: Is there likely to be recurrence of this problem the longer you follow these patients?

Dr. Essani: That is certainly possible, and that is the reason why we will be following the patients for a longer period of time. However, fissure recurrence has been reported to occur most frequently within the first 6 months.

Abdominal Insufflation With CO_2 Causes Peritoneal Acidosis Independent of Systemic pH

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We have shown that the inflammation-attenuating effects of CO_2 pneumoperitoneum during laparoscopy are not due to changes in systemic pH. However, acidification of peritoneal macrophages in an in vitro CO_2 environment has been shown to reduce LPS-mediated cytokine release. We tested the hypothesis that the peritoneum is locally acidotic during abdominal insufflation with CO_2—even when systemic pH is corrected. Rats (n = 20) were anesthetized and randomized into two groups: continued spontaneous ventilation (SV) or intubation and mechanical ventilation (MV). All animals were then subjected to abdominal insufflation with CO_2. Mean arterial pH among SV rats decreased significantly from baseline after 15 and 30 minutes of CO_2 pneumoperitoneum (7.329 → 7.210 → 7.191, $P < 0.05$), while arterial pH among MV rats remained relatively constant (7.388 → 7.245 → 7.316, P = NS). In contrast, peritoneal pH dropped significantly from baseline and remained low for both groups during CO_2 abdominal insufflation (SV 6.74 → 6.41 → 6.40, $P < 0.05$; MV 6.94 → 6.45 → 6.45, $P < 0.05$). In a second experiment, rats (n = 10) were randomized to receive abdominal insufflation with either CO_2 or helium. Abdominal insufflation with helium did not significantly affect peritoneal pH (7.10 → 7.02 → 6.95, P = NS), and the decrease in pH among CO_2-insufflated animals was significant compared with helium-insufflated animals ($P < 0.05$). Peritoneal pH returned to baseline levels in all groups within 15 minutes of desufflation in both experiments. A significant local peritoneal acidosis occurs during laparoscopy which is specifically attributable to the use of CO_2 and which is independent of systemic pH. These data provide additional evidence that localized peritoneal acidosis is central to the mechanism of CO_2-mediated attenuation of the inflammatory response following laparoscopic surgery. (J GASTROINTEST SURG 2005;9:1245–1252)

KEY WORDS: Carbon dioxide, pH, acidosis, pneumoperitoneum, laparoscopy

The laparoscopic approach to surgery of the alimentary tract is now well accepted for appropriate procedures. Patients undergoing minimally invasive abdominal surgery benefit from less postoperative pain, shorter postoperative ileus, shorter hospital stays, a more rapid return to preoperative activity, and superior cosmesis compared with their laparotomized counterparts.[1–4] Because clinical data have shown that the release of inflammatory mediators is less following laparoscopy than following conventional open surgery,[5,6] the molecular mechanisms underlying the improved results observed following laparoscopic surgery have become an area of active investigation.

Work from our laboratory has shown that peritoneal insufflation with CO_2 blunts the hepatic expression of acute phase genes in laparoscopic models of perioperative sepsis.[7,8] Furthermore, we have recently shown that abdominal insufflation with CO_2, but not helium or air, significantly increases survival among animals with LPS-induced sepsis and that the protective effect of CO_2 pneumoperitoneum is even capable of "rescuing" animals from abdominal sepsis that have already undergone a laparotomy.[9]

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We have also provided evidence suggesting that the mechanism of CO_2 insufflation–specific reduction of the inflammatory response involves interleukin (IL)-10–mediated downregulation of tumor necrosis factor (TNF)-α.[9,10]

Finally, we have shown that the attenuation of inflammation when CO_2 pneumoperitoneum is used during laparoscopy are not due to changes in systemic pH.[11] However, macrophages harvested from CO_2-insufflated peritoneal cavities, as well as peritoneal macrophages acidified in an in vitro CO_2 environment, exhibit blunted proinflammatory cytokine release profiles in response to endotoxin stimulation. Therefore, we hypothesized that the peritoneum becomes locally acidotic during pneumoperitoneum—even when systemic pH is corrected. Additionally, we hypothesized that this effect occurs because of the unique biological properties of CO_2, and thus does not occur when pneumoperitoneum is achieved with a biologically inert gas such as helium.

MATERIAL AND METHODS

General Procedures

All procedures were part of an animal protocol reviewed and approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), 10–12 weeks old and weighing 250–300 g, were housed in cages where standard chow and water were available ad libitum. The animal housing environment was maintained at a temperature of 22 °C with a 12-hour light/dark cycle. The rats were acclimatized to their environment for 3–5 days upon arrival and then fasted overnight prior to intervention. All procedures were performed under aseptic conditions. Anesthesia was induced in an isoflurane chamber for all animals. Maintenance vaporized isoflurane was delivered through a nosecone and, eventually, through an endotracheal tube in the mechanically ventilated animals. Rats randomized to receive mechanical ventilation were intubated with a 14-gauge angiocatheter under endoscopic vision using a 3-mm laparoscope as a laryngoscope as previously described.[12] Ventilator settings for this group were a tidal volume of 2.5 mL and a respiratory rate of 100 breaths/min (minute ventilation = 250 mL/min or approximately 900 ml/kg/min). Catheters for arterial and venous blood sampling made from polyethylene tubing with an outer diameter of 0.965 mm and an internal diameter of 0.58 mm flushed with heparinized saline were placed in the right femoral arteries and left femoral veins under direct vision through 1-cm groin incisions. Arterial and venous blood was analyzed using a portable handheld blood gas analyzer (iStat; Abbott, East Windsor, NJ). Pneumoperitoneum was achieved by delivering either carbon dioxide (CO_2) or helium through an 18-gauge angiocatheter placed percutaneously through the abdominal wall. Insufflation pressure was maintained at 4 mm Hg using a laparoscopic insufflator (Olympus America Inc., Melville, NY). Peritoneal pH was measured using an Accumet AB15 Basic benchtop pH meter (Fisher Scientific International Inc., Hampton, NH). An MI-508 esophageal pH microelectrode (Microelectrodes Inc., Bedford, NH) was placed in a dependant portion of the peritoneal cavity posterior to the liver such that the tip of the catheter was constantly bathed in the small amount of peritoneal fluid present there. The electrode was positioned through the abdominal wall via a percutaneously placed 14-gauge angiocatheter. An MI-402 reference electrode (Microelectrodes Inc.) was inserted into the rectum through the anus. The system was calibrated before each animal by immersing the tips of the pH and reference electrodes in sterile commercially prepared buffer solutions (Fisher Scientific, Fair Lawn, NJ) of pH 7.0 and pH 4.0. All animals were euthanized via anesthetic overdose at the end of the experiments. Figure 1 shows the setup for these procedures.

Effect of Mechanical Ventilation on Vascular-Peritoneal pH Gradient

Rats (n = 20) were anesthetized and randomized into two groups: continued spontaneous ventilation or intubation and mechanical ventilation. Femoral artery and vein catheters were inserted and a peritoneal pH probe was placed behind the liver as described above. Rats randomized to the mechanical ventilation group were intubated and mechanically ventilated. Baseline pH measurements were obtained (t = 0 minutes), and all animals were then subjected to abdominal insufflation with CO_2 at 4 mm Hg for 30 minutes. Additional pH measurements were obtained after 15 minutes of insufflation (t = 15 minutes), at the end of 30 minutes of insufflation (t = 30), 15 minutes following desufflation (t = 45 minutes), and 30 minutes following desufflation (t = 60 minutes).

Effect of CO_2 or Helium Gas on Vascular-Peritoneal pH Gradient

Rats (n = 10) were anesthetized and then randomized to receive abdominal insufflation with either

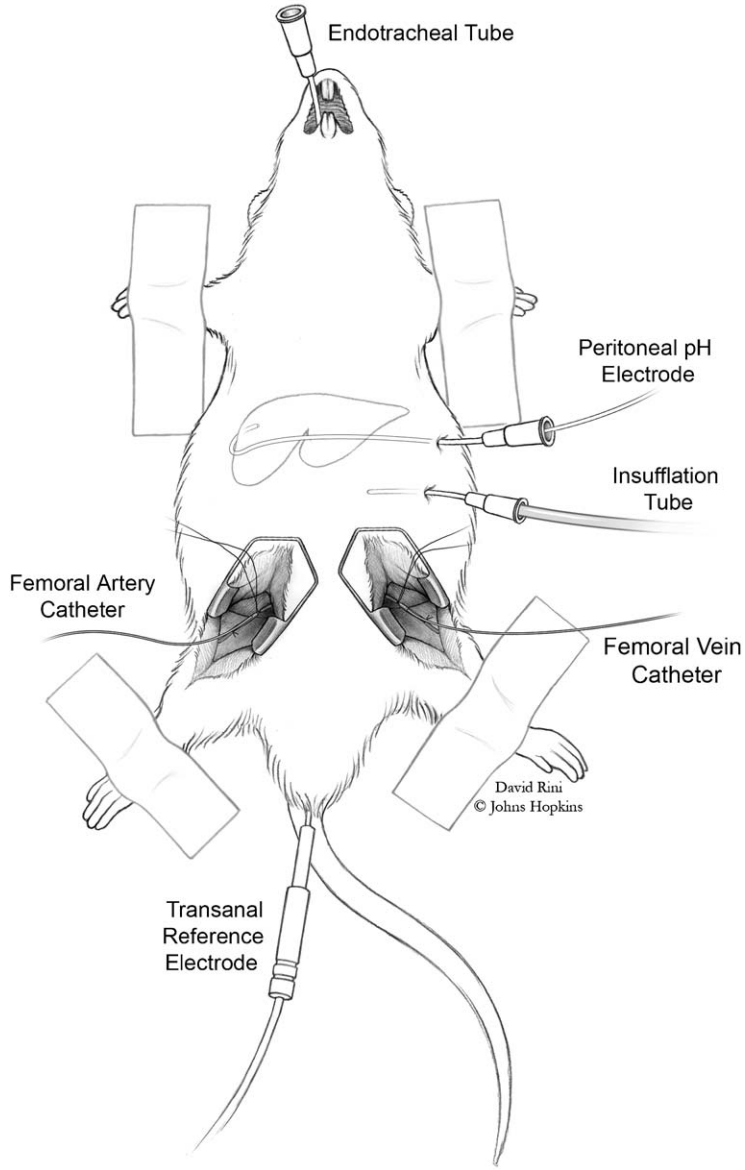

Fig. 1. Setup for rodent pneumoperitoneum, mechanical ventilation, arterial and venous blood sampling, and peritoneal pH monitoring.

CO_2 or helium gas. Femoral artery catheters and peritoneal pH probes were inserted as described above. All animals maintained spontaneous ventilation, receiving vaporized isoflurane through a nosecone. pH measurements were again obtained at baseline (t = 0 minutes), after 15 minutes of insufflation (t = 15 minutes), at the end of 30 minutes of insufflation (t = 30), 15 minutes following desufflation (t = 45 minutes), and 30 minutes following desufflation (t = 60 minutes).

Data Analysis

Mean arterial blood, venous blood, and peritoneal pH measurements after insufflation were compared with average baseline parameters using the Student's *t* test. Mean change in arterial blood and peritoneal pH following insufflation with CO_2 was compared with the mean change following insufflation with helium using the Student's *t* test. Differences between groups were considered significant when $P \leqslant 0.05$. Analysis was performed using Excel software (Microsoft Corporation, Seattle, WA).

RESULTS

In order to determine the effect of pneumoperitoneum with and without mechanical ventilation on both peritoneal and systemic acid-base status, pH

was measured in three compartments (arterial, venous, and peritoneal) before, during, and after 30 minutes of abdominal insufflation with CO_2 (Fig. 2). Mean arterial pH among spontaneously ventilated rats decreased significantly from baseline after 15 and 30 minutes of CO_2 pneumoperitoneum (7.329 ± 0.065 → 7.210 ± 0.022 → 7.191 ± 0.056, $P < 0.05$ for both time points, mean ± SD), while arterial pH among mechanically ventilated rats remained relatively constant (7.388 ± 0.022 → 7.245 ± 0.136 → 7.316 ± 0.087, P = NS for both time points, mean ± SD). In contrast, peritoneal pH dropped significantly from baseline and remained low for both groups during CO_2 abdominal insufflation (spontaneous ventilation 6.74 ± 0.32 → 6.41 ± 0.40 → 6.40 ± 0.42, $P < 0.05$ for both time points, mean ± SD; mechanical ventilation 6.94 ± 0.25 → 6.45 ± 0.26 → 6.45 ± 0.24, $p < 0.05$ for both time points, mean ± SD). Peritoneal pH returned to baseline levels in both groups within 15 minutes of desufflation. Venous pH also dropped significantly from baseline and remained low for both groups during insufflation with CO_2 (spontaneous ventilation 7.323 ± 0.023 → 7.094 ± 0.007 → 7.078 ± 0.004, $P < 0.05$ for both time points, mean ± SD; mechanical ventilation 7.386 ± 0.040 → 7.148 ± 0.044 → 7.166 ± 0.056, $P < 0.05$ for both time points, mean ± SD).

To confirm that the peritoneal pH effects observed with pneumoperitoneum were due specifically to the biologic activity of CO_2 rather than the mechanical effects of abdominal insufflation, the effect on pH of CO_2 pneumoperitoneum was compared with that of helium pneumoperitoneum (Fig. 3). Abdominal insufflation with helium did not significantly affect peritoneal pH (7.10 ± 0.06 → 7.02 ± 0.09 → 6.95 ± 0.13, P = NS for both time points, mean ± SD). A significant decrease in peritoneal pH over time among CO_2-insufflated animals was again observed (7.16 ± 0.04 → 6.63 ± 0.04 → 6.44 ± 0.26, $p < 0.05$ for both time points, mean ± SD), and this decrease was also found to be significant compared with the helium-insufflated animals ($P < 0.05$ for both time points). Peritoneal pH returned to baseline levels among animals insufflated with both CO_2 and helium within 15 minutes of desufflation.

DISCUSSION

A great deal of evidence now contests the once generally accepted notion that smaller incisions alone account for the observed differences between laparoscopic and conventional approaches to surgery of the abdominal viscera. Pneumoperitoneum has been shown to alter host physiology both through the mechanical effects of abdominal distention/pressure[10,13–17] and through the unique biological activity of carbon dioxide gas.[7–11,18–21] While CO_2 pneumoperitoneum clearly has specific effects on the body's response to inflammation and injury, the mechanism connecting CO_2 insufflation at one end with altered immune function at the other is still relatively disjointed.

One potential target of the mechanism underlying CO_2-pneumoperitoneum–mediated attenuation of

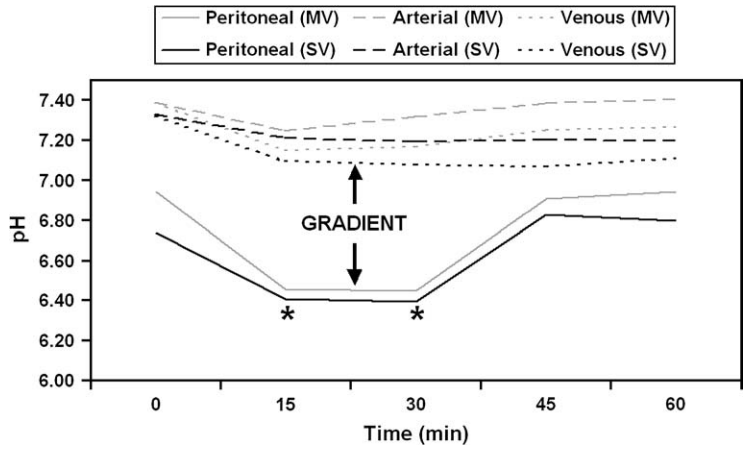

Fig. 2. Arterial (*dashed lines*), venous (*dotted lines*), and peritoneal (*solid lines*) pH before, during, and after 30 minutes of abdominal insufflation with CO_2 among spontaneously ventilated SV (*black lines*) and mechanically ventilated MV (*gray lines*) rats. A vascular-peritoneal pH gradient develops rapidly following induction of pneumoperitoneum and resolves equally quickly following desufflation. *$P < 0.05$ for peritoneal pH among both spontaneously ventilated and mechanically ventilated animals compared with preinsufflation baseline parameters.

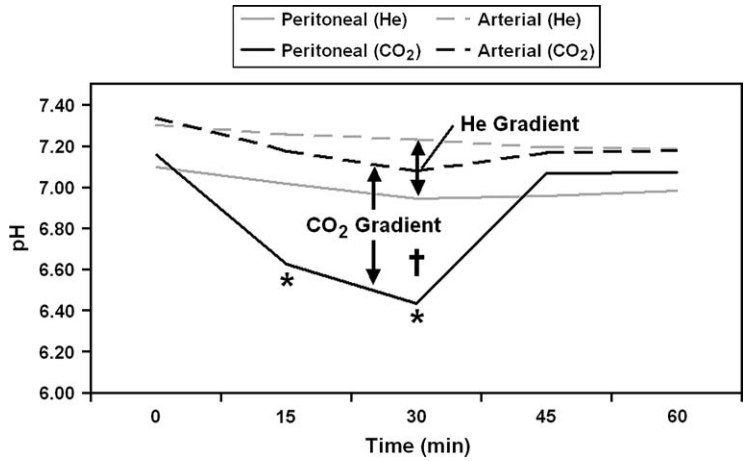

Fig. 3. Arterial (*dashed lines*) and peritoneal (*solid lines*) pH before, during, and after 30 minutes of abdominal insufflation with CO_2 (*black lines*) or helium (He, *gray lines*) among spontaneously ventilated rats. The significant vascular-peritoneal pH gradient that develops immediately following insufflation with CO_2 does not occur with helium. $^*P < 0.05$ for peritoneal pH among CO_2-insufflated animals compared with preinsufflation baseline. $^\dagger P < 0.05$ for change in both arterial and peritoneal pH following insufflation with CO_2 compared with the change in these parameters following insufflation with helium.

the inflammatory response relates to the effects of CO_2 absorption on acid-base chemistry. While it is well established that systemic absorption of CO_2 leads to increased carbonic acid production in the blood ($CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+$),[19,22–30] this effect was initially dismissed as having little clinical significance given that patients undergoing laparoscopy are virtually always mechanically ventilated and can therefore have this hypercarbic load eliminated through increased minute ventilation.[12,31–35] Indeed, for many surgeons, the inability to correct significant aberrations in systemic pH is reason alone to convert from laparoscopy to an open procedure (or at least to temporarily desufflate the abdomen). However, pH is believed to be buffered so tightly in biological systems precisely because even subtle changes in pH can have profound effects on molecular physiology through changes in protein structure and function. With regard to laparoscopy, data from West et al show that macrophages acidified with CO_2 both in vitro (in culture) and in vivo (during pneumoperitoneum) produce significantly less TNF in response to in vitro lipopolysaccharide (LPS) stimulation compared with exposure to air or helium[36] and that maintenance of normal intracellular pH is required for LPS-stimulated macrophage TNF release.[37] One unavoidable feature of the experimental models used in these experiments is that an effective means of systemic CO_2 elimination is not provided for. However, the data from these experiments still strongly suggest that local cellular acidosis may be important with regard to the function of peritoneal macrophages during and after laparoscopy.

Therefore, because clear evidence of local peritoneal acidosis in the presence of systemically normalized acid-base status is essential to a pH-based mechanism of pneumoperitoneum-mediated inflammation attenuation and because the vascular-peritoneal pH gradient during laparoscopy has not been clearly defined, in the current study we sought to characterize the effects of pneumoperitoneum and ventilation on concurrent peritoneal and systemic pH. Furthermore, to demonstrate what portion of these effects is due specifically to carbon dioxide, we compared CO_2 insufflation to helium insufflation in our model of laparoscopy. We found that while the arterial acidosis that ensues following commencement of CO_2 pneumoperitoneum is corrected with mechanical ventilation and increased minute ventilation, the significant peritoneal acidosis that occurs during CO_2 abdominal insufflation remains regardless of whether the additional CO_2 load is eliminated and the arterial pH corrected. Furthermore, we confirmed that the peritoneal pH effects observed with pneumoperitoneum are due specifically to the biologic activity of CO_2 rather than to the mechanical effects of abdominal insufflation that would occur with any gas (helium in our experiment). The sharp increase in pH to baseline peritoneal levels following termination of insufflation with CO_2 indicates that the peritoneal acidosis of laparoscopy is a transient phenomenon. The persistently low venous pH observed following CO_2 pneumoperitoneum in our

study was likely a consequence of caudal venous stasis both from femoral vessel occlusion and the pressure of abdominal insufflation.

The current study confirms that local peritoneal acidosis—present as a pH gradient relative to the arterial circulation even in ventilated, systemically pH-normalized animals—is a potential and likely candidate piece in the mechanism underlying CO_2-pneumoperitoneum–specific attenuation of the inflammatory response. Future work should aim to verify that the immunomodulatory effects of CO_2-specific peritoneal acidosis can be replicated by recreating the acidic peritoneal environment with a non-gaseous acid. Furthermore, the specific intracellular mechanism whereby acidosis inhibits macrophage release of proinflammatory cytokines requires in depth investigation. Finally, the role of peritoneal acidosis in mediating other benefits of laparoscopy such as decreased postoperative pain, decreased ileus, etc., should be studied.

CONCLUSIONS

We have shown that a significant local peritoneal acidosis occurs during laparoscopy with CO_2. This effect is transient—occurring only during active abdominal insufflation—and is specifically attributable to the use of carbon dioxide. Furthermore, the vascular-peritoneal pH gradient is independent of minute ventilation and systemic pH. These data provide additional evidence that localized peritoneal acidosis is central to the mechanism of CO_2-mediated attenuation of the inflammatory response following laparoscopic surgery.

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Discussion

Dr. Stanley Ashley (Boston, MA): This is a nice study, it is convincing data, and it has an interesting hypothesis that I guess seems so simple that it makes it a little hard for us to believe that the reduction in peritoneal pH with CO_2 is responsible for many of the beneficial effects of laparoscopy. I have a couple of questions about the implications of your findings.

First, I know there have been some studies using other gases, helium or argon, for example. Has anybody ever really demonstrated clinically that there are differences in how people do when you use such inert gases as opposed to CO_2 for a laparscopic operation? One would expect the benefits of laparoscopy to be less.

The second question relates to what your findings mean for where we use laparoscopy. You have demonstrated attenuation of the inflammatory response to something like LPS, but that doesn't mean that if you really had a bacterial challenge that it wouldn't also suppress the immune response to that. So should we stop using CO_2 for perforated appendices or other acute infections?

And then the third question is does this mean that if we were not doing laparoscopy, if we were about to do a laparotomy, should we insufflate the abdomen with CO_2 ahead of time and would that anti-inflammatory effect last through a laparotomy and reduce the negative effects of it?

Thank you for the opportunity to comment.

Dr. Hanly: Thank you, Dr. Ashley. Regarding your first question, there have been a number of studies that have actually looked at the clinical use of other gases. As we all know, CO_2 has become the predominant gas, and that is principally because of the increased risk of venous gas embolism associated with some of the other gases. The nice thing about CO_2, of course, is that because dissolved CO_2 is so easily buffered in the bloodstream, it is more quickly absorbed and is less likely to come out of solution in the circulation. Furthermore, there have also been studies that have looked at immunologic differences between gases, and, indeed, there are data showing that CO_2 has specific immunologic advantages in humans. Other gases that have been studied include air, helium, nitrous oxide, argon, and nitrogen, among others.

The question regarding how these data should influence our choice about when to use laparoscopy is a very good one. When we initially started looking at this phenomenon, we found a much more accentuated difference with the CO_2 groups when there was a more ignited inflammatory response. So obviously the models that we are using are fairly robust with regard to the amount of inflammation that is generated. This suggests that the CO_2 effect is probably much more important in patients who have a large systemic inflammatory response, whether that is from infection or from extensive retroperitoneal dissection, etc.

Regarding whether or not our data suggest that we should insufflate patients for 30 minutes before doing a laparotomy, this is obviously something we have thought a lot about, and the same question

could be asked about septic patients in the ICU, for instance. Obviously we don't think that the data are mature enough yet to begin insufflating patients as a treatment for sepsis and inflammation, but there are data that suggest that the effect of CO_2 on the peritoneal macrophage is relatively enduring, and can last for up to 5 or 6 hours. So it is conceivable that if you could "turn off" the peritoneal macrophages for the period of time during which they are most exposed to surgical stress, you might be able to prevent negative downstream effects of the inflammatory cascade.

Dr. Gerald Larson (Louisville, KY): A simple question about the chemistry. What is the pH of CO_2 in the bottles, and if that is not acidic at that point, is it the chemical reaction when it combines with water that generates the hydrogen?

Dr. Hanly: As my freshman chemistry professor always used to say, "ions don't fly," so, by definition, you cannot measure the pH of a gas. However, I am sure that you all remember learning about Le Chatelier's principle from your undergraduate chemistry courses. Essentially, you have the CO_2 in pressure over a liquid, and as that CO_2 is dissolved in the liquid, it pushes the reaction equation toward carbonic acid and the release of protons. So, yes, the acid generated results from the dissolved CO_2.

Dr. Natalie Joseph (Philadelphia, PA): Since this does modulate our immune response, how does this impact laparoscopy when it is used, for example, in malignancy?

Dr. Hanly: That is obviously a very important question, and some of the early data, of course, were concerningly suggestive that laparoscopy might actually be deleterious in the setting of malignancy. The theory was that the altered immune response gave cancer cells a better chance to grow postoperatively, and this idea was used to explain the relatively high rates of port site metastases observed in the early reports of laparosopic surgery for cancer. However, I think that some of the more recent data we have seen—at least clinically with regard to laparoscopic colon cancer surgery, for example—suggest that laparoscopic approaches to oncologic surgery are not a problem as long as care is taken to handle tissues delicately and use wound protectors, etc. And, of course, there are research groups who are specifically dedicated to looking at this very question.

Barrett's Epithelium After Antireflux Surgery

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Barrett's epithelium (BE), defined as endoscopically visible, histologically proved intestinal-type epithelium in the esophagus, is considered the ultimate consequence of long-standing gastro(duodeno)esophageal reflux disease (GERD). Recent reports suggest that effective antireflux therapy may promote the regression of this metaplastic process. This study aimed to establish whether antireflux surgery (laparoscopic fundoplication) can induce any endoscopic and/or histologic changes in BE. Thirty-five consecutive cases of BE (11 short-segment [SBE] and 24 long-segment [LBE]) were considered. All patients underwent extensive biopsy sampling before and after surgery (mean follow-up, 28 months; range, 12–99 mo). In all cases, (a) intestinal metaplasia (IM) extension (H&E), (b) IM phenotype (high-iron diamine [HID]), and (c) Cdx2 immunohistochemical expression were histologically scored in the biopsy material obtained before and after fundoplication. After surgery, a significant decrease in IM extension and a shift from incomplete- to complete-type IM were documented in SBE. No significant changes occurred in the LBE group in terms of IM extension or histochemical phenotype. A drop in the immunohistochemical expression of Cdx2 protein was also only documented in the SBE group. Antireflux surgery significantly modifies the histologic phenotype of SBE, but not of LBE. (J GASTROINTEST SURG 2005;9:1253–1261)

KEY WORDS: Barrett's epithelium, intestinal metaplasia, Barrett's mucosa, gastro-esophageal reflux

Barrett's epithelium (BE) is currently defined as endoscopically visible, histologically proved, intestinal-type epithelium in the tubular esophagus; BE is divided into two topographical variants (short versus long BE segments) according to the (endoscopically assessed) distance between the gastroesophageal junction and the upper limit of the BE (<3 and $\leqslant 3$ cm).[1]

BE is the final consequence of long-standing gastro(duodeno)esophageal reflux disease (GERD) and it has been associated with a 30-fold risk of esophageal adenocarcinoma.[2,3] The current treatment for BE (proton pump inhibitors [PPIs] or antireflux surgery) is aimed to the subsidiary control of the GERD-related symptoms and prevention of complications; at the present, the accomplishment of the ultimate purpose of reversing metaplastic transformation (and reducing cancer risk) is considered utopistic.[4]

This conviction has been based mainly on endoscopic data, however, without paying specific attention to any posttreatment histologic modifications. More recent follow-up studies, considering both the endoscopic and the histologic assessment of the posttherapy modifications in BE, suggest that effective antireflux surgery may promote the regression of intestinal metaplasia (IM) (while esophageal islands of gastric-type epithelium can still be histologically demonstrated).[5,6]

The extension of BE can range from a focal lesion (restricted to isolated glandular cells) to the extensive transformation of native mucosa, and it is generally

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accepted that a greater extent of esophageal intestinalization coincides with a higher risk of neoplastic transformation. As for the biological typing of the metaplasia, two main histologic/histochemical phenotypes of IM have been described: (a) complete IM (also known as small-intestinal type, or type I, or sialomucin-secreting IM) and (b) incomplete IM (also known as colonic-type or type I/II or sulfomucin-secreting). Incomplete IM (particularly type III IM) is the variant most closely related to the onset of adenocarcinoma.[7] As a consequence, both the extension and the phenotype of intestinalized glands can be considered as markers of cancer risk.

The natural history of IM is only partially understood, but there is evidence that IM is associated with a new expression of transcriptional factors (Cdx1/Cdx2) resulting in the onset/maintenance of an enterocytic phenotype. In humans, Cdx2 expression in normal tissue is restricted to intestinal-type epithelium and is considered a suitable marker of intestinal immunophenotype (in the esophagus as well as in the stomach).[8]

The present study was designed to investigate the histologic changes induced in BE by antireflux surgery by assessing its extension, histochemical phenotype, and Cdx2 immunohistochemical expression in a series of patients before and after antireflux surgery.

MATERIAL AND METHODS

Patient Population

From 1994 to 2003, all BE patients undergoing laparoscopic fundoplication at our institution were invited to join a follow-up study protocol involving clinical, functional, endoscopic, and histologic assessments. Patients with high-grade noninvasive neoplasia (NiN) were ruled out.

Clinical Assessment and Physiologic Studies

GERD symptoms were recorded using a standard questionnaire. Severity and frequency of heartburn, acid regurgitation, pain, and dysphagia were scored and final scores were calculated by adding the severity of each symptom (0 = none, 2 = mild, 4 = moderate, 6 = severe) to its frequency (0 = never, 1 = occasionally, 2 = once a month, 3 = every week, 4 = twice a week, 5 = daily).

Esophageal manometry was performed before and 6 months after surgery, and whenever a patient had recurrent symptoms, using a pneumohydraulic perfusion system. The lower esophageal sphincter (LES) pressure was calculated by averaging the pressures recorded by four side holes positioned at the same level, 90° apart, withdrawing the catheter twice using a motorized pull-through technique at a constant speed of 1 mm/sec, from the stomach to the esophageal body, passing through the high-pressure zone (so the LES pressure was the average of eight pressure recordings). The LES pressure was calculated as the mid-expiratory pressure at the respiratory inversion point. Abdominal and overall LES lengths were calculated as the average distance from the point where the pressure trace rises steadily by at least 2 to 3 mm Hg with respect to the intragastric baseline pressure, the respiratory inversion point (abdominal part), and the point where the pressure trace falls below the esophageal baseline pressure (overall length). Amplitude, duration, and coordination of esophageal contractions generated by swallowing 10 ml of water were also recorded along the esophageal body.[9]

Twenty-four-hour pH monitoring of the distal esophagus was used to evaluate abnormal GERD preoperatively and 6 months after the operation, and whenever the patient had recurrent symptoms, by positioning a glass electrode 5 cm above the upper border of the LES, according to the standard procedure used at our laboratory and described elsewhere.[10] The glass probe was connected to a portable solid state monitor (Digitrapper; Medtronics) and the acid reflux parameters assessed were total percentage of the time when the pH was less than 4, percentage of the time when the pH was less than 4 while upright, percentage of the time when the pH was less than 4 while supine, number of episodes with a pH less than 4, number of episodes with a pH less than 4 lasting more than 5 minutes, and duration of the longest episode with a pH less than 4.

Bile reflux was monitored with a fiberoptic sensor connected to a portable spectrophotometer (Bilitec 2000; Medtronics). The 0.2 absorbance threshold was assumed to indicate bile reflux (the absorbance scale ranged from 0 to 1). To avoid any influence of food, patients were fed with a standardized 1000 ml of banana- or vanilla-flavored liquid diet (Nutridrink® Nutricia, Holland) with proper absorbance characteristics. Data were collected in a portable data trapper and then downloaded onto a computer and analyzed in much the same way as for pH recordings.[11]

Endoscopy

Endoscopy was performed under light sedation with midazolam. Any redness and velvety texture in the esophagus were assumed to indicate nonnative esophageal mucosa, but it was classified as BE only after histologic confirmation (H&E, high-iron

diamine [HID]) of the presence of IM. The following measurements were taken: (a) distance (in cm) from the incisor to the end of the tubular esophagus (i.e., proximal margin of the gastric folds = gastroesophageal junction [GEJ]); (b) distance (cm) from the incisor to the proximal margin of the red, velvety-textured area; and (c) length of BE, defined as the distance between the proximal margin of the BE (histologically confirmed) and the end of the tubular esophagus.[12] Hiatal hernia was considered as the portion of stomach between the diaphragmatic pinch-cock and the GEJ.

BE was distinguished as short (<3 cm) or long (>3 cm) segment. In patients with suspected long-segment BE (LBE), multiple biopsy samples were taken every 2 cm, starting at the distal end; in cases of short-segment BE (SBE), one or more biopsy samples were taken, depending on the length and extension of the BE segment. BE was classified as circular (covering the whole circumference of the esophagus), with "flames" (when it spread mainly like long flames) or "islands" (when it was surrounded by normal squamous epithelium). Any esophagitis, ulcers, or strictures were recorded. Esophagitis was classified according to the Los Angeles system.[13] For the purpose of this study, we considered the endoscopy performed immediately before surgery and the last follow-up endoscopy.

Histology

Biopsy samples were fixed in 10% buffered formalin, embedded in paraffin and stained with H&E. BE was defined as the presence of fully developed goblet cells. The histochemical phenotype of the intestinal metaplasia was assessed by high-iron diamine (HID). According to Jass and Felipe, IM was classified as type I (goblet cells secreting only sialomucins; synonyms: complete-type or mature IM), type II (goblet cells secreting both sialomucins and sulfomucins; synonyms: incomplete-type or immature IM), and type III (only sulfomucins in both goblet and columnar cells; synonyms: incomplete-type or immature IM).[14] The samples were tested for *Helicobacter pylori* using the modified Giemsa stain.

All biopsy samples were evaluated by the same pathologist (M. Cassaro) expert in upper gastrointestinal diseases, who was unaware of whether the biopsy samples had been taken before or after surgery. The pathologic parameters evaluated in the whole set of biopsy samples obtained before and after surgery were (1) IM extension and (2) IM phenotype. IM was semiquantitatively scored as the percentage of intestinalized glands detected in each set of biopsy samples (0 = no IM; 1 = intestinalized glands covering 1% to 30% of the biopsy samples; 2 = IM covering 30% to 70% of the biopsy samples; 3 = IM exceeding 70% of the biopsy samples). For IM phenotype, when different types of IM coexisted in the same set of biopsy samples, type III was considered dominant over type II, and type II over type I.

Conventional Definitions

Excluding any specific reference to the lesion's biological behavior, the following conventional nomenclature was used to describe the clinicopathologic outcome of the BE: (a) "reversion" when no intestinalized glandular mucosa was documented in any of the postsurgical biopsy samples. This meant both gastric-type mucosa without IM, and native-type esophageal squamous epithelium; (b) "partial regression" meant a decreasing prevalence of intestinalized glands in the whole set of biopsy samples obtained at follow-up endoscopy; (c) "persistence (with no changes)" when the extension of IM detected at follow-up endoscopy remained the same as before surgery; and (d) "progression" when the prevalence of intestinalized glands detected at follow-up endoscopy was higher than in the presurgical biopsy set.

According to the Padova international classification, NiN (i.e., dysplasia) was classified as low and high grade.[15]

Immunohistochemistry

The immunohistochemical study was performed in 32 of the original 35 patients (in 3 patients, no more tissue samples were available after performing the histochemical reactions). Sections were dewaxed using xylene and alcohol. Antigens were retrieved in citrate buffer at pH 6 and sections were immunostained using the prediluted anti-Cdx2 antibody and the Vectastain Elite ABC kit (Vector laboratories, Burlingame, CA). The color was then developed using the DAB/Ni peroxidase substrate kit (Vector laboratories). Normal large bowel mucosa was used as a positive control. The sections stained with Cdx2 were evaluated for any nuclear staining in goblet cells or nongoblet columnar cells. Any nuclear staining for Cdx2 was considered positive.[16]

Clinical Outcome

Based on the above-mentioned clinical, physiologic, and endoscopic criteria, surgery was considered as having failed when patients had recurrent symptoms with proved abnormal acid exposure in the distal esophagus and/or recurrent severe esophagitis.

Statistical Analysis

Data were expressed as medians and (ranges or 10th and 90th percentiles, as appropriate). Pairwise comparisons were drawn using the Mann-Whitney test. Fisher's two-tailed exact test was used to compare categorical data. Stepwise logistic regression was used to identify independent predictors of BE regression. A P value less than 0.05 was considered significant.

RESULTS

Between 1994 and 2003, 45 patients with BE underwent laparoscopic antireflux surgery at our institution. This represents 13% of all 354 laparoscopic antireflux procedures performed during said period of time. Thirty-five of these patients (28 men, 7 women; median age, 52 years; range, 20–72) completed at least 1 year of follow-up and formed the study group. The median clinical and endoscopic follow-up was 28 months (range, 12–99). Thirteen patients (37%) had a follow-up greater than 1 and less than 2 years; 13 patients (37%) had a follow-up greater than 2 and less than 4 years, and 9 patients (26%) had a follow-up greater than 4 years.

Surgical Outcome

Thirty-one patients (89%) had a laparoscopic floppy-Nissen fundoplication, three had a laparoscopic Collis-Nissen due to a short esophagus, and one had a Toupet partial fundoplication due to ineffective esophageal motility. All but one had primary surgical repair. Postoperative mortality was zero, and there were no major complications. The mean hospital stay was 4 days (range, 2–16).

Symptomatic and Physiologic Outcome

Before surgery, the patients' symptoms had lasted a mean 27 months (range, 2–300). Table 1 shows the preoperative and postoperative symptom scores: a significant drop in the score was observed for each of the symptoms considered except dysphagia, which was unaffected by the operation (two patients had persistent postoperative dysphagia requiring endoscopic pneumatic dilations). Preoperatively, 74% of patients were chronically on PPI therapy; after surgery, only 7 patients (18%) continued to take PPIs ($P < 0.01$).

Table 1. Preoperative and postoperative symptoms in Barrett's epithelium patients

Symptoms	Before surgery	After surgery	P
Total	16 (16–42)	0 (0–18)	<0.01
Heartburn	8 (3–11)	0 (0–3)	<0.01
Regurgitation	4 (3–10)	0 (0–3)	<0.01
Chest pain	0 (0–11)	0 (0–8)	<0.01
Dysphagia	0 (0–11)	0 (0–9)	NS

Values given as No. (range).

Esophageal manometry was performed in all patients before surgery and in 31 of 35 (89%) repeated the test 6 months afterward. Figure 1 illustrates the presurgical and postsurgical manometric characteristics of the LES: both sphincter lengths and pressures increased significantly after surgery ($P<0.05$), as did the amplitude of esophageal contractions 5 cm above the LES (pre: 45 mm Hg., range: 14–106; post: 52 mm Hg., range: 9–144; $P < 0.01$).

Acid exposure in the distal esophagus was assessed before the operation by 24-hour pH monitoring in all patients, and 31 of 35 patients (89%) repeated the test 6 months later. Figure 2 shows the preoperative and postoperative total acid exposure of the distal esophagus. On a single-patient basis, 4 patients (all with LBE) had recurrent GERD: the times from surgery to recurrence were 4, 9, 24, and 60 months.

Bile monitoring was done preoperatively and postoperatively in 15 patients. Global exposure of the distal esophagus to bile over 24 hours decreased from 3.7% (range, 0–41) before the operation to 0 (range, 0–16) afterward ($P < 0.05$).

Endoscopy

Patients had a median of 2 (range, 1–11) endoscopies before and after surgery (range, 1–9). Hiatal hernia was found preoperatively in 27 patients (77%). After surgery, two patients had persistent hernia and recurrent GERD. Table 2 summarizes the changes induced by surgery on endoscopic esophagitis.

Before surgery, 24/35 patients (69%) had LBE and 11 (31%) had SBE. After surgery, all but one patient still had endoscopic evidence of red velvety areas above the GEJ (Table 3).

Histologic Outcome

A median of five biopsy samples (range, 2–17) were obtained from patients with LBE before and after surgery, while a median of 4 biopsy samples (range, 1–7) were collected from patients with SBE.

1. BE extension (i.e., IM extension)

   After surgery, a reversion of BE was demonstrated in six patients (17%), all with SBE: histologic examination found gastric-type mucosa in only five of them, while a native squamous-type epithelium was documented in one.

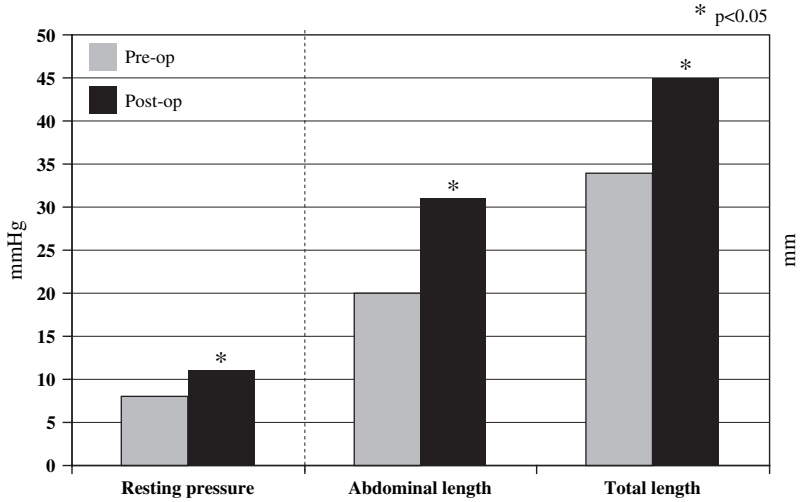

Fig. 1. Preoperative and postoperative manometric characteristics of the LES: sphincter lengths and pressure were significantly increased after surgery ($P < 0.05$). In detail, the preoperative median measurements were LES resting pressure: 8 (10th and 90th percentiles, 5–15) mm Hg; LES overall length: 34 (24–58) cm; LES abdominal length: 20 (12–40) cm. Postoperative median measurements were LES resting pressure: 11 (8–19) mm Hg; LES overall length: 45 (35–63) cm; LES abdominal length: 31 (17–48) cm. Preoperative and postoperative values are statistically different ($P < 0.05$).

Partial regression of IM was recorded in 11 patients (31%), and persistence with no changes in 13 (37%). Histology revealed a progression in five patients (four with LBE and one with recurrent GERD). On the whole, complete reversion or partial histologic regression of IM was recorded in 48% of patients.

2. IM phenotypes (type I [i.e., mature] versus type II-III [i.e., immature] IM)

Considering the whole set of biopsy samples at the initial endoscopy, the immature IM type was more prevalent in LBE than in SBE (10 of 24 versus 1 of 11), although the difference was not statistically significant.

The IM phenotypes found before and after surgery in both SBE and LBE are shown in Table 4. No type III IM was detected in SBE patients after antireflux surgery.

There were no cases of complete reversion of IM after surgery among the patients with LBE. As for the IM phenotypes, sampling after surgery demonstrated a marginal reduction in the prevalence of type II-III IM (18 of 21 patients with LBE segment had type II/III IM preoperatively versus 15 of 21 postoperatively). Two cases of low-grade NiN were detected at presurgical endoscopy: one of these was also demonstrated after surgery, while the other disappeared. None of the patients developed high-grade NiN or adenocarcinoma.

At univariate analysis, histologic reversion/partial regression of IM was associated with SBE and a prevalence of type I IM ($P < 0.05$); at multivariate analysis, reversion/partial regression was associated only with SBE ($P < 0.01$).

In the initial biopsies, 16 of 32 patients had a nuclear immunoreaction to Cdx2 antigen. In the

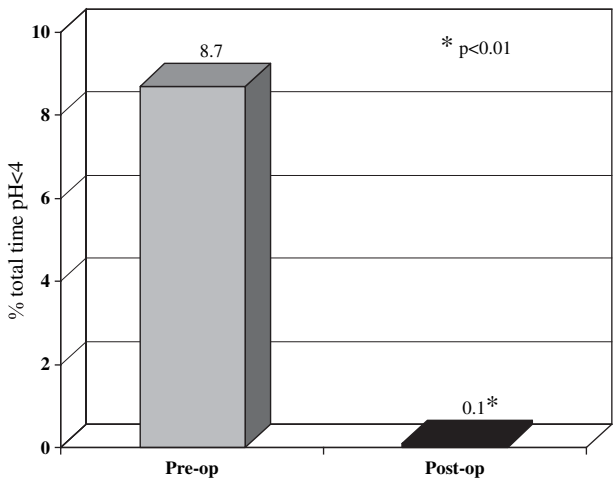

Fig. 2. Percentage total time pH less than 4 in the distal esophagus: preoperative values are 8.65 (10th and 90th percentiles, 2.1–18.73); postoperative values are 0.1 (0–6.95). Four LBE patients had recurrent GERD on 24-hour pH monitoring. Preoperative and postoperative values are statistically different ($P < 0.05$).

Table 2. Changes induced by surgery in endoscopic esophagitis

Grade	Before surgery	After surgery	*P*
No esophagitis	22 (63%)	32 (91%)	0.05
Esophagitis	13 (37%)	3 (9%)	0.02
A: Mucosal break <5 mm in length	3 (9%)	1 (3%)	
B: Mucosal break >5 mm	3 (9%)	1 (3%)	
C: Mucosal break continuous between >2 mucosal folds	4 (11%)	0	
D: Mucosal break >75% of esophageal circumference	3 (8%)	1 (3%)	

follow-up biopsy samples, no changes in Cdx2 expression were seen in LBE, while a lower prevalence of said positive immunoreaction was found in cases of SBE (8 of 11 preoperatively versus 5 of 11 postoperatively). Cdx2 expression was negative in all but one patient, in whom a complete reversion of the BE was histologically documented.

Discussion

BE derives from the esophageal mucosa's long-standing exposure to gastrointestinal juices and intestinalization of the native squamous epithelium has been linked to the morphogenesis of esophageal adenocarcinoma. BE is generally considered irreversible, but the intestinalized glandular epithelium has been seen to disappear completely in a minority of BE patients. Focusing on LBE, an extensive review documented complete BE reversion in 13 of 340 patients (4%), while a partial regression was observed in 12%. BE persisted or even progressed in the vast majority of subjects (83% of patients), however.[3] More recent studies have suggested that eliminating GERD in cases of SBE may result in the intestinalized glands disappearing.[6,17,18]

In patients with GERD, laparoscopic fundoplication leads to a consistent reduction in the exposure of the esophageal mucosa to acid/bile juices, so effective antireflux surgery improves both the endoscopic esophagitis score and GERD-related symptoms.

In the present study, manometry showed that fundoplication increases LES pressure and length, restoring the functional barrier against GERD. As a result, 24-hour esophageal acid and bile juice monitoring showed a significant reduction in esophageal refluxate. Restoring the esophageal luminal environment to normal prompted an improvement in subjective clinical symptoms, associated with a significant decrease in the intake of PPIs. The postsurgical clinical and endoscopic follow-up also showed that the improvements in esophagitis score and clinical symptoms were durable.

Table 3. Endoscopic length of velvety mucosa in short and long Barrett's epithelium (SBE and LBE) patients

	Before surgery		After surgery	
	Median	Range	Median	Range
SBE (n = 11)	2	1–3	1	0–2
LBE (n = 24)	8	3.5–12	6	3.5–12

Most earlier studies on the effects of antireflux surgery focused on changes in the endoscopic length of BE, which is known to be affected by high interobserver and intraobserver variability. To minimize this shortcoming, the present study investigated postsurgical changes in BE phenotype by adopting an extensive presurgical and postsurgical histologic assessment, involving all biopsies being evaluated by the same pathologist, who was given no clinical information.

The results of our study support the hypothesis that effective fundoplication results in a complete reversion of IM in about 50% of cases of SBE. Even in patients whose BE persisted, the comparison between presurgical and postsurgical biopsies demonstrated (a) a significantly reduced extension of IM and (b) a significant change in IM phenotype, from the types more prone to evolve into cancer to the variant associated with a low risk of neoplastic progression (i.e., modification of immature to mature IM).

The findings of our study must be weighed against the likelihood of sampling errors (i.e., of IM being overlooked). While sampling errors cannot be completely ruled out, the following considerations make them unlikely. First, the theory that BE is difficult to biopsy after antireflux surgery is a misconception. The squamocolumnar junction usually lies at or above the top of the fundoplication, which enables the red, velvet-like epithelium to be readily visualized and sampled. In the present series, said epithelium remained endoscopically detectable after surgery in all but one patient.

The number of "reverted" SBE cases is much the same in this as in previous studies, ranging from

Table 4. Preoperative and postoperative intestinal metaplasia (IM) phenotype in short Barrett's epithelium segments and long Barrett's epithelium segments

Short Barrett's epithelium segments			Long Barrett's epithelium segments	
Before surgery	**After surgery**	**IM phenotype**	**Before surgery**	**After surgery**
0/11 (0%)	6/11 (54.5%)*	Absent	0/24 (0%)	0/24 (0%)
8/11 (73%)	4/11 (36.3%)	Type I	4/24 (16.6%)	7/24 (29%)
2/11 (18%)	1/11 (9%)	Type II	10/24 (41.6%)	9/24 (37.5%)
1/11 (9%)	0/11 (0%)	Type III	10/24 (41.6%)	8/24 (33.3%)

*$P < 0.05$ pre- versus post-surgery.

54% to 65%,[6,18,19] supporting the hypothesis that eliminating GERD in cases of SBE can elicit a biological process leading to phenotypic changes in the cells. In all patients whose IM was no longer detectable after surgery, Cdx2 was no longer evident either. Since Cdx2 is an extremely sensitive marker of the presence of IM, this reinforces the histologic observation and offers a hint as to how IM may be reverted. Although the biological events leading to esophageal mucosa intestinalization are still unknown, it may be that the gene(s) responsible for epithelial differentiation (from squamous to intestinal metaplastic epithelium and vice versa) can be "switched on or off," depending on the presence of environmental agents (and acid/bile reflux, in particular). In the present study, the drop in the immunohistochemical expression of Cdx2 protein detected at follow-up biopsy would be consistent with this assumption.

In LBE patients, follow-up failed to document any significant change in the IM's extension or histochemical phenotype. The different outcome of surgery in LBE and SBE could be explained by two alternative hypotheses: either (a) the natural history of BE differs in LBE and SBE patients or (b) LBE and SBE are different stages of the same disease (the short variant coinciding with an early stage, the long segment with more advanced disease), in which case LBE would be a more consolidated form of esophageal metaplasia, less prone to reversion (even after abolishing GE reflux).

In conclusion, surgery is effective in controlling reflux, reducing symptoms and improving esophagitis in patients with BE; the extension of IM is reduced, its phenotype changes to more mature forms, and—in SBE—there is a strong tendency towards a complete phenotypic and genotypic reversion.

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Discussion

Dr. Tom DeMeester (Los Angeles, CA): Dr. Zaninotto, I congratulate you on an excellent presentation, the type of presentations we have become accustomed to receiving from your group. From my review of the manuscript, the work was carefully done and nicely illustrated.

Dr. Zaninotto has told us that he has followed 35 patients with Barrett's, 11 short segment (<3 cm) and 24 long segment (>3 cm), for two years, and has evaluated them for evidence of regression by a variety of measurements. One such measurement of regression was the extent of the intestinal metaplasia grading by the amount of goblet cells per high-power field in the biopsy; the second was the type of mucus formed by the goblet cells, mature and immature, as evidence of a phenotypic change; and the third was the level of expression of the Cdx gene.

We know the density of goblet cells varies throughout the segment of Barrett's esophagus; that is, there are many more goblet cells in the upper end than in the bottom end of a Barrett's segment. My first question is, how did you assure you were getting an accurate sample in order to state that the goblet cell density was reduced?

The most important finding of Dr. Zaninotto showed that complete regression occurred only in short segment Barrett's. There was no regression in the long segment variety. This principle has been well established, and your work confirms that short segment Barrett's can be reversed. In our experience the goblet cells disappear, but the cardiac mucosa remains. Can you tell us if you had similar retention of cardiac mucosa in those who regressed? Did evidence of regression persist on a second endoscopy? In other words, was there a substantial effect? Why do you think patients with the long segment do not regress?

Your findings on the Cdx expression are interesting and show that surgery can be a form of gene therapy. Do you have any evidence for the type of exposure, acid or bile, that induces the gene expression?

You had 5 patients in whom Barrett's progressed after an antireflux operation, 4 with long segment Barrett's. One had recurrence of reflux, and we can understand why he progressed, but the others, why did they progress? Was the Collis gastroplasty done in these patients? That would be important because the gastric segment can still result in increased acid exposure.

I enjoyed the paper. You have provided us with a large amount of information coupled with good insight and discussion. Thank you.

Dr. Zaninotto: Thank you, Dr. DeMeester, for your kind comments. You raised a number of important questions and I'll try to answer all of them. The first question was how we assessed the changes in what we called the "extension" of intestinal metaplasia. Intestinal metaplasia (IM) is patchy, and the presence of metaplastic glands can vary along the Barrett's epithelium: to avoid this problem, all the biopsy samples from a given patient were examined together and IM was semi-quantitatively scored for the pooled set of biopsies.

As for the second question about the phenotype of IM before and after surgery (i.e., presence of complete-type 1 or mature IM, versus incomplete-type 2/3 or immature IM), in the Barrett's epithelium setting, we do not have convincing evidence of a significantly higher risk of adenocarcinoma in type 2/3 than in type 1 IM. In our definition of "regression" we adopted the criteria used in the model of gastric oncogenesis, according to which, type 1 IM is considered more "benign" and less prone to progress to cancer than type 2/3 IM.

The third question addresses the issue of IM disappearing after surgery. In all but one patient, at least two post-surgical endoscopies demonstrated the presence of velvet mucosa (histologically confirmed as cardiac-type mucosa without IM).

Professor DeMeester asked us to try and explain the different behavior documented in patients with the short versus long Barrett's mucosa (BM) segments. I'm afraid we can offer no plausible explanation for such divergent clinical-pathologic outcomes, and the hypothesis that a more "stabilized" IM (associated with long-segment) takes longer to regress remains a mere hypothesis. This consideration may also answer the next question Professor DeMeester made.

As for the last question (whether progression of BM was associated with recurrent or persistent acid reflux as in Collis Nissen), the functional study only demonstrated recurrent reflux in some of the patients considered. The number of observations does not entitle us to draw any final conclusions, however.

Imaging of Acute Mesenteric Ischemia Using Multidetector CT and CT Angiography in a Porcine Model

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Acute mesenteric ischemia, a frequently lethal disease, requires prompt diagnosis and intervention for favorable clinical outcomes. This goal remains elusive due, in part, to lack of a noninvasive and accurate imaging study. Traditional angiography is the diagnostic gold standard but is invasive and costly. Computed tomography (CT) is readily available and noninvasive but has shown variable success in diagnosing this disease. The faster scanning time of multidetector row CT (M.D.CT) greatly facilitates the use of CT angiography (CTA) in the clinical setting. We sought to determine whether M.D.CT-CTA could accurately demonstrate vascular anatomy and capture the earliest stages of mesenteric ischemia in a porcine model. Pigs underwent embolization of branches of the superior mesenteric artery, then imaging by M.D.CT-CTA with three-dimensional reconstruction protocols. After scanning, diseased bowel segments were surgically resected and pathologically examined. Multidetector row CT and CT angiography reliably defined normal and occluded mesenteric vessels in the pig. It detected early changes of ischemia including poor arterial enhancement and venous dilatation, which were seen in all ischemic animals. The radiographic findings—compared with pathologic diagnoses— predicted ischemia, with a positive predictive value of 92%. These results indicate that M.D.CT-CTA holds great promise for the early detection necessary for successful treatment of acute mesenteric ischemia. (J GASTROINTEST SURG 2005;9:1262–1275)

KEY WORDS: Mesenteric ischemia, computed tomography, angiography, animal model

Acute mesenteric ischemia, the process of insufficient blood supply to the bowel, carries consequences ranging from a transient, totally reversible insult to a lethal event. The prevalence of mesenteric ischemia is increasing in the United States as the population ages. It is estimated that nearly 1% of patients presenting with acute abdominal pain have ischemic intestinal disease,[1–3] and it may be responsible for 0.1% of all hospital admissions.[1] The lethality of the disease is well documented, with some series reporting mortality rates exceeding 60%.[4–7]

A critical factor for survival of acute mesenteric ischemia is early diagnosis and intervention. In cases of superior mesenteric artery (SMA) embolism in which surgery is performed once the bowel is infarcted, the mortality rate nearly doubles from 35% to 68%.[8] The mean duration of ischemia preceding surgery for noninfarcted bowel is 13 hours, compared with 21 hours for those with infarction.[9] Thus, the window of opportunity for intervention is narrow and prompt diagnosis is crucial for patient survival. Nevertheless, this goal remains elusive, due

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in part to the lack of an accurate diagnostic imaging tool.

Although angiography is considered the standard of reference for the diagnosis of mesenteric ischemia, it is not widely used because it is invasive, time-consuming, and costly. Computed tomography has been historically used with variable success for evaluating small bowel ischemia. It can help detect ischemic changes in the affected small bowel loops and mesentery. Changes that have been reported include bowel wall thickening and edema, submucosal hemorrhage, increased or decreased enhancement of the bowel wall, mesenteric stranding or fluid, and pneumatosis intestinalis.[10–13] However, these morphologic descriptions are not adequate for the early detection of reversible bowel ischemia. A recent study reported that spiral CT demonstrated a sensitivity and specificity of 64% and 92%, respectively, for diagnosing mesenteric ischemia.[14]

Multidetector CT, combining multiple rows of detectors and faster gantry rotation with narrow collimation, increases scanning speed and virtually eliminates motion and respiratory artifact. These factors allow for improved visualization of the small-bowel wall and distal branches of the mesenteric vessels. Faster scanning also allows for more accurate timing of intravenous contrast bolus administration so that the data can be obtained during both the arterial and venous phases. The resulting improved quality of the three-dimensional reformatted images has allowed for significant advances in CT angiography in the cerebral vessels,[15] pulmonary arteries,[16] and hepatic,[17] renal,[18] and pancreatic vessels[19] of the abdomen. Application of this method to the mesenteric vessels and evaluation of small bowel ischemia has also been reported.[20] Although these studies elegantly demonstrate how CT angiography can image acute mesenteric ischemia, its utility as an accurate diagnostic tool must still be demonstrated.

Here we describe the use of multidetector CT angiography (M.D.CT-CTA) in a porcine model of mesenteric ischemia. Branches of the SMA were selectively identified under fluoroscopic guidance. SMA branch occlusion was performed by injection of microspheres and gel foam and confirmed by angiogram. Standard laboratory analyses were sent preischemic insult and prior to surgical excision. Pigs were imaged using 64-slice helical M.D.CT at 1, 3, or 6 hours postembolization to assess qualitative and quantitative markers of ischemia. These radiologic findings were compared to the findings at surgical exploration and to the gross and microscopic pathological diagnoses.

We report that: (1) M.D.CT-CTA was able to accurately define the porcine arterial and venous mesenteric anatomy. (2) M.D.CT-CTA with three-dimensional reconstruction protocols was able to accurately identify and define the occluded arteries when compared to standard angiography. (3) M.D.CT-CTA was able to detect the early changes of mesenteric ischemia, including poor arterial enhancement and venous dilatation. These two findings were seen in all ischemic animals. (4) The radiographic findings, when compared to microscopic pathologic diagnoses, were used to predict ischemia with a positive predictive value of 92% and a negative predictive value of 80%.

MATERIALS AND METHODS

Animal Preparation, Embolization, and Angiography

Approval was obtained from our institution's subcommittee on research animal care, and animal care was provided in accordance with the *Guide for the Care and Use of Laboratory Animals*.[21] Each pig, weighing between 35 and 40 kg, was placed under general anesthesia with inhaled isoflurane. An endotracheal tube was placed and intravenous access was obtained in an auricular vein.

Angiography of one of the branches of the mesenteric artery was performed using a modified Seldinger technique. A mesenteric arteriogram was obtained by injecting 20 ml of nonionic iodinated contrast material (Ultravist 300, Berlex Laboratories, Montville, NJ). The vessel was embolized using 700–900 micron polyvinyl alcohol particles and gel foam (Contour SE Microspheres, Boston Scientific, Natick, MA) until stasis was achieved in the branch vessel. The catheter was then pulled back to the ostium of the mesenteric artery and an arteriogram was obtained to confirm vessel occlusion.

CT Imaging

Following predetermined time points of 1, 3, or 6 hours after embolization, each pig underwent a dual-phase scanning of the upper abdomen, in the craniocaudal direction, using a 64-slice M.D.CT scanner (SOMATOM Sensation 64, Siemens Medical Systems, Erlangen, Germany). An 18–20-gauge cannula was placed in an auricular vein, and a noncontrast-enhanced CT of the abdomen was performed from the dome of the diaphragm to the level of pubic symphysis, using a detector collimation of 0.6 mm, slice thickness of 3 mm, and a pitch of 0.75. For initiating the arterial phase scanning, the time to peak aortic enhancement was first estimated by a minitest bolus of 15 ml of iodinated contrast injected at 4 ml/second. Finally, about 2ml/kg of 300 mg I/ml of iodinated contrast (Isovue 300, Bracco Diagnostics,

Princeton, NJ) was power-injected at a rate of 4 ml/second, followed by 30 ml of saline flush, also injected at 4 ml/second, and arterial phase scanning was performed. Venous phase scanning followed after a delay of 50 seconds. No oral contrast was administered at any time. Four additional, nonembolized pigs underwent the exact same scanning protocol as the control subjects.

For the arterial and venous phase imaging, a detector collimation of 0.6 mm, slice thickness of 1 mm, pitch of 0.75, and tube rotation of 0.5 second were selected. Other parameters were kept constant for each phase of scanning, such as kVp of 120 and effective mAs of 200–240. Source images were reconstructed at 50% overlap. The reconstructed images were processed to create two-dimensional and three-dimensional maps for the mesenteric arterial and venous system using multiplanar reformation (MPR) and maximum intensity projection (MIP) techniques.

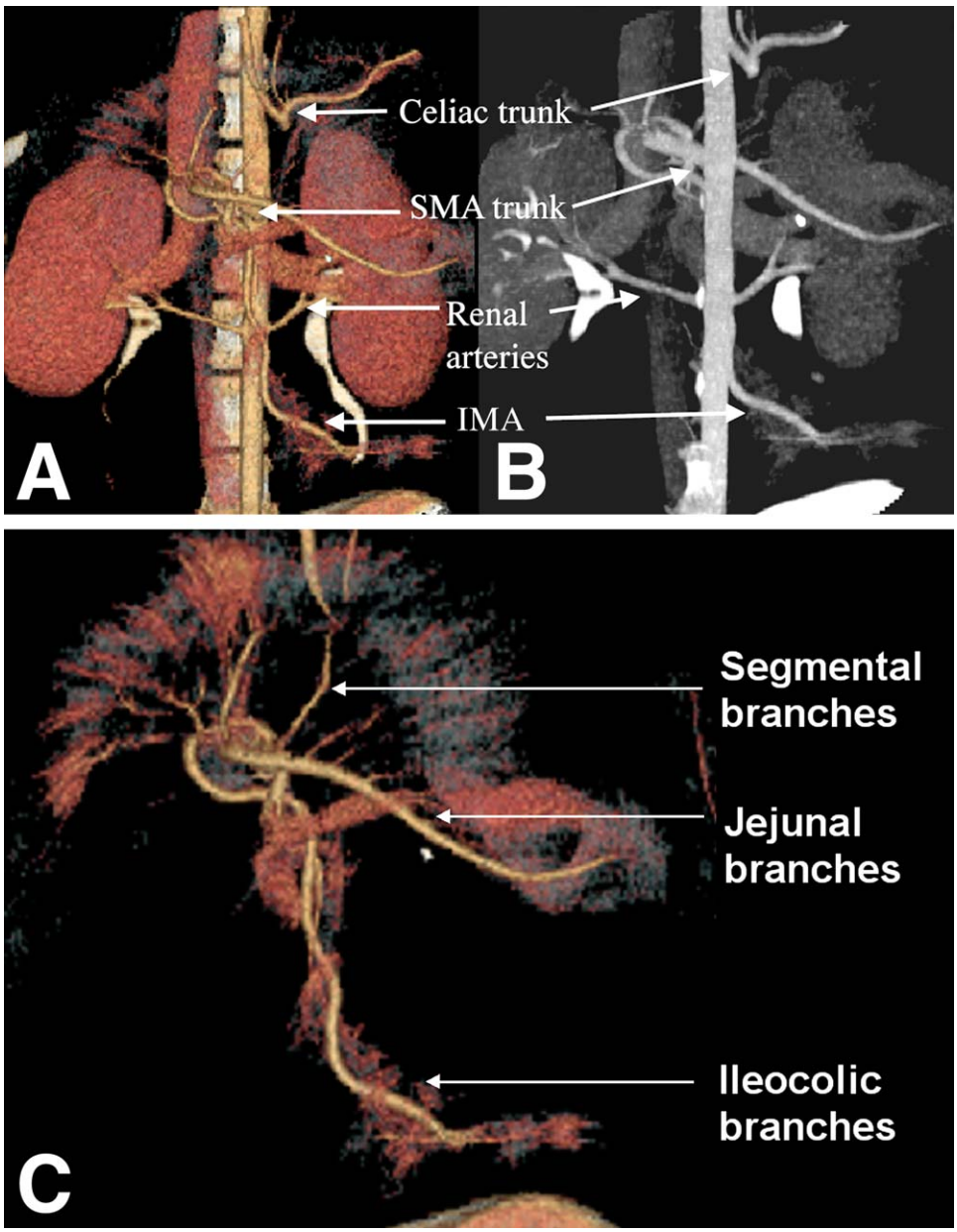

Fig. 1. Normal arterial anatomy in control animal, as visualized by M.D.CT-CTA. Major aortic branches can be clearly seen. (**A**) MIP three-dimensional reconstruction with volume rendered technique. (**B**) MPR two-dimensional reconstruction. (**C**) MIP three-dimensional reconstruction with volume rendered technique reconstruction of SMA anatomy.

Surgery and Pathological Analysis

After the final CT scan, pigs underwent surgical exploration. During laparotomy, the location and severity of bowel ischemia was determined by bowel wall color, the degree of dilation present, inflammatory changes, and the degree of vascular cyanosis present. The diseased bowel segment was then photographed and resected prior to euthanasia. The bowel segments were fixed in formalin and subsequently analyzed for both macroscopic and microscopic pathological changes in three regions: proximal jejunum; the segment comprising distal jejunum and proximal ileum; and distal ileum. A pathologist used a 5-point scale (1 = normal; 2 = mucosal congestion; 3 = loss of superficial glandular architecture; 4 = loss of all glandular architecture, including deep crypts, with necrosis of the muscularis mucosae; 5 = transmural necrosis, including muscularis propria) to rank the level of ischemia in each segment; when heterogeneity was present, the most ischemic value was recorded.

CT Data Analysis

All images were transferred to a postprocessing workstation (Advantage Windows; GE Healthcare Information Technologies, Milwaukee, WI) where they were analyzed in a consensus mode by two radiologists who remained blinded to the type of embolization procedure used on each animal. A subjective assessment was performed focusing on the presence of the following signs: reduced small-bowel wall enhancement, mural thickening, mesenteric stranding or fluid, congestion of small mesenteric veins, ascites, and pneumatosis intestinalis.

For the quantitative assessment of small-bowel perfusion, enhancement measurements were performed in multiple small (mean diameter, 1.5 mm) elliptical regions of interest in the small-bowel wall within three separate, predefined regions: the proximal jejunum, the segment comprising the distal jejunum and proximal ileum, and the distal ileum. Based on the measurements taken for these three areas of small-bowel wall, the mean enhancement was calculated for each data set in Hounsfield units (HU). A bowel segment was graded as one of the following: normal (25–45 HU), ischemic insult (<25 HU), or ischemic insult with bowel hyperemia (>45 HU).

The regions of presumed bowel injury recorded by the radiologists and those resulting from the pathological analysis were compared, and radiologic predictions were rated as true-positive, false-positive, false-negative, or true-negative for statistical purposes.

RESULTS

M.D.CT-CTA Identifies Normal Porcine Arterial and Venous Anatomy

In four control pigs that were tested, M.D.CT-CTA was able to demonstrate normal mesenteric

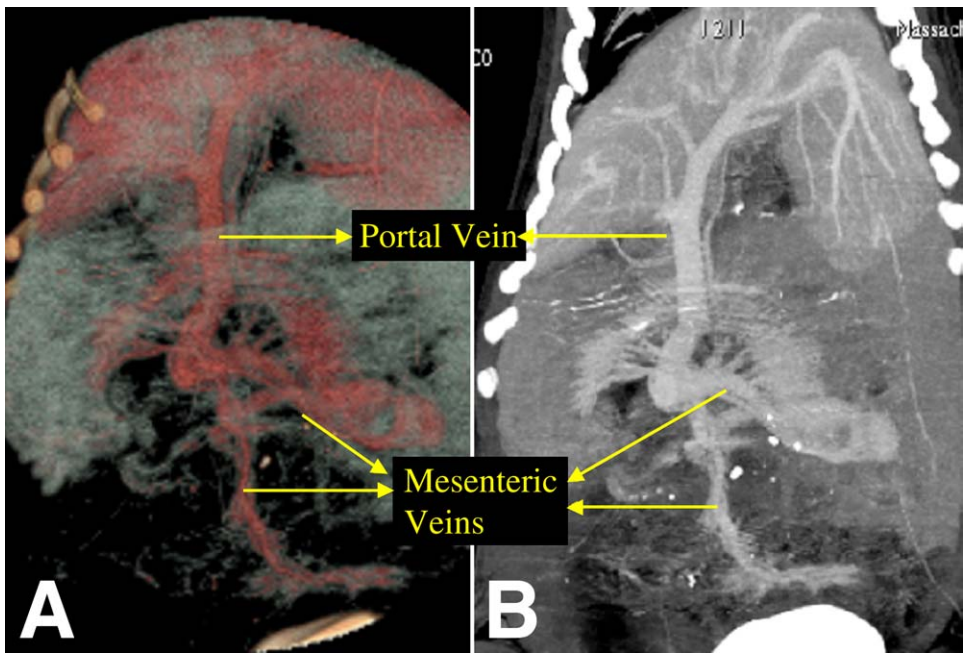

Fig. 2. Normal portal and mesenteric venous anatomy as visualized in control pig by M.D.CT-CTA with (**A**) three-dimensional volume rendered technique and (**B**) MPR two-dimensional reconstructions.

vascular anatomy—both arterial supply (Fig. 1) and venous drainage (Fig. 2) of the bowel were readily identified. MPR two-dimensional reconstruction and MIP three-dimensional reconstruction both reliably identified normal abdominal vascular anatomy. The major branches of the aorta, including the celiac trunk, the SMA, the IMA, and the renal arteries, were all visualized using these reconstruction techniques. Using MIP reconstruction, all major named branches of the SMA, including the jejunal and ileocolic branches, could easily be identified. Furthermore, second-order segmental branches could also be identified readily (Fig. 1, *C*). Normal portal and mesenteric venous anatomy could also be clearly delineated using the MIP and MPR reconstruction techniques; in this manner, the superior mesenteric, inferior mesenteric, and segmental drainage were easily identified (Fig. 2).

Perfusion of healthy bowel was determined by the degree of enhanced signal following contrast injection. Pre-IV contrast values were determined from multiple points in the three bowel regions of interest—the proximal jejunum, the distal ileum, and the segment comprising distal jejunum and proximal ileum. The average signal of nonenhanced bowel was found to be 40 HU (range, 36–44 HU) in all areas of the small bowel, with no variation detected between any of the three regions of interest (Fig. 3, *A*).

The maximum bowel wall enhancement was identified at 60 seconds following injection of intravenous iodinated contrast material (Fig. 3, *B*). At this time point, control animals showed an average contrast enhancement of 70 HU (range, 65–85 HU). Thus, healthy, perfused bowel yielded a mean change in enhancement of 30 HU (range, 25–45). This was consistently found throughout all bowel segments and in all control pigs. These values of healthy, perfused bowel were later used to set objective benchmarks for radiologically diagnosed ischemia.

M.D.CT-CTA Reliably Defines and Identifies Occluded Arterial Anatomy

Six pigs underwent catheter-assisted embolization of SMA branches and were assessed by M.D.CT-CTA

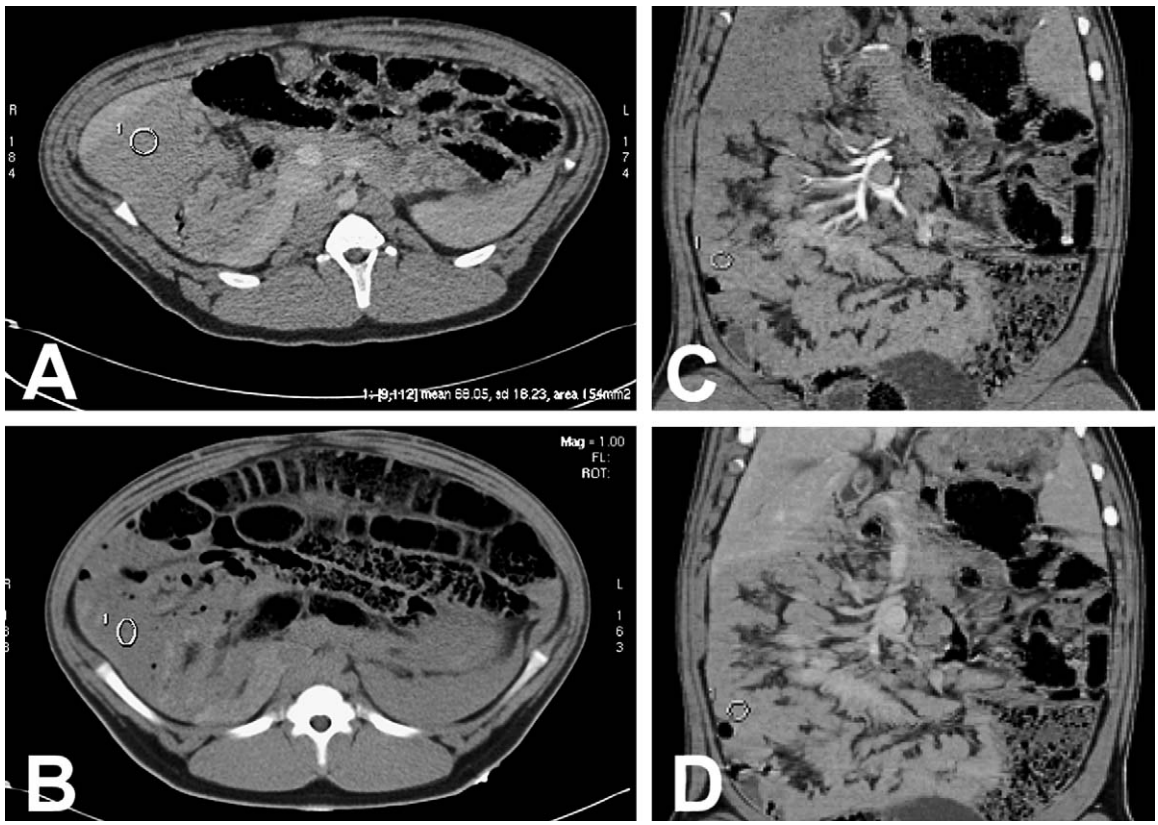

Fig. 3. Axial and coronal CT images of control pigs demonstrating baseline enhancement of the small bowel. (**A**) Postcontrast axial CT image shows jejunal enhancement of 67 HU following iodinated contrast administration. (**B**) Precontrast axial image shows enhancement in the jejunum loops of 41.9 HU. (**C**) Arterial phase coronal reformatted image shows 67 HU enhancement in the small bowel. (**D**) Venous phase coronal reformatted image shows 70 HU enhancement in the small bowel.

to determine if the location of arterial occlusion correlated with standard angiography. In each pig, the branch vessel was embolized, and then the same catheter was used to perform an arteriogram and to confirm vessel occlusion (Fig. 4, *A*, *B*). The vascular anatomy was then studied at designated time points—1, 3, and 6 hours—by M.D.CT-CTA with MIP reconstruction. In all cases, this technique reliably identified the same occluded vessel visualized with traditional angiography (Fig. 4, *C*, *D*).

Catheter-Assisted Arterial Embolization Induces Mesenteric Ischemia

During laparotomy, the location and severity of bowel ischemia was determined by bowel wall color,

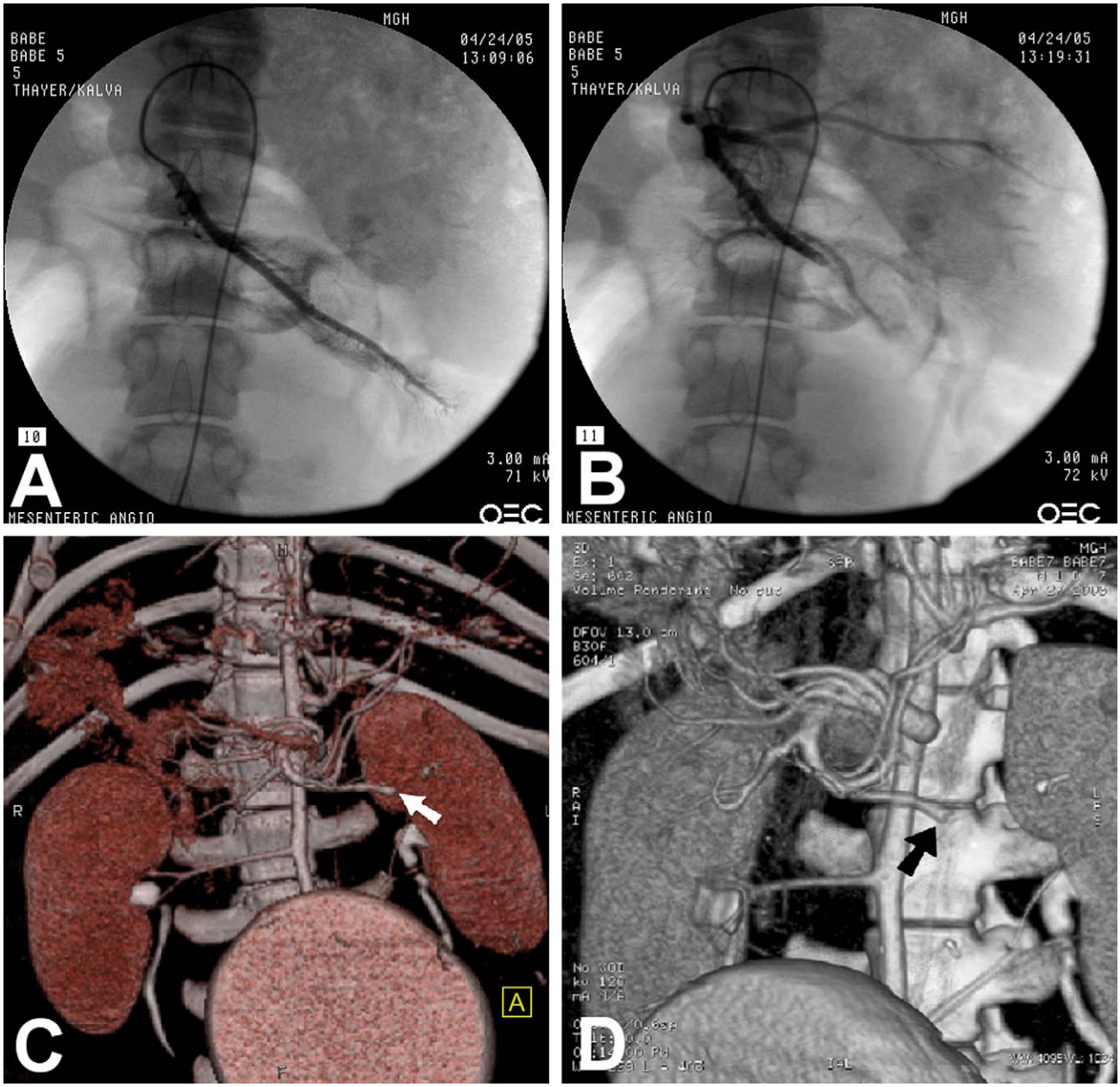

Fig. 4. Accurate identification and detection of occluded arterial anatomy by M.D.CT-CTA. (**A**) Fluoroscopic angiography demonstrates normal filling pattern of jejunal branch of superior mesenteric artery. (**B**) Angiography postembolization reveals occluded jejunal branch. (**C**) M.D.CT-CTA using volume rendered technique reconstruction demonstrates the occlusion of the jejunal branch of the SMA (*white arrow*). (**D**) M.D.CT-CTA using MIP three-dimensional reconstruction demonstrates the occlusion of the jejunal branch of SMA (*black arrow*).

the degree of dilation present, inflammatory changes, and the degree of vascular cyanosis present. Ischemic segments were photographed (Fig. 5) and saved for gross and microscopic pathologic analysis. For each pig, the bowel was divided into three anatomic regions—proximal jejunum; the segment comprising distal jejunum and proximal ileum; and distal ileum—and the grade of ischemia was determined for these regions. A pathologist (M.M.-K.) was asked to histologically evaluate the three regions of interest and grade the injury on a 5-point scale, ranging from normal to transmural necrosis. All occlusion time points resulted in ischemic damage to the small intestine (Fig. 6; Table 1).

The overall level of bowel ischemia in each pig was based on the ischemic grade of the most severely affected segment. Based on this criterion, the overall degree of ischemic insult roughly correlated with the duration of arterial occlusion, as shown in Table 1. Occlusions of 1 hour resulted in bowel injuries ranging from mucosal congestion (Grade 2) to superficial loss of glandular architecture (Grade 3). Occlusions of 3 hours resulted in injuries ranging from Grade 3 to total loss of glandular architecture in the mucosa (Grade 4). Finally, occlusions of 6 hours resulted in injuries ranging from Grade 4 to transmural necrosis with myocyte degeneration and total loss of glandular architecture in the mucosa (Grade 5).

Early Changes in Mesenteric Ischemia are Detectable by M.D.CT-CTA

After embolization of either the jejunal or the ileocolic branches of the superior mesenteric artery, each pig was assigned to remain ischemic for a specific duration of time prior to scanning. Two pigs were selected for each time point: 1 hour, 3 hours, or 6 hours. Following the prespecified ischemic period, each animal underwent analysis by precontrast and postcontrast M.D.CT-CTA.

Initially, a qualitative radiologic assessment was performed, looking for the standard indicators of mesenteric ischemia. Blinded radiologists were asked to comment on the accepted assessments for bowel ischemia: the state of the mesenteric vasculature, bowel wall thickening and edema, submucosal hemorrhage, increased or decreased enhancement of the bowel wall, mesenteric stranding or fluid, ascites, and pneumatosis intestinalis. In all six ischemic animals, even at the latest time points, bowel wall thickening, stranding, and pneumatosis were not identified. Ascites was only detected in one animal.

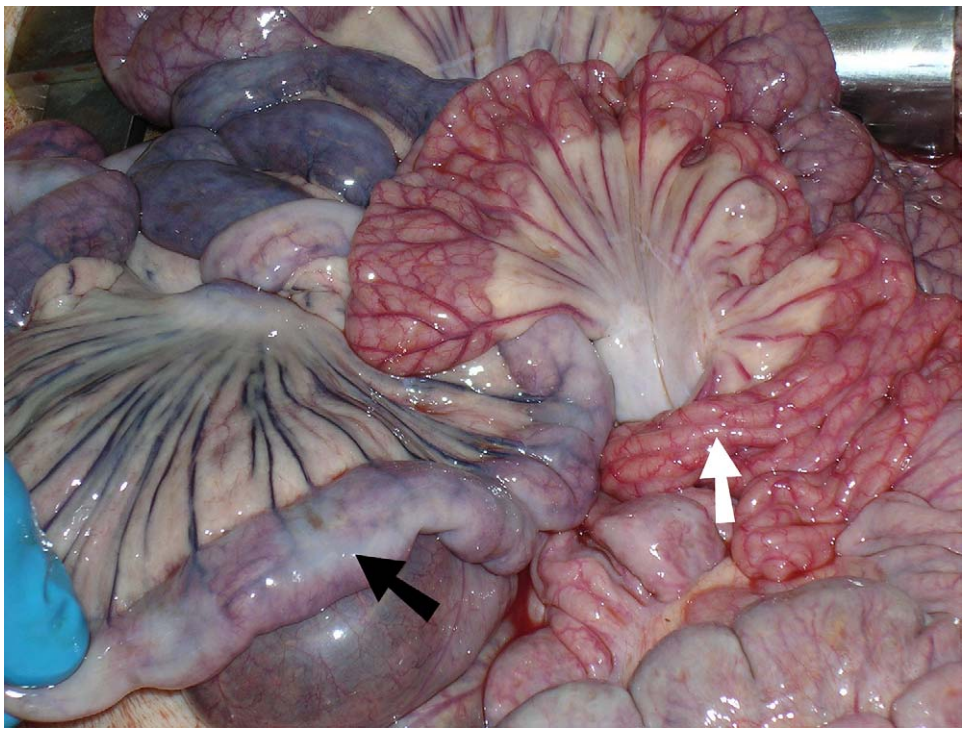

Fig. 5. Six-hour ischemic bowel segment as visualized at laparotomy. Ischemic bowel (*black arrow*) demonstrates cyanosis, devascularization, and darkening of the mesenteric vessels. Surrounding healthy bowel (*white arrow*) is, by contrast, well-vascularized and pink.

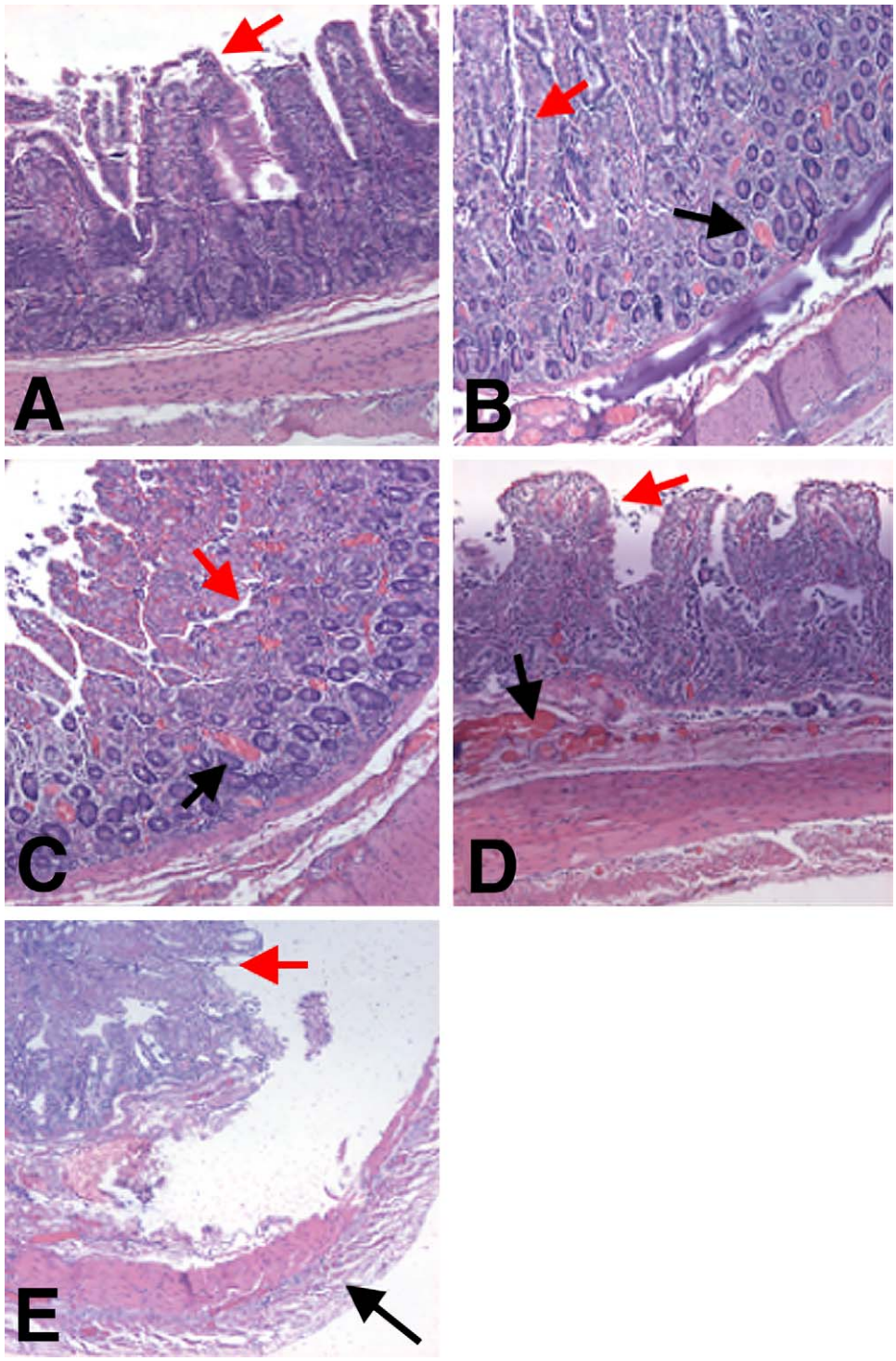

Fig. 6. Histopathology of ischemic bowel, with five representative ischemic grades. Villous structures represented by *red arrows*. (**A**) Grade 1. Normal. No vascular congestion is present, and both the villous architecture and muscular layer are preserved. (**B**) Grade 2. Villous architecture is preserved, with some mucosal congestion and dilated capillaries (*black arrow*). (**C**) Grade 3. Mucosa is congested (*black arrow*) with loss of superficial glandular architecture, but deep villous architecture is preserved. (**D**) Grade 4. The mucosa is completely involved, with loss of all superficial and deep glandular architecture, but the muscular layer is preserved. (**E**) Grade 5. There is total loss of glandular architecture, and the muscularis propria shows degeneration, fragmentation, and myocyte death, all indicative of transmural infarction (*black arrow*).

Table 1. Pathological evaluation of bowel segments at time of laparotomy

Duration of ischemia	No. Pig	Proximal jejunum	Distal jejunum/ proximal ileum	Distal ileum	Overall level of ischemia
1 h	2	1	**2**	**2**	**2**
1 h	6	1	**3**	**3**	**3**
3 h	1	1	**3**	**3**	**3**
3 h	5	1	**2**	**4**	**4**
6 h	3	**2**	**3**	**5**	**5**
6 h	4	1	**4**	**4**	**4**

A 5-point scale of ischemia was used (1 = normal; 2 = mucosal congestion; 3 = loss of superficial glandular architecture; 4 = loss of all glandular architecture, including deep crypts, with necrosis of the muscularis mucosae; 5 = transmural necrosis, including muscularis propria). When heterogeneity was present, the most severe ischemic value was recorded. Ischemic values emphasized in bold face.

However, all ischemic animals, including those imaged only 1 hour after embolization, showed a significant decrease in arterial phase enhancement following contrast administration (Fig. 7), as well as marked venous dilatation (Fig. 8). Thus, these two signs appear to be early and sensitive indicators of bowel wall ischemia. Because decreased arterial enhancement was a sensitive indicator of perfusion in all six ischemic animals, and because it can be easily identified by physicians with a broad spectrum of clinical training, we chose this sign for quantitative characterization.

We therefore considered the measured enhancement values before and after the administration of intravenous contrast as indicators of poor arterial flow to the small bowel. Using the range of normal bowel wall enhancement obtained from the study of control animals, predetermined cutoff points were set to define the level of enhancement indicative of ischemia. A bowel segment demonstrating 25–45 HU change in small-bowel attenuation following contrast injection was considered to be normal. On the other hand, a change of less than 25 HU in attenuation was considered consistent with poor perfusion and ischemia. At the same time, a change in attenuation greater than 45 HU was considered consistent with an ischemic insult causing vascular congestion and hyperemia.

Every embolized pig was found to be ischemic as defined by these criteria, while control pigs were not (data not shown). Areas of diminished enhancement in the arterial phase were readily identified in the supplied segments of occluded arteries (Fig. 7), and these findings were validated by the gross pathological findings at laparotomy (Fig. 6). These radiographic findings were present as early as 1 hour following embolization, before there was a measurable change in serum ischemic markers. They were also seen at the later time points, when markers such as potassium, alkaline phosphatase, and creatine kinase were elevated (data not shown).

M.D.CT-CTA Reliably Predicts the Presence of Mesenteric Ischemia

We finally sought to quantify how reliably the measured enhancement levels predicted mesenteric ischemia in the 18 bowel segments studied (3 segments/pig × 6 pigs). The radiologic prediction for each bowel segment was compared to the corresponding gold standard pathological diagnosis, and each prediction was rated as true-positive, false-positive, false-negative, or true-negative for statistical purposes.

The histopathologic findings are summarized in Table 1, the radiologic findings in Table 2, and Table 3 shows a comparison of the two data sets. Briefly, these data show that M.D.CT angiography correctly predicted ischemia in 12 of 13 bowel segments with pathologically defined ischemia (PPV = 92%). The one false-negative was seen in a 6-hour pig that developed low-level ischemia (Grade 2) in the proximal jejunum and was radiologically classified as normal (Grade 1). Likewise, 12 of 13 segments that were predicted to be ischemic by M.D.CT-CTA were ultimately found to demonstrate pathologic signs of ischemia (sensitivity = 92%). The one false-positive was seen in a 3-hour pig that was found on pathologic examination to have normal proximal jejunum, but it was radiologically predicted to have early ischemia and hyperemia based on >45 HU enhancement. The negative predictive value and specificity were both calculated as 80% (Table 3).

DISCUSSION

Acute mesenteric ischemia is an often devastating disease that is currently the leading cause of the acute abdomen in medical intensive care unit patients[22] and in hemodialysis patients.[23] As the incidences of cardiac and thromboembolic disease increase worldwide, we may expect the same for mesenteric ischemia. Despite the importance of early detection in preventing the high mortality associated with bowel infarction, it continues to pose a diagnostic challenge to clinicians. Angiography remains the diagnostic gold standard, but its implementation is costly and it is not available in all clinical settings. While traditional CT imaging has improved the diagnosis of many abdominal diseases, it has been

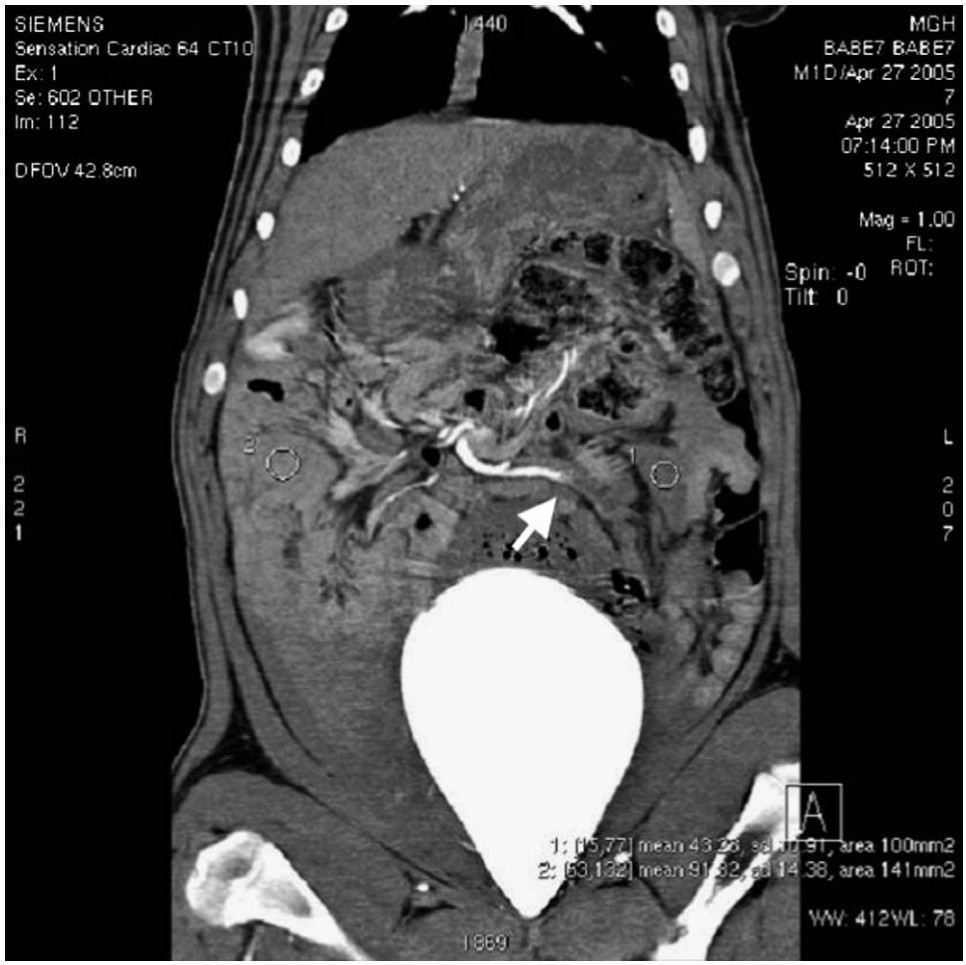

Fig. 7. Coronal reformatted contrast-enhanced CT image of pig abdomen, 6 hours postembolization, demonstrates poor enhancement of distal jejunum at 43 HU (**1**) as compared to 91 HU in the distal ileum (**2**). Occlusion of jejunal branch (*white arrow*) of SMA supplying the nonviable bowel is visualized.

shown to have a sensitivity of only 64% for the diagnosis of mesenteric ischemia.[14] Because of the ability of M.D.CT-CTA to visually represent arterial anatomy and perfusion, it may represent a great improvement over single-detector CT in imaging this disease.

We therefore aimed to study the M.D.CT imaging of acute mesenteric ischemia of the small bowel in a porcine model. First, we demonstrated M.D.CT-CTA readily identifies both normal and occluded vascular anatomy, and the two radiologic signs that appeared to correlate best with the presence of ischemia were venous dilatation and abnormal postcontrast arterial enhancement. These findings were present before the onset of serum chemistry abnormalities that are often characteristic of bowel ischemia.

Using the predetermined criteria of hypo-enhancement (<25 HU) and hyper-enhancement (>45 HU) yields both a sensitivity and positive predictive value of 92%, suggesting that these may ultimately be useful parameters for clinical practice. Whereas areas of severe, irreversible ischemia should logically demonstrate significantly lower enhancement than areas of reversible, less severe ischemia, we were unable to statistically distinguish ischemic grades 3, 4, and 5 based on radiology alone. Bowel that took on a hyperemic character, that is, a change in signal intensity of >45 HU, was not identified in any late ischemic lesions (Grades 3–5). Thus, it appears that M.D.CT-CTA is a highly sensitive indicator of bowel perfusion and ischemia, and it can be used to detect mesenteric ischemia at its earliest stages as well as throughout its progression from early hypo-perfusion to severe, nonviable bowel ischemia. However, our results do not support the use of M.D.CT-CTA to definitively exclude the diagnosis of mesenteric ischemia in the case of a negative radiographic finding, as the negative predictive value was found to be only 80%.

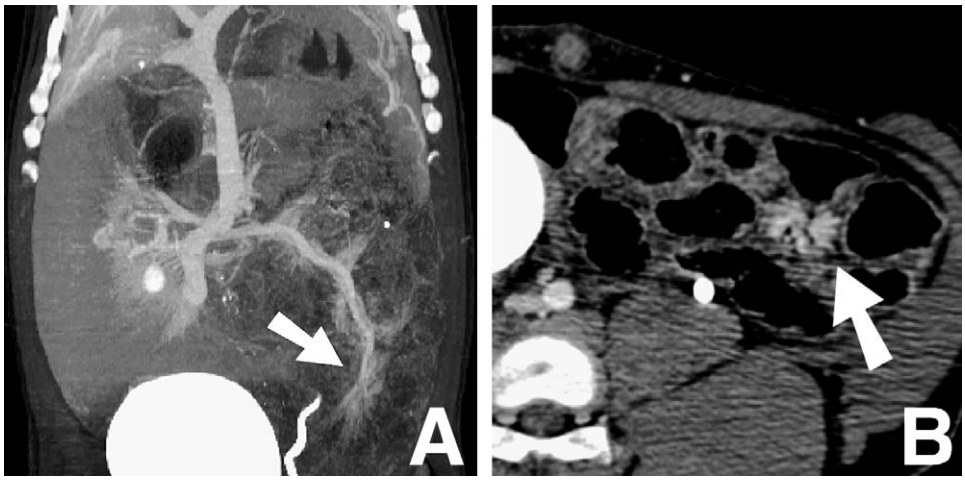

Fig. 8. Venous dilatation (*white arrows*) noted in coronal (**A**) and axial (**B**) images by M.D.CT-CTA (postcontrast injection) at the site of arterial occlusion, indicating ischemia.

The small number of animals studied, and therefore the small number of bowel segments examined, limited the overall statistical significance. Another difficulty was the ability to distinguish various segments of the pig bowel radiographically. While porcine intestinal anatomy closely resembles that of humans, it does differ radically in a few ways; namely, the jejunum and ileum are often transposed, and ileal loops can be difficult to identify on CT. As a result, differentiating much of the pig jejunum and ileum is often not possible; we thus limited our study to only those segments that could reliably be distinguished from one another: the proximal jejunum, the distal ileum, and the intervening segment of distal jejunum and proximal ileum. Future studies may better elucidate the anatomic definition of ischemic segments.

Despite these weaknesses, M.D.CT-CTA appears to represent a great improvement over single-detector CT scanning in its ability to diagnose acute mesenteric ischemia. One of the drawbacks of traditional imaging modalities is that they only identify the end-organ damage resulting from low-perfusion states, such as pneumatosis, mural wall thickening, mesenteric stranding or fluid, and ascites. In contrast, M.D.CT-CTA appears to detect the initial perfusion defects, as opposed to end-organ tissue damage, that are characteristic of this process. It thus

Table 2. Qualitative and quantitative assessment of ischemic bowel segments

Duration of ischemia	No. Pig	Proximal jejunum	Distal jejunum/ proximal ileum	Distal ileum	Qualitative assessment by radiologist
1 h	2	35	**22**	**6**	Distal jejunum and proximal ileum are the worst affected. Venous dilatation is clearly present in the distal jejunum and proximal ileum.
1 h	6	29	**15**	**24**	Proximal jejunum appears normal. Some areas of ileum appear ischemic.
3 h	1	**73**	**62**	**4.1**	Very significant injury, involving a long segment of distal jejunum, entire ileum, and cecum. Only a small segment of proximal jejunum looks viable. The rest of the bowel appears nonviable. Venous dilatation primarily in jejunum.
3 h	5	41	**20**	**0.4**	Distal jejunum looks ischemic, proximal ileum looks ischemic, but not to the point of nonviability. Some venous dilatation present in the proximal jejunum.
6 h	3	31	**4.6**	**0.5**	A long segment of involvement, from the distal jejunum through distal ileum. Very poor enhancement in some areas, likely ischemic/infarcted with nonviability.
6 h	4	40	**12**	**7**	Ileum is most significantly affected. Hyperemia and venous dilatation exist in some areas of jejunum.

Average degree of arterial phase enhancement change in bowel segments over pre contrast imaging by M.D.CT-CTA, listed in Hounsfield units. Boldface signifies segments deemed to be ischemic based on degree of arterial enhancement (>45 HU or <25 HU).

Table 3. Radiographic findings compared with pathologic diagnoses

	Ischemia (+)	Ischemia (−)	Total
Scan (+)	12	1	13
Scan (−)	1	4	5
Total	13	5	18

may represent a sensitive, early indicator of mesenteric ischemia, which is critical in the prevention of irreversible tissue changes and in improving surgical and patient outcomes.

M.D.CT-CTA also has several advantages over traditional angiography. First, it requires less time to mobilize all members of the care team, and acquire data by M.D.CT-CTA, than angiography requires. Second, this technique may be more cost-effective; it requires fewer personnel and less technical expertise, and the M.D.CT equipment may be used for other CT-based imaging studies. Third, because of its lower degree of invasiveness and associated morbidity, it may be performed with a lower index of clinical suspicion, and it thus may represent an ideal screening modality.

This imaging technique may have broad applications in any given clinical situation. Because diminished perfusion is characteristic of all forms of acute mesenteric ischemia, M.D.CT-CTA may be useful in detecting not only the embolic ischemia demonstrated here, but also ischemia from other etiologies such as venous thrombosis, low-flow states (nonocclusive mesenteric ischemia), or acute-on-chronic SMA ischemia. Although there are many facets of M.D.CT-CTA analysis, such as the MIP and MPR reconstructions that can readily identify compromised vascular anatomy, this diagnostic tool is not limited to complex and time-consuming image processing algorithms. Rather, the simple quantification of decreased bowel enhancement has a 92% positive predictive value in this disease, it is easily attainable in most emergency rooms, and it can be easily identified by or communicated to a surgeon.

CONCLUSION

In summary, we have shown that M.D.CT-CTA can be reliably used to diagnose mesenteric ischemia in a porcine model. Venous dilatation and abnormal arterial enhancement were found to be consistent signs of ischemia, as early as 1 hour following arterial occlusion. Based on our quantitative data, arterial phase enhancement of less than 25 HU was generally associated with severe ischemia, and enhancement of greater than 45 HU, which is believed to be a sign of vascular congestion, was seen in less severe ischemia. These parameters taken together predict ischemia with 92% sensitivity and positive predictive value. Any results extrapolated from this animal study must first be validated in clinical trials, but the relatively high sensitivity and positive predictive value of these tests suggest an encouraging avenue for future diagnosis of this disease in human patients.

We thank Arthur Foubert for his technical assistance in handling and operating on the pigs.

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Discussion

Dr. Stanley Ashley (Boston, MA): This is a very nice animal study suggesting that CT might be used for diagnosis in an area where it has not been used previously, mesenteric ischemia. The MGH has proven the utility of CT in the diagnosis of appendicitis, and this study suggests that, in another difficult area, it is going to be useful as well. I have a couple of questions.

First, what is the pathophysiology of the venous dilatation that you saw in the ischemic segments? That is not something that I think about occurring with mesenteric ischemia, and I wonder what that means.

The second relates to the model. A large number of these patients have nonocclusive disease. Microspheres were good for reproducing the pathophysiology of thrombosis or embolus but do you have any data about this technique's utility in low-flow states?

Finally, the data would suggest that you could actually make a distinction between viable and nonviable bowel, preoperatively. One of the big dilemmas in these patients is whether to revascularize, with the inherent morbidity, versus just going ahead and resecting the segment. Do you think this technique might allow us to make that decision a little better?

Dr. Rosow: The first question regards the pathophysiology of venous dilatation. In the earliest stages of ischemia, as seen on CT, we were able to see congestion of the small veins as an indication of early tissue death. From a radiologic standpoint, venous dilatation seems to represent this early ischemic state.

The second question regards the nonocclusive variety. We didn't create a nonocclusive state to yield ischemia, as the end products are relatively similar. We do speculate, because our findings are based on the Houndsfield enhancement unit alone, that even in nonocclusive states, one would be able to see a similar reduction in the amount of arterial phase enhancement.

Our study was not designed to determine whether or not someone can preoperatively assess viability versus nonviability. The results do suggest that there is an objective set of criteria that can be used to determine this. However, our data were used to determine ischemia versus nonischemia, as opposed to early ischemia (viable) versus severe ischemia (nonviable). A greater degree of statistical power is required to make that assessment.

Dr. John Christein (Rochester, MN): I think you are doing important work here in an attempt identify this early group of ischemic bowel patients who are, in fact, salvageable. However, when we see these patients in the emergency department with vague abdominal pain, usually they are just run through the scanner by the ED physician, usually before a surgical consult and typically with standard IV contrast and cuts. Is that completely different than the M.D.CT you are using, and if it is, is there a price difference or technology difference to the M.D.CT scanner?

Dr Rosow. It is actually a fairly similar protocol. It requires simple injection of intravenous contrast, as well as no oral contrast administration. The price difference, I think, would only come in the purchasing of the equipment. Once the multidetector CT scanner is in place, we use a very similar protocol in terms of the computing power and technical help that is required.

Dr. Thomas Howard (Indianapolis, IN): This may be a follow-up to the first question, but with regard to motion artifact, to get those very, very good images that you showed with three-dimensional reconstructions in small peripheral vessels, what was required technically? Is the pig anesthetized and immobilized, how fast are the images obtained, and is technique able to be translated to a clinical scenario where a patient may or may not be able to cooperate with that type of study?

Dr. Rosow: Yes, the pigs are anesthetized and intubated; therefore, perhaps motion artifacts aren't represented as significantly as they could be. However, respiratory artifacts were taken into consideration using this method, so the amount of precision

required to get these angiographic findings using the multidetector protocol is significantly enhanced. However, you are able to do the same protocol on a 16-detector CT as well. It does not require just a 64-detector CT.

Dr. Michael Sarr (Rochester, MN): Great study. Even I could see those occluded vessels. What about using smaller vessels? I am sure you are going to go on and occlude progressively smaller vessels. And are you blinding your radiologists to looking at the small occluded vessels?

Dr. Rosow: In answer to the first question, we have considered using occlusion of the smaller vessels. It is more of a limitation of the technique at this point; really, I think in terms of the availability of the size of the microspheres that can be used for embolization. The radiologists were completely blinded to whether or not the pig was ischemic versus not ischemic, and as to the branch of the artery that was selected for embolization as well. So, they had no knowledge prior to reading of these scans which artery had been embolized.

Regulation of Amino Acid Arginine Transport by Lipopolysaccharide and Nitric Oxide in Intestinal Epithelial IEC-6 Cells

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As a precursor for nitric oxide (NO) synthesis and an immune-enhancing nutrient, amino acid L-arginine plays a critical role in maintaining intestine mucosal integrity and immune functions in sepsis. However, the relationship between intestinal arginine transport and NO synthesis in sepsis remains unclear. In the present study, we investigated the effects of lipopolysaccharide (LPS) and NO on the arginine transport in cultured rat intestinal epithelial IEC-6 cell. Near-confluent IEC-6 cells were incubated with LPS (0–50 μg/ml) in serum-free Dulbecco's modified Eagles's medium, in the presence and absence of the NO donor sodium nitroprusside (SNP, 0–500 μmol/L) and the inducible nitric oxide synthase (iNOS) inhibitor *N*-ω-nitro-L-arginine (NNA, 0–1000 μmol/L) for various periods of time (0–48 hours). Arginine transport activity, arginine transporter CAT1 mRNA and protein levels were measured with transport assay, Northern blot analysis, and Western blot analysis, respectfully. LPS increased arginine transport activity in a time- and dose-dependent fashion. Prolonged incubation of LPS (24 hours, 25 μg/ml) resulted in a 3-fold increase of arginine transport activity (control: 28 ± 5; LPS: 92 ± 20 pmol/mg/min, $P < 0.05$), with the System y^+ as the predominant arginine transport system, and a 2-fold increase of System y^+ *CAT1* mRNA and transporter protein levels ($P < 0.05$). LPS increased the arginine transport System y^+ maximal velocity (V_{max}, control: 1484 ± 180; LPS: 2800 ± 230 pmol/mg/min, $P < 0.05$) without affecting the transport affinity (K_m, control: 76 ± 8; LPS: 84 ± 14 μmol/L, p = NS). The LPS-induced arginine transport activity was blocked by sodium nitroprusside (SNP) (control: 25 ± 6; LPS: 97 ± 26*; SNP: 22 ± 0.4$^+$; LPS+SNP: 33 ± 10.3$^+$ pmole/mg/min, $^*P < 0.01$ and ^+p = NS, compared with control). In contrary, the LPS-induced arginine transport activity was further augmented by NNA (control: 18 ± 3.2; LPS: 59 ± 2.7*; NNA: 26.3 ± 5.8; LPS + NNA: 127 ± 18$^+$ pmol/mg/min; $^*P < 0.01$ compared with control and $^+P < 0.01$ compared with control or LPS). LPS-stimulates arginine transport activity in IEC-6 cells via a mechanism that involves increase of transport System y^+ mRNA levels and transporter protein levels. The LPS-stimulated arginine transport activity is regulated by the availability of nitric oxide. (J GASTROINTEST SURG 2005;9:1276–1285)

KEY WORDS: Lipopolysaccharide, nitric oxide, arginine, undifferentiated intestinal epithelia

Amino acid arginine is a semiessential amino acid in human and weaning rats.[1] It has received much attention in the past decades mostly for being the sole precursor for nitric oxide (NO) synthesis; the latter regulates numerous biological functions.[2,3]

Intestinal epithelial membrane arginine transport is essential in maintaining arginine homeostasis as dietary arginine accounts for 80% of arginine production.[4] Like all other amino acids, intestinal arginine absorption occurs via discrete amino acid transporter systems.[5] The absorbed arginine is utilized either locally with enterocyte or systemically via systemic circulation in various tissues. Major arginine metabolic pathways in enterocyte include[6]

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(1) arginine converted to NO via nitric oxide synthetase (NOS) and (2) arginine degraded to urea or ornithine by arginases.

Sepsis and septic mediators including endotoxin up-regulate NO production, stimulate inducible nitric oxide synthetase (iNOS), and stimulate cytokines production.[7–10] It has been proposed that NO production is associated with sepsis-related gut injury, bacterial translocation, and loss of gut barrier functions.[7,11–13] In various tissue, inducible NO production depends on extracellular arginine and the membrane arginine transport serves as a rate-limiting step in regulating NO production.[14–16]

The purpose of this in vitro study was to study the effect of lipopolysaccharide (LPS) on membrane arginine transport activity and transporter function in the undifferentiated intestinal epithelial cell line (IEC-6) crypt cells, and the relationship between intracellular NO and this LPS-induced arginine transport.

MATERIAL AND METHODS

IEC-6 Cell Cultures

The rat crypt intestinal epithelial IEC-6 cell line was obtained from American Type Culture Collection (Rockville, MA) at passage 13. Cells were routinely maintained in T-150 flasks in a 37°C humidified incubator in 5% CO_2/air. Cells were routinely grown in Dulbecco's modified Eagle medium (DMEM) containing 25 mmol/L glucose and 0.4 mol/L sodium bicarbonate, supplemented with 5% fetal bovine serum (FBS), 4 mmol/L glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 μg/L insulin. The stock IEC-6 cells were passaged weekly following treatment with 0.05% trypsin and 0.02% EDTA. Cells were re-seeded at a density of 4.5×10^6 cells per T-150 flask for future subculturing, seeded into six-well cluster Costar tissue culture plates at a density of 1×10^5 cells per well for Northern blot or Western blot analysis, or seeded in the 24-well cluster Costar tissue culture plates at a density of 1×10^4 cells per well for transport experiments. Near-confluent cells (day 5, passages 20–40) were used for experiments. The day of seeding was designated as day 0. The growth medium was changed every 2 days, and cultures were inspected daily using a phase contrast microscope.

Cell Treatments

Prior to treatments, cell monolayer was washed three times with serum-free media and reincubated in serum-free media (i.e., DMEM supplemented only with penicillin and streptomycin, lacking FBS) containing LPS (0–50 μmol/L) for various periods of time (0–48 hours).

Cells were also treated with NO donor sodium nitroprusside (SNP, 0–1 mmol/L) or NOS inhibitor *N*-ω-nitro-L-arginine (NNA, 0–1 mmol/L). IEC-6 cells remained viable (viability > 99% by dye exclusion) during the treatment period (48 hours).

L-Arginine Transport Measurements

L-Arginine transport activity was measured at 37°C ± 1.0°C. Following pre-treatment of cells with various agents (described above), cells were rinsed three times with "transport buffer" (37°C) comprised of 137 mmol/L choline Cl, 10 mmol/L HEPES/Tris buffer (pH 7.4), 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 2.5 mmol/L $CaCl_2$, and 10 mmol/L leucine. Transport was initiated by simultaneously adding 1 ml of this buffer also containing L-[^{3}H]arginine (2 μCi/ml, 1 μmol/L to 10 mmol/L) into each transport well (24 wells/plate). Each transport plate contained both the control and treatment groups. Cell culture plates were continuously shaken by an orbital shaker (1 Hz) during the uptake period. Uptake was arrested by discarding the transport buffer and washing cells three times with ice-cold transport buffer lacking substrate. Radioactivity of isotope, extracted from the cells with 1 ml 1N NaOH and neutralized with acetic acid, was assayed by liquid scintillation spectrometry. Protein in the NaOH extract was measured using the Bio-Rad protein assay. Initial rates of transport activity were determined during the linear transport period (2 minutes), with zero time points serving as blanks. Transport rates are expressed as nmoles of arginine per minute per milligram of cell protein. System y^+ arginine transport was arginine transport measured in choline Cl uptake buffer that was not inhibited by leucine.

Northern Blot Analysis of System y^+ *CAT1* mRNA

Following pretreatment of cells with various agents (described earlier), cells were rinsed three times with phosphate buffer solution (PBS). Total RNA was isolated from control and treated IEC-6 cells using the "Totally RNA" isolation kit (Ambion, Austin, TX). Total RNA (10 μg) was separated on a 1% formaldehyde gel and transferred to Gene Screen membrane (New England Nuclear, Boston, MA) in 20× standard saline citrate. The membrane was hybridized with an antisense oligonucleotide probe specific to rat *CAT1* (5′-AGTGCCAATG GACATGAGGTCCACCA-3′)[17] and then stripped and rehybridized with an oligonucleotide probe

specific for 18S ribosomal RNA (5′-GTTATTG CTCAATCTCGGGTG-3′). Autoradiographs were scanned with a laser densitometer and the *CAT1* signal normalized to 18S RNA. The *CAT1* probes were 3′ end-labeled using terminal transferase and ^{32}P-dATP, and the 18S probe was 5′ end-labeled using T_4 polynucleotide kinase and ^{32}P-ATP.

Western Blot Analysis of System y^+ CAT1 protein

Following pretreatment of cells with various agents (described above), cells were rinsed three times with phosphate buffer solution. IEC-6 whole-cell lysate was obtained by incubated cells in lysis buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 1.0 mmol/L EGTA, 100 mmol/L NaF, 0.2 mmol/L Na_3VO_4, 1 mmol/L phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin). Equal amounts of protein from control and treated cells were separated on an SDS-PAGE gel and transferred to Immobilon-P transfer membrane (Millipore, Medford, MA). The transfer membrane was then hybridized with polyclonal System y^+ CAT1 protein antibodies (developed in the authors' laboratory) overnight at 4°C and rehybridized with horseradish peroxidase–conjugated secondary antibody (1:2000). Protein was detected using the ECL system (Amersham, Piscataway, NJ).

Statistical Analysis

All experiments were conducted at least in triplicate (including the zero-time blanks), and all experiments were confirmed using at least two independently generations of stock cells. Experimental means are reported ±SD. Comparisons of means were made by ANOVA with pairwise multiple comparisons by the Newman-Keuls method at $P < 0.05$. Transport kinetic parameters were obtained by fitting data to the Michaelis-Menten equation by nonlinear regression analysis using the Enzfitter computer program (Biosoft, Cambridge, UK).

RESULTS

Effect of LPS on Arginine Transport Time Course and Dose Response

To test the effect of LPS on the arginine transport activity, arginine transport was measured in the IEC-6 cells after the cells had been incubated in LPS (0–50 μg/ml) for various periods of time (minutes to 48 hours). LPS stimulated the arginine transport activity in a time-dependent manner. At least 12 hours of continuous incubation was required for LPS to exhibit stimulatory effect on the total arginine transport activity (Fig. 1). A peak stimulation of 50% was observed at 24 hours and the stimulation persisted for at least 48 hours (Fig. 1). The System y^+–mediated arginine transport activity was stimulated as early as 6 hours, peaked at 24 hours (3-fold), and persisted for 48 hours (Fig. 2). LPS stimulated the arginine transport activity in a dose-dependent manner. Significant stimulation was first observed at LPS concentration greater than 5 μg/ml. A 3-fold increase and a 4-fold increase in arginine transport were observed at [LPS] = 25 and 50 μg/ml (Fig. 3),

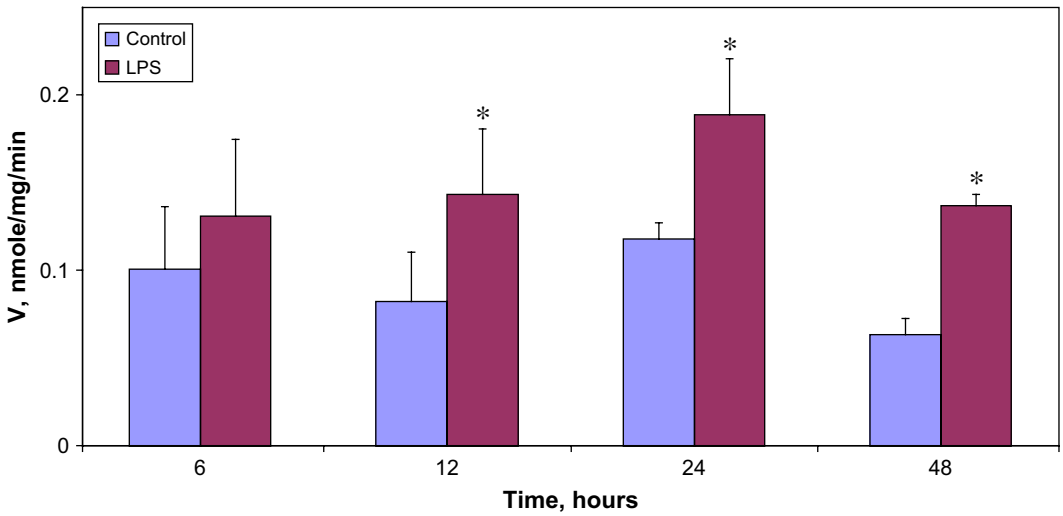

Fig. 1. Effect of lipopolysaccharide (LPS) on arginine transport activity. Na^+-independent transport activity of L-arginine (5 μmol/L) measured in IEC-6 cells incubated in LPS (0–50 μg/ml) for various periods of time (minutes to 48 hours). Transport values are mean ± SD (n = 9, *$P < 0.05$).

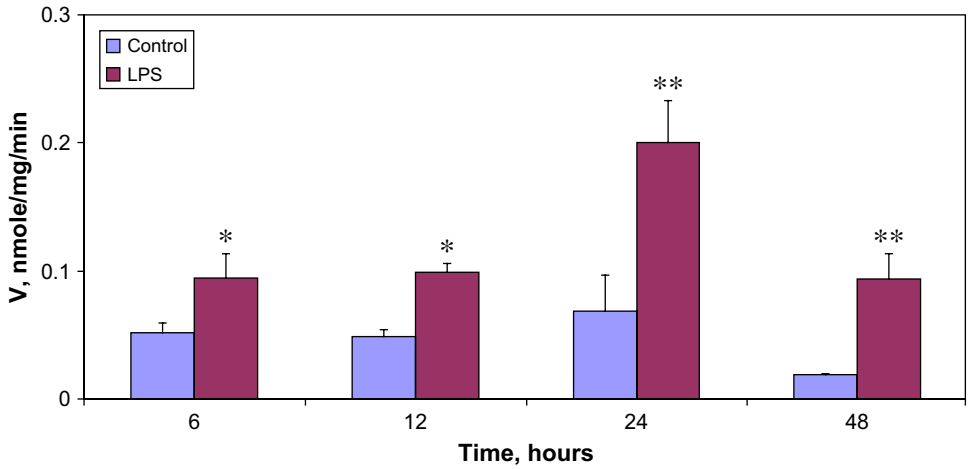

Fig. 2. Effect of lipopolysaccharide (LPS) on System y^+–mediated arginine transport activity. Na^+-independent System y^+ transport activity of L-arginine (5 μmol/L) measured in IEC-6 cells incubated in LPS (0–50 μg/ml) for various period of time (minutes to 48 hours). Transport values are mean ± SD (n = 9, *$P < 0.05$, **$P < 0.01$).

respectfully. A 24-hour LPS (25 μg/ml) treatment was selected for the subsequent experiments in this study.

Effect of LPS on the System y^+ Transporter *CAT1* mRNA, Protein Levels, and the Transport Kinetics

To assess the effect of LPS on the arginine transport System y^+ transporter gene *CAT1* level, *CAT1* mRNA levels were measured in the control and LPS-treated cells. *CAT1* mRNA level was increased nearly 3-fold after 24 hours of continuous LPS treatment (CAT1/18S relative levels: 0.17 ± 0.02 control versus 0.43 ± 0.01 LPS, $P < 0.001$) (Fig. 4).

To assess the effect of LPS on the arginine transport System y^+ transporter CAT1 protein levels, CAT1 protein levels were measured in the control and LPS-treated cells using the polyclonal anti–System y^+ antibody developed in this lab. The System y^+ transporter protein levels were increased 3-fold after 24 hours of continuous LPS treatment (relative levels: 0.18 ± 0.01 versus 0.48 ± 0.03 LPS, $P < 0.001$) (Fig. 5).

The System y^+–mediated arginine transport kinetics was then assessed. Transport of arginine of

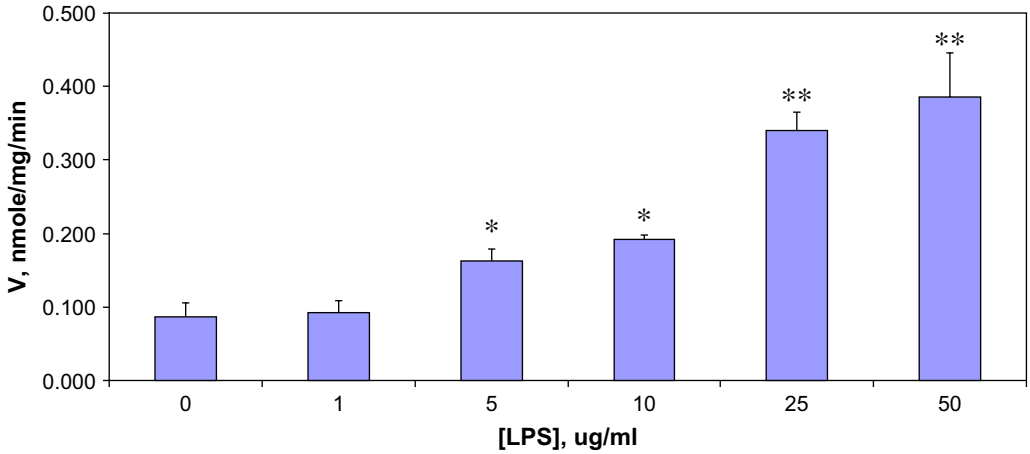

Fig. 3. Dose response of lipopolysaccharide (LPS) on System y^+–mediated arginine transport activity. Na^+-independent System y^+ transport activity of L-arginine (5 μmol/L) measured in IEC-6 cells incubated in LPS (0–50 μg/ml) for 24 hours. Transport values are mean ± SD (n = 9, *$P < 0.05$, **$P < 0.01$).

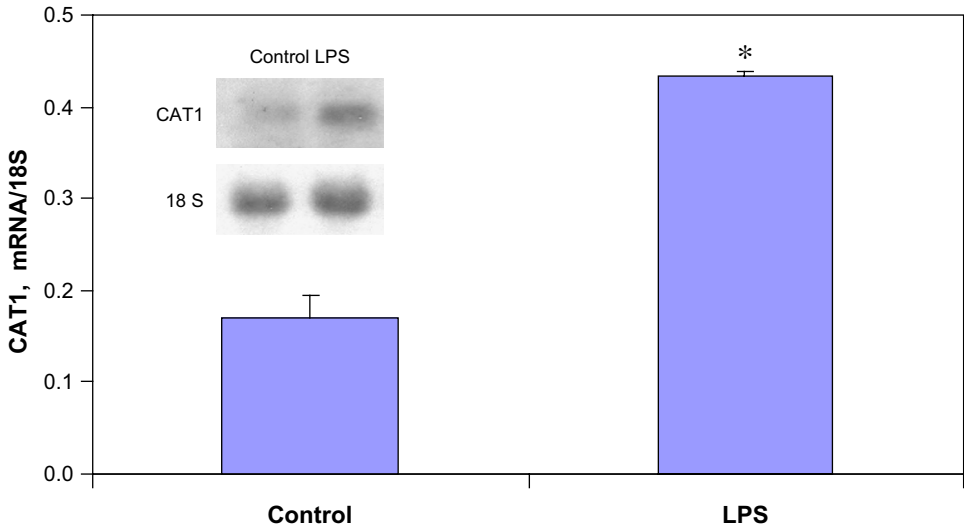

Fig. 4. Effect of lipopolysaccharide (LPS) on System y^+ transporter *CAT1* mRNA levels. Northern blot of arginine transporter CAT1 levels measured in IEC-6 cells incubated in LPS (0–25 μg/ml) for 24 hours. *Inset*: representative Northern blot. CAT1/18S values are mean ± SD (*$P < 0.001$).

various concentrations (1 μmol/L to 10 mmol/L) was measured in the control and LPS-treated cells (Fig. 2). LPS treatment stimulated the arginine transport maximal velocity (V_{max}, 1.48 ± 0.18 nmol/mg/min control versus 2.80 ± 0.23 nmol/mg/min LPS, $P < 0.05$). However, LPS did not significantly alter the transporter apparent affinity (K_m, 76 ± 8 μmol/L arginine control versus 84 ± 14 μmol/L arginine LPS, p = NS) (Fig. 6).

These data suggest that LPS stimulation of glutamine transport involves elevation of transporter mRNA levels and transporter protein levels as well as functional transporter units.

Effect of Nitric Oxide Donor Sodium Nitroprusside on the LPS-Induced System y^+ Arginine Transport Activity and Transporter mRNA Levels

To assess the effect of NO availability on the LPS-induced arginine transport activity, arginine transport was measured in the control and LPS-treated cells in

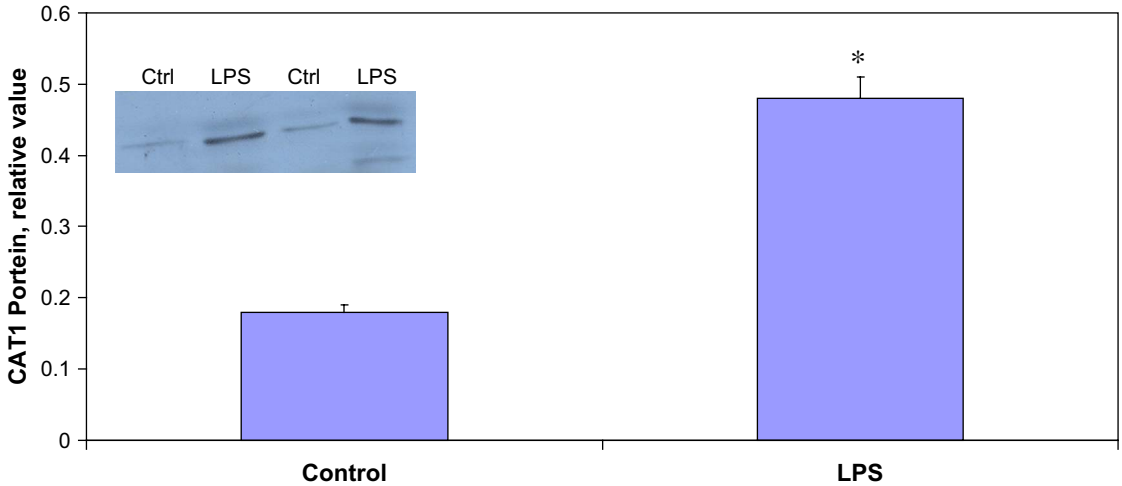

Fig. 5. Effect of lipopolysaccharide (LPS) on System y^+ transporter CAT1 protein levels. Western blot of arginine transporter CAT1 levels measured in IEC-6 cells incubated in LPS (0–25 μg/ml) for 24 hours. *Inset*: representative Western blot. Relative protein values are means ± SD (* $P < 0.001$).

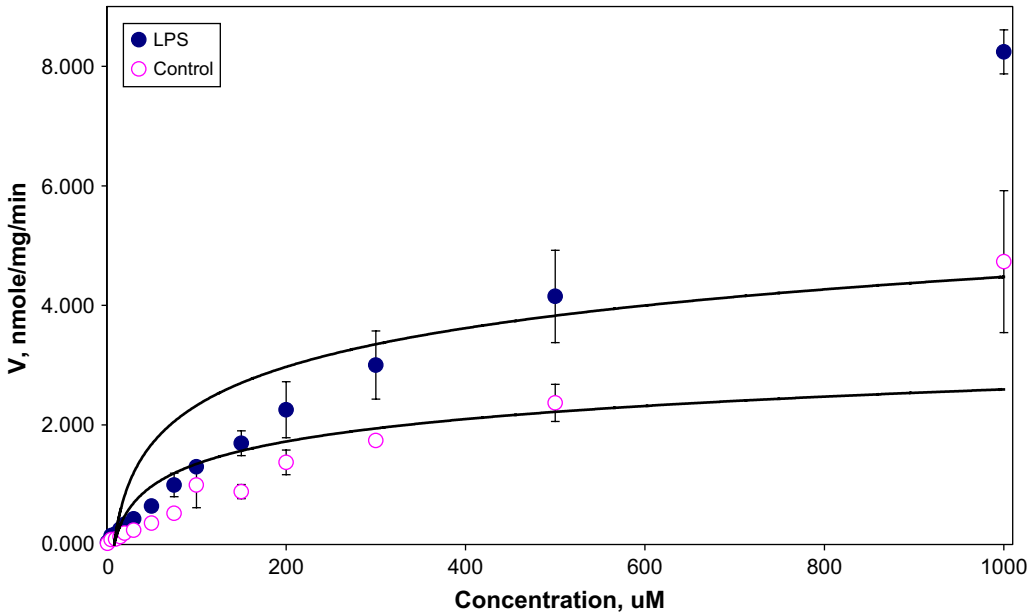

Fig. 6. Effect of lipopolysaccharide (LPS) on System y^+ arginine transport kinetics. Na^+-independent System y^+ transport activity of L-arginine (1–1000 μmol/L) measured in IEC-6 cells incubated in LPS (0–25 μg/ml) for 24 hours. Transport values are mean ± SD (n = 6).

the absence or presence of NO donor SNP (0–500 μmol/L) for 24 hours. SNP completely abolished the LPS-induced arginine transport activity without affecting the arginine transport in control cells. This inhibition starts at a concentration as low as 50 μmol/L (Fig. 7). Similarly, SNP reduced the LPS-induced System y^+ transporter gene *CAT1* mRNA levels (Fig. 8).

These data suggest that availability of intracellular is sufficient to prevent the LPS-induced increase of membrane arginine transport.

Effect of Nitric Oxide Synthetase Inhibitor *N*-ω-Nitro-L-Arginine on the LPS-Induced System y^+ Arginine Transport Activity and Transporter mRNA levels

To assess the role of NO synthesis on the LPS induced-arginine transport activity, arginine transport was measured in the control and LPS-treated cells in the absence or presence of NO synthetase (NOS) inhibitor NNA (0–1000 μmol/L) for 24 hours. NNA did not affect the LPS-induced arginine transport activity even at a high concentration of 1000 μmol/L (Fig. 9). Similarly, NNA had no effect on the LPS-induced System y^+ transporter gene *CAT1* levels (Fig. 10).

These data suggest that inhibition of NOS does not prevent the LPS-induced increase of membrane arginine transport.

DISCUSSION

The objective of this study was to investigate in vitro regulation of intestinal epithelial membrane arginine transport by LPS and the relationship among the LPS-induced arginine transport, cellular NO availability and NOS.

Small intestinal epithelia undergo spontaneous proliferation and differentiation along the villous axis: the undifferentiated crypt cells become more differentiated as they move toward the villous tip, and the more differentiated villous cells display higher absorptive capacity.[18–20] Intestinal epithelial cells absorb intestinal luminal nutrients via discrete transport systems.

Arginine is a semiessential amino acid and an immune-enhance nutrient. The majority of arginine production in humans has a dietary source with endogenous arginine synthesis accounting for 10–15% under normal conditions.[4] Luminal arginine transport across jejunal epithelial membrane into enterocytes, via discrete amino acid transport systems, is essential in maintaining host arginine homeostasis. The absorbed arginine is either utilized within the intestinal epithelia or released into systemic circulation to be utilized by other organs. Sepsis and its mediators such as endotoxin and cytokines alter a wide range of intestinal functions such cytokine production, NO production, intestinal barrier dysfunction, and membrane amino acid absorption.

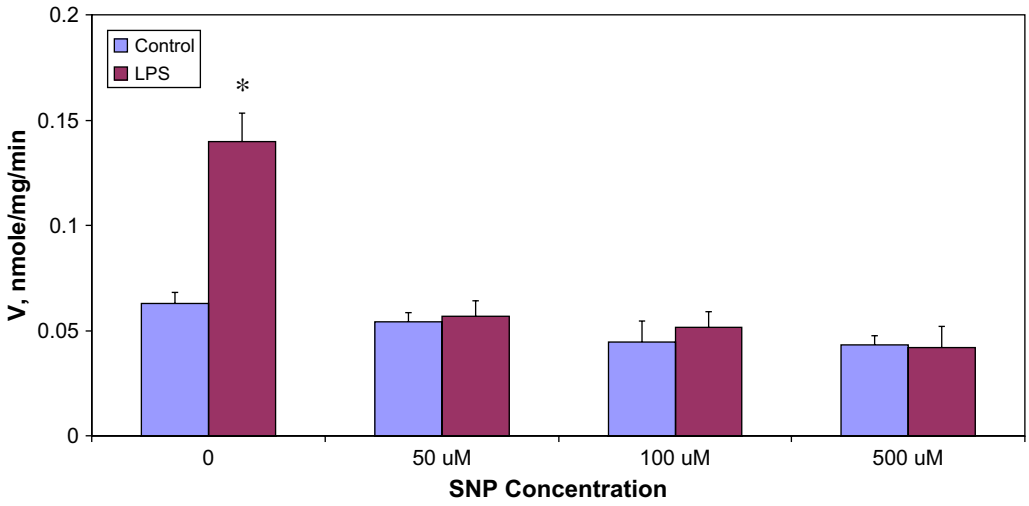

Fig. 7. Effect of sodium nitroprusside (SNP) on the lipopolysaccharide (LPS)-induced System y^+–mediated arginine transport activity. System y^+ transport activity of L-arginine (5 μmol/L) measured in IEC-6 cells incubated in LPS (0–50 μg/ml) for 24 hours ± SNP (0–500 μmol/L). Transport values are mean ± SD (n = 6, $^*P < 0.01$).

In this study, we studied the effect of LPS on the membrane arginine transport and how one of the local arginine metabolites, NO, influences this arginine membrane transport. IEC-6 cell line, derived from rat small intestine crypt cells, undergoes proliferation and grows as a homogeneous population under standard cell culture conditions.[21] Arginine transport across IEC-6 plasma membrane occurs via passive diffusion and Na^+-independently facilitated transport Systems y^+, L, y^+L, and b^{o+}. At a low arginine concentration (5 μmol/L), passive diffusion accounts for less than 1% of the total transport, Na^+-independent System y^+ transport accounts for 70% of the transport, and other Na^+-independent systems account for the remaining 30% of the total transport activity.

The present study is the first to study the regulation mechanism of arginine transport in undifferentiated crypt cells by endotoxin. LPS stimulated the membrane transport activity in a time- and dose-dependent

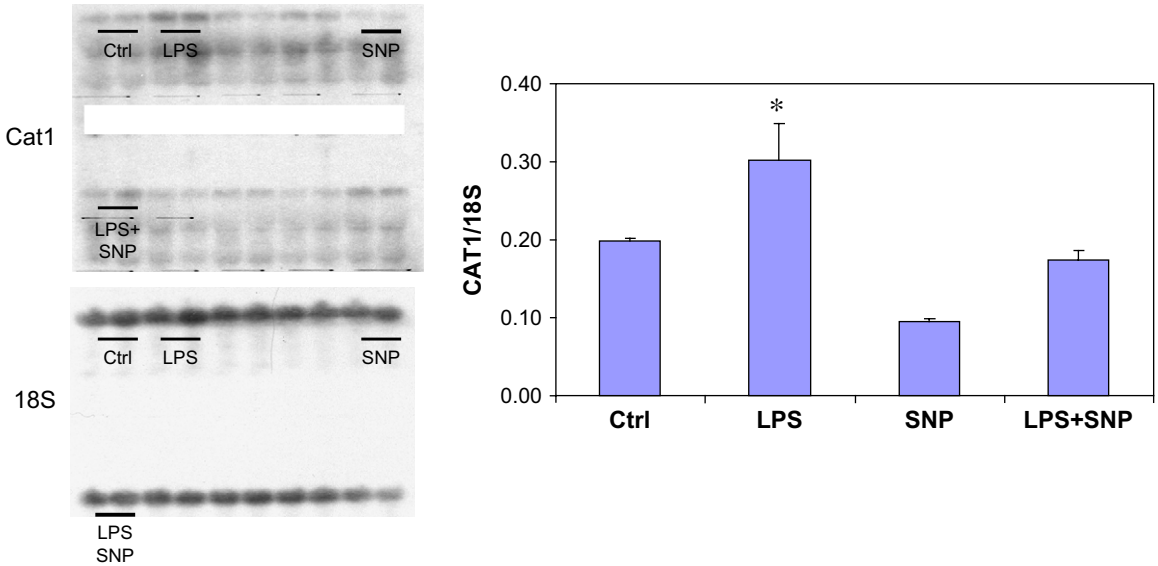

Fig. 8. Effect of sodium nitroprusside (SNP) on the lipopolysaccharide (LPS)-induced System y^+ transporter *CAT1* mRNA levels. Northern blot of arginine transporter CAT1 levels measured in IEC-6 cells incubated in LPS (0–25 μg/ml) for 24 hours ± SNP (0–500 μmol/L). *Inset*: representative Northern blot. CAT1/18S values are mean ± SD ($^*P < 0.001$).

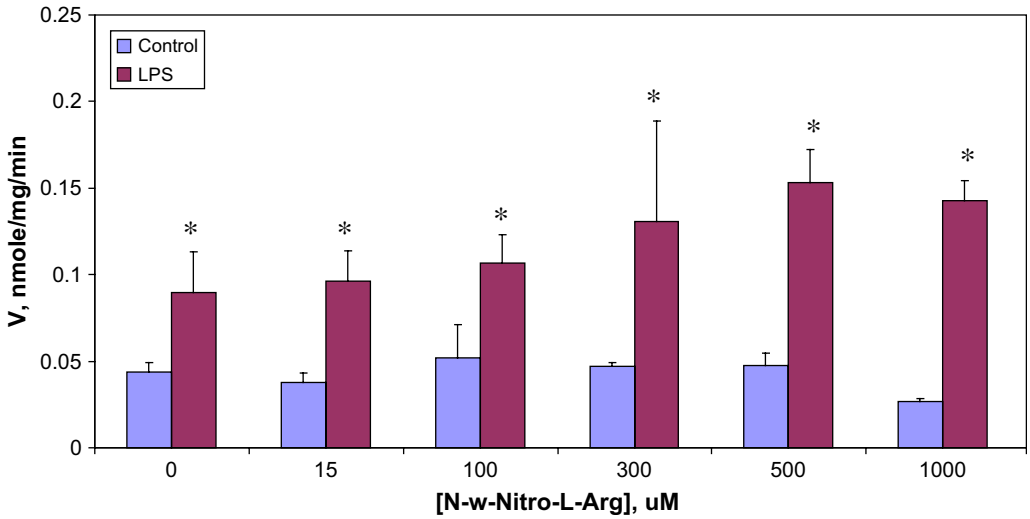

Fig. 9. Effect of *N*-ω-nitro-L-arginine (NNA) on the lipopolysaccharide (LPS)-induced System y^+-mediated arginine transport activity. System y^+ transport activity of L-arginine (5 μmol/L) measured in IEC-6 cells incubated in LPS (0–50 μg/ml) for 24 hours ± NNA (0–1000 μmol/L). Transport values are mean ± SD (n = 6, $^*P < 0.01$).

manner. As shown in Figs. 1 and 2, prolonged LPS exposure was required for LPS to stimulate both the total arginine transport activity (12 hours of exposure) and the System y^+–mediated arginine transport activity (6 hours of exposure). The majority of the LPS-induced arginine transport is mediated by the arginine System y^+. The arginine transport is relatively sensitive to LPS. LPS starts to exhibit stimulatory effect at a concentration as low as 5 μmol/L. The long interval between the LPS exposure and transport activity changes suggests that LPS activates arginine transport via a chronic mechanism. LPS elevates the specific System y^+ transporter CAT1 mRNA and transporter protein levels (Figs. 4, 5). Figure 6 represents the Michaelis-Menton transformation of arginine transport kinetics. Kinetic analysis shows that maximal capacity V_{max} was increased by LPS while transport affinity K_m was unaffected. Taken together

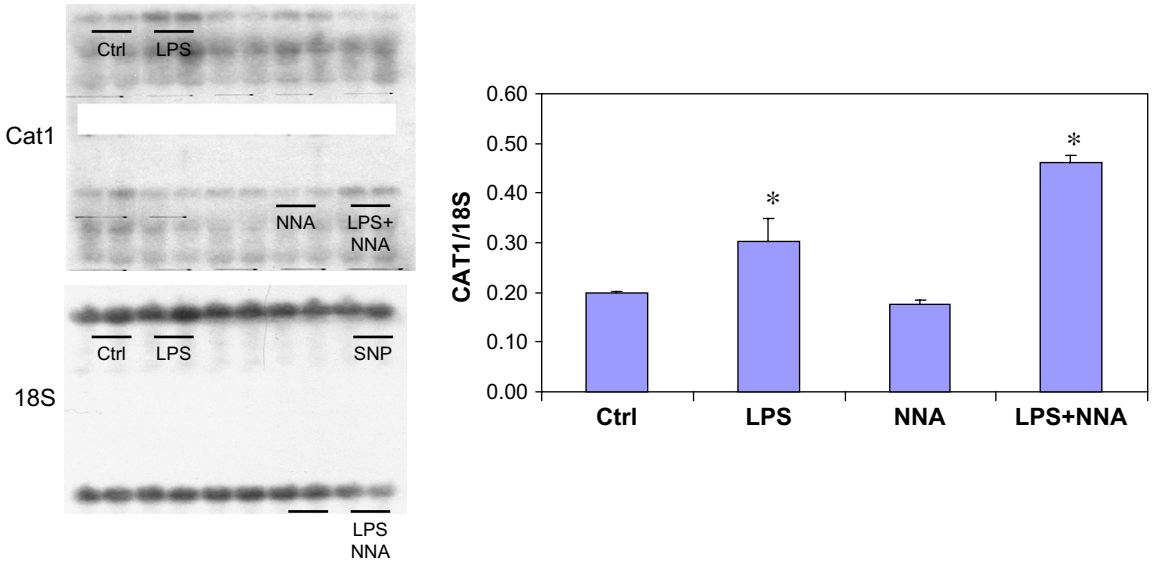

Fig. 10. Effect of *N*-ω-nitro-L-arginine (NNA) on the lipopolysaccharide (LPS)-induced System y^+ transporter *CAT1* mRNA levels. Northern blot of arginine transporter *CAT1* levels measured in IEC-6 cells incubated in NNA (0–1000 μmol/L) for 24 hours ±SNP (0–500 μmol/L). *Inset*: representative Northern blot. CAT1/18S values are mean ± SD ($^*P < 0.001$).

with the increased arginine transporter CAT1 mRNA and transporter protein, the data suggest that LPS stimulates arginine uptake by increasing functional copies of System y^+ transport units rather than modifying transport affinity. The elevation of transporter CAT1 mRNA and transporter protein after LPS treatment (Figs. 4, 5) indicates that LPS stimulates System y^+ arginine transport activity by either specifically enhancing the transcription and the translation of the System y^+ transporter or stabilizing the transcribed mRNA and/or newly synthesized protein.

The absorbed arginine in enterocyte is either utilized within the cells or released to systemic circulation. Intracellular arginine can either be degraded to urea or ornithine or converted to NO by iNOS. Forsythe et al.[7] reported LPS stimulated NO production in IEC-6 cells. Because arginine is the exclusive precursor for NO synthesis, it would be interesting to see how NO affects the arginine transport. As shown in Figure 7, the presence of NO donor SNP[22] abolished the LPS-induced arginine transport activity at a concentration as low as 50 μmol/L. Similarly, SNP abolishes the LPS-induced arginine transporter mRNA levels. These data demonstrate that the availability of NO is sufficient to control the increase arginine demand in septic event. Intracellular NO synthesis is carried by NOS, a process that is inhibited by arginine analogue NNA.[23] As shown in Figures 8 and 9, NNA had no effect on the LPS-induced arginine transport activity and the LPS-induced arginine transporter *CAT1* mRNA level even at a very high concentration of 1 mmol/L. Combined with the SNP data, these data suggest that NO availability, not the NO synthesis process, is critical in regulating the LPS-induced aginine transport.

CONCLUSION

LPS stimulates intestinal undifferentiated epithelial arginine transport activity by increasing the levels of arginine transporter *CAT1* mRNA, CAT1 protein, and functional transporter units. This LPS stimulation of arginine transport is regulated by the availability of intracellular NO.

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Discussion

Dr. Frank Moody (Houston, TX): I appreciate the opportunity to discuss this very elegant piece of work. The results are very clear; when you expose these well-described epithelial cells to lipopolysaccharide, then you increase the movement of arginine into the cell, no question about that. If you give a nitrogen donor nitroprusside, then you inhibit the increased movement into the cell. Inhibition of iNOS reverses this outcome. But lacking are measurements of the abundance of the message, the concentration of the protein, or biologic evidence that in fact nitric oxide was produced in this system.

Now, I am aware of the paper that shows that it is, but I am sure you have some measurements of this in your own system, and so that is my first question.

The second question relates to what happens when you expose the cells in their native environment to lipopolysaccharide. You are aware of Rosemary Kozar's work at the University of Texas in Houston in which using an ischemia/reperfusion rat model in which the intestinal wall is perfused with its intact blood supply and the lumen is perfused with arginine, there is a marked reduction in the mucosal barrier with severe injury micoscopically. My question is, have you carried out your studies in an in vivo model?

Your work with arginine has significant clinical relevance in view of the current controversy as to whether this amino acid may in fact be harmful in patients with sepsis. Should we keep arginine within immune-enhancing diets, because there is a subgroup of patients in intensive care units, usually medical units, which weakens my argument, I guess, but who do not do well, it is thought, because of arginine in the diet. So I would appreciate your discussion on that point.

Dr. Pan: To answer your first question, actually we haven't measured nitric oxide production in our lab. There are many labs that have observed a significant increase of nitric oxide production when they treat IEC-6 cells with lipopolysaccharide.

Your second question is a very important and complex one. As we know, the whole intestine is composed of various types of cells and tissues. These cells and tissues respond to stimuli differently. That was one of the main reasons why we chose IEC-6 cells because they are homogeneous cells and because these undifferentiated cells consumed most of the arginine absorbed. To refer to Rosemary Kozar's work, if we have a segment of intestine, you have a combination of differentiated cells and undifferentiated cells. So it would be very difficult to delineate what percent of the arginine that transports across the brush border membrane goes to nitric oxide, what percent of them will be converted to urea, what percent of them will go to a systemic system. These percentages may have significant clinical impact. There are emerging data that suggest another product, peroxynitrite, may play an important role in regulating intestinal function. Some people suggest that NO may be beneficial and peroxynitrite may be detrimental to intestinal epithelia. But it is not known when tissue decides to produce NO or peroxynitrite when exposed to stimuli.

Dr. Moody: And arginine in an immune-enhancing diet, is that a good thing or a bad thing?

Dr. Pan: I personally believe it is a good thing; there have been many studies and clinical trials to show its potential benefits. However, better understanding of the biological bases and better controlled trials are needed to fully appreciate arginine's function in the immune-enhancing diet.

Dr. Jody Gookin (Raleigh, NC): By what means are you ascribing arginine transport to the y^+ system versus the other systems?

Dr. Pan: The classification of arginine transport study was done many years ago. Traditionally it is characterized by the inhibition profile, the transport kinetics, and pH sensitivity, and more recently, molecular biology. In our case, the CAT1 mRNA codes for the System y^+ in the intestine.

Dr. Gookin: What about the CAT2B transporter?

Dr. Pan: The CAT2 transporter is in the liver, brain, macrophage, and muscle.

Dr. Gookin: No one has ever looked at it for intestinal epithelium, so how do you know that it is not there?

Dr. Pan: CAT1 is the one in the intestine and CAT2A and B are isoforms found in other tissues.

CA 19-9 Levels Predict Results of Staging Laparoscopy in Pancreatic Cancer

Andreas Karachristos, M.D., Ph.D., Nikolaos Scarmeas, M.D., M.Sc., John P. Hoffman, M.D.

Laparoscopy has emerged as an important staging procedure for determining resectability of pancreatic cancer. However, a small fraction of patients with pancreatic cancer benefit from its use and therefore the routine application of laparoscopy remains controversial. We hypothesized that serum CA 19-9 levels may identify patients who will or will not benefit by laparoscopy. We retrospectively reviewed our database of 63 patients with pancreatic adenocarcinoma who underwent staging laparoscopy and correlated findings with CA 19-9 levels. Overall, laparoscopy identified metastatic disease in 12 patients (19%). None of those required any further operation. The resectability rate (patients who underwent resection after laparoscopy) was 73.5%. There was one false-negative laparoscopy (1.6%). Patients with higher CA 19-9 levels had significant higher odds of having metastasis identified by laparoscopy (odds ratio, 1.83; 95% confidence interval, 1.03–3.24; $P = .04$). There was no patient with CA 19-9 levels below 100 U/ml in whom metastatic disease was identified during laparoscopy: 18 patients (28.6%) with CA 19-9 levels below this cutoff point had negative laparoscopy and could have avoided the procedure had this cutoff been used for screening. This would have increased the laparoscopy yield to 26.7%. In patients with adenocarcinoma of the pancreas, low CA 19-9 levels predict low probability of metastatic disease; in those patients, laparoscopy can be spared. On the contrary, patients with elevated CA 19-9 have an increased probability of metastatic disease, and these patients may benefit from diagnostic laparoscopy. (J GASTROINTEST SURG 2005;9:1286–1292)

KEY WORDS: CA 19-9, laparoscopy, pancreatic cancer

Pancreatic cancer is the fourth leading cause of cancer mortality in the United States, with a death-to-incidence ratio of approximately 0.99. However, actuarial 5-year survival of appropriately selected patients after resection is 21%.[1] Diagnostic laparoscopy is an important staging procedure to determine resectability in pancreatic cancer. In an early report, Cuschieri et al[2] analyzed the use of laparoscopy in diagnosis, staging, and assessment of operability of the disease. Several recent studies have documented the value of laparoscopy in obviating unnecessary laparotomy in patients with metastatic[3,4] and locally advanced pancreatic cancer.[5] In these studies, staging laparoscopy (SL) revealed unsuspected metastasis or unrecognized advanced disease in 31%–35% of patients. Furthermore, it has been used as adjunct in choosing the appropriate palliative method[6] and in selecting among locoregional chemoradiation and systemic chemotherapy in patients with incurable pancreatic cancer.[7,8] Nevertheless, several authors are reluctant to recommend routine use of SL in all potentially resectable pancreatic cancers, citing a modest diagnostic yield of 14% when sophisticated imaging modalities are used.[9,10]

Although the tumor-associated antigen CA 19-9 was initially described as a marker for colorectal cancer,[11] it later became vident that its most important clinical applications are related to pancreatic cancer.[12,13] Since then, CA 19-9 is recognized as the first tumor marker that successfully aids in the diagnosis of pancreatic cancer with reported sensitivity of 68%–94% and specificity of 76%–100%.[14] The CA 19-9 monoclonal antibody has as its epitope the blood group carbohydrate antigen sialyl Lewis[α]. This can be expressed on either

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glycoproteins or glycolipids, and specifically in pancreatic cancer, CA 19-9 detects the epitope expressed on circulating mucins.[14] Apart from its diagnostic implications, it has been proved useful in monitoring the clinical course and prognosis of this disease,[15] as well as predicting the response to chemotherapy or chemoradiation therapy.[16,17] Furthermore, it has been suggested that CA 19-9 may improve selection of surgical candidates[18] and may predict the outcome of resected patients.[19,20]

The hypothesis of this study is that preoperative determination of serum CA 19-9 levels may predict the results of SL. By doing so, it may help identify subgroups of patients in whom SL may not be necessary and it may therefore increase the laparoscopy's diagnostic yield.

MATERIAL AND METHODS

We retrospectively reviewed the Fox-Chase Cancer Center database of patients diagnosed with pancreatic cancer who successfully underwent diagnostic laparoscopy. Patients with radiologic evidence of metastatic disease were excluded as well as patients with diagnosis of distal bile duct cancer, tumor of the ampulla of Vater, and duodenal cancers. Only patients with pathology-confirmed pancreatic adenocarcinoma were included. Seventy-one patients were identified between 1996 and 2003. After exclusion of patients with undetectable CA 19-9 levels (nonsecretors) and those without preoperative determination of CA 19-9, 63 patients were selected. These patients form the basis of this study. Data collected include patient's age and gender, date of diagnosis, imaging data, date of operation, operative records, pathology and cytology reports, tumor location and size, serial preoperative CA 19-9 and bilirubin levels, and chemoradiation data.

Computed tomography (CT) for evaluation of pancreas cancer is performed with a spiral scanner, with double contrast using 3-mm pancreas sections. Patients with CT performed in outside institutions were evaluated for adequacy and, if necessary, CT was repeated. Diagnostic laparoscopy at Fox-Chase Cancer Center is performed as part of our staging protocol. According to CT results, two groups of patients were identified: group 1 included patients with potentially resectable tumors. If there was no evidence of metastatic deposits on laparoscopy, an exploratory laparotomy was performed and, if appropriate, resection followed. Group 2 comprised potentially resectable patients with localized tumors in close proximity to the mesenteric or portal vein. These patients underwent diagnostic laparoscopy and, if negative, were entered in a protocol of gemcitabine-based neoadjuvant chemoradiation.[21] After completion of the preoperative treatment, patients were restaged by CT and, if free of metastasis, they were explored. All patients identified as metastatic received systemic chemotherapy. Laparoscopy was performed in a standard fashion: Pneumoperitoneum was established with an open technique and a 30-degree laparoscope was inserted through a 10-mm periumbilical incision. All peritoneal surfaces are carefully inspected including the abdomen, diaphragm, omentum, bowel mesentery, and pelvis. An additional 5-mm instrument was inserted through a right upper quadrant incision to facilitate evaluation of the undersurface of the liver and bowel mesentery. All suspicious lesions were biopsied using True-Cut needle or biopsy forceps. We do not routinely biopsy lymph nodes or evaluate local tumor extension. Normal saline 500–1000 ml was instilled upon entering the peritoneal cavity, before any tissue manipulation. After gentle abdominal agitation, the fluid is allowed to dwell and subsequently is aspirated and sent for standard cytology examination.

A CA 19-9 radioimmunoassy kit (Abbott Laboratories, Chicago, IL) was used to determine CA 19-9 levels. The recommended normal value by the manufacturer is 37 U/ml. If several CA 19-9 values were available, the level closest before SL was selected (within 2 weeks). Serum CA 19-9 was collected concurrently with complete liver function tests, including bilirubin.

We used χ^2 to examine the association of laparoscopic findings (positive versus negative) with categorical variables such as gender, tumor site (body-tail versus head-uncinate), peritoneal cytology findings (positive versus negative), potential resectability based on CT findings (group 1 versus group 2), and results of laparoscopy based on CT findings. We used t test to examine the association of laparoscopic findings or other categorical variables with continuous variables such as age, gender, CA 19-9, bilirubin, tumor size (cm), and tumor location. Because of the skewed distribution of CA 19-9 and bilirubin variables, we used them in the form of logarithms (which were normally distributed).

We used logistic regression analyses to examine whether CA 19-9 (predictor) was associated with laparoscopic findings (outcome). The models simultaneously adjusted for age (years), gender (male as the reference), bilirubin, pancreatic tumor site (body-tail versus head-uncinate, with head-uncinate as the reference), and CT-assessed pancreatic tumor

size (cm). We controlled for bilirubin levels, because it is documented that increased cholestasis falsely elevates CA 19-9; this is likely due to decreased capacity of the cholestatic liver to degrade and excrete CA 19-9.[12,22]

RESULTS

Sixty-three patients were included in the present study. Twenty-seven (43%) were considered potentially resectable by CT (group 1), and 36 (57%) patients were categorized as potentially resectable with unfavorable location (group 2). The median CA 19-9 levels were not significantly different between the two groups. Patient characteristics are shown in Table 1. Overall, SL identified 12 patients with histology-confirmed metastasis (19%). From those, seven patients had liver only, two had liver and peritoneal, one had liver, peritoneal, and gastric, one had metastatic deposits on the round ligament along with peritoneal nodules, and one had peritoneal only (Table 2). Seven patients with metastasis were identified from group 1 (25.9%) and five from group 2 (13.9%); this difference was not statistically significant. One patient had a false-negative laparoscopy (1.6%). The patient had a resectable by CT tumor. Laparoscopy was negative, and upon exploration, a small (<5 mm) liver metastasis was palpated at segment 8. None of the 12 patients whose metastatic disease was revealed by laparoscopy required any further operation. No complications attributed to laparoscopy per se were noticed, and patients for whom this was the only procedure were usually discharged the following day. Laparoscopy revealed metastasis in 17.7% (9 of 51) of patients with tumor located either at the head or the uncinate process and 25% of those whose tumor was either at the body or tail of the gland ($P = .03$).

Overall, 36 patients (73.5%) were resected after SL. Two patients refused resection after laparoscopy and were excluded from the analysis. The resectability rate after laparoscopy in group 1 (those with resectable disease by CT) was 83.3%. Two patients from the second group developed metastasis during neoadjuvant therapy. The resectability rate in group 2 (potentially resectable by CT) was 67.7%.

Table 1. Patient characteristics

Patients	Group 1 (n = 27)	Group 2 (n = 36)
Age (mean yr)	66.4	66.4
Female (%)	44.4	63.9
Tumor location (%)		
Head	77.8	66.7
Uncinate process	11.1	8.3
Body	11.1	16.7
Tail		8.3
Size (mean cm)	3.5	3.7
CA 19-9 (median U/mL)	326.5	324

Table 2. Metastasis identified by laparoscopy

Laparoscopic Findings	Group 1 (n = 27)	Group 2 (n = 36)
Metastasis	7	5
Liver	7	5
Peritoneal	2	3
Mesenteric nodule	1	—
Gastric	—	1
Round ligament	1	—

CA 19-9 values ranged from 7 to 113,870 U/ml (median, 324). There was no significant difference in median values between groups (group 1, 326.5; group 2, 324). None of the patients with CA 19-9 of 100 or less had metastasis revealed by laparoscopy (Fig. 1). Eighteen patients (28.6%) had CA 19-9 levels below this cutoff point. In group 1, 25.9% (7 of 27) of patients had CA 19-9 levels of 100 or less, and 30.55% (11 of 36) in group 2 had CA 19-9 levels of 100 or less. Patients with higher CA 19-9 had higher probability of having laparoscopically identified metastasis, even when simultaneously controlling for age, gender, bilirubin, tumor site, and size (Table 3). For each additional logarithmic unit of CA 19-9, there was 1.8 times higher odds of detecting metastasis during laparoscopy. Tumor site was also significant in the model: compared with patients with CT-assessed tumor location at the head or uncinate process, patients in whom tumor was located at the body or tail had approximately 15 times higher chances of having metastatic disease during laparoscopy. There was no patient with CA 19-9 levels below 100 U/ml in whom metastatic disease was identified during laparoscopy (sensitivity, 100%; specificity, 64%). There was a single patient with false-negative laparoscopy results who had a preoperative CA 19-9 level of 18.9. After excluding the patients with metastatic disease identified by laparoscopy, 51 patients were either explored immediately or entered into a protocol of neoadjuvant chemoradiation. Ultimately, 77.8% (14 of 18) of those with CA 19-9 levels below 100 and 66.7% (22 of 33) of those with CA 19-9 levels above 100 underwent resection (Fig. 2).

Results of peritoneal washings were available in 52 patients (82.5%): 55.6% (5 of 9) of those with metastatic disease identified by laparoscopy had evidence of malignant cells in the aspirate, in

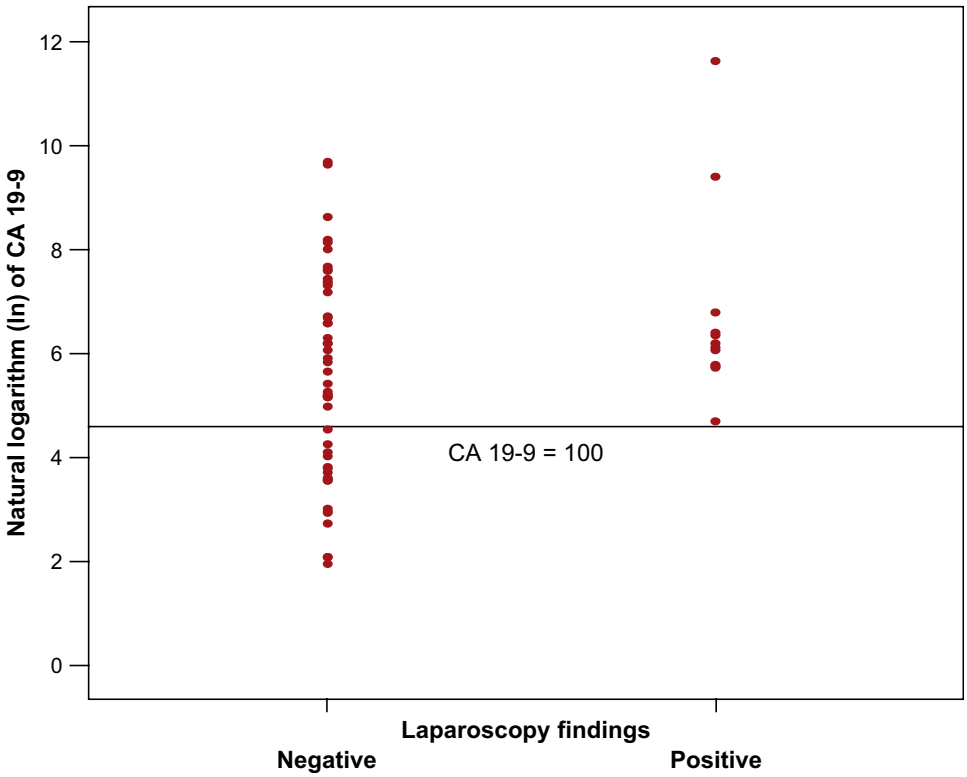

Fig. 1. CA 19-9 levels by laparoscopic findings.

contrast with only 11.6% (5 of 43) of those with negative laparoscopy ($P = .008$). Subsequently, 67.5% (27 of 40) of those with negative peritoneal cytology underwent resection in contrast with only 20% (2 of 10) with positive peritoneal cytology ($P = .011$).

DISCUSSION

CT has become the standard imaging modality in determining resectability of pancreatic cancer.

Table 3. Logistic regression analyses, predicting laparoscopically identified metastatic disease by CA 19-9 (logarithmically transformed), controlling for location (head/uncinate as the reference), age, gender (male as the reference), bilirubin (logarithmically transformed), and size (in centimeters)

	Odds ratio	95% Confidence interval	*P*
CA 19-9	1.83	1.03–3.24	.04
Site	15.59	1.41–172.13	.03
Age	0.93	0.85–1.01	.09
Gender	0.77	0.10–5.67	.79
Bilirubin	0.84	0.35–2.01	.69
Size	0.79	0.36–1.72	.56

However, even high-quality helical CT scanners using standardized pancreatic protocol may "understage" a significant number of patients with potentially resectable or locally advanced disease. In a recent study,[23] 18 of 98 (18%) patients with localized disease had visible metastatic disease by SL, to either the liver (12%) or the peritoneum (6%). Specifically, the incidence of undetectable metastasis was 18% in the subgroup of patients with potentially resectable cancers and 24% in those with locally advanced disease. The diagnostic yield of SL was higher in patients with distal lesions (35%) compared with those with central tumors (18%). Multiple studies support the ability of SL to spare unnecessary laparotomies in patients with occult extrapancreatic spread. The experience from Massachusetts General Hospital reveals that SL demonstrated metastasis invisible by CT in 24% of patients, and this increased to 31% when SL findings were combined with positive peritoneal cytology.[3,24] Again, detection of metastatic lesions was more common in patients with distal mass (36%) than in those with proximal tumors (17%). In their latest report, the resectability rate for patients with localized disease after SL was 74.2%. Extensive, multiport laparoscopy has been demonstrated to facilitate diagnosis of distal metastatic deposits, as

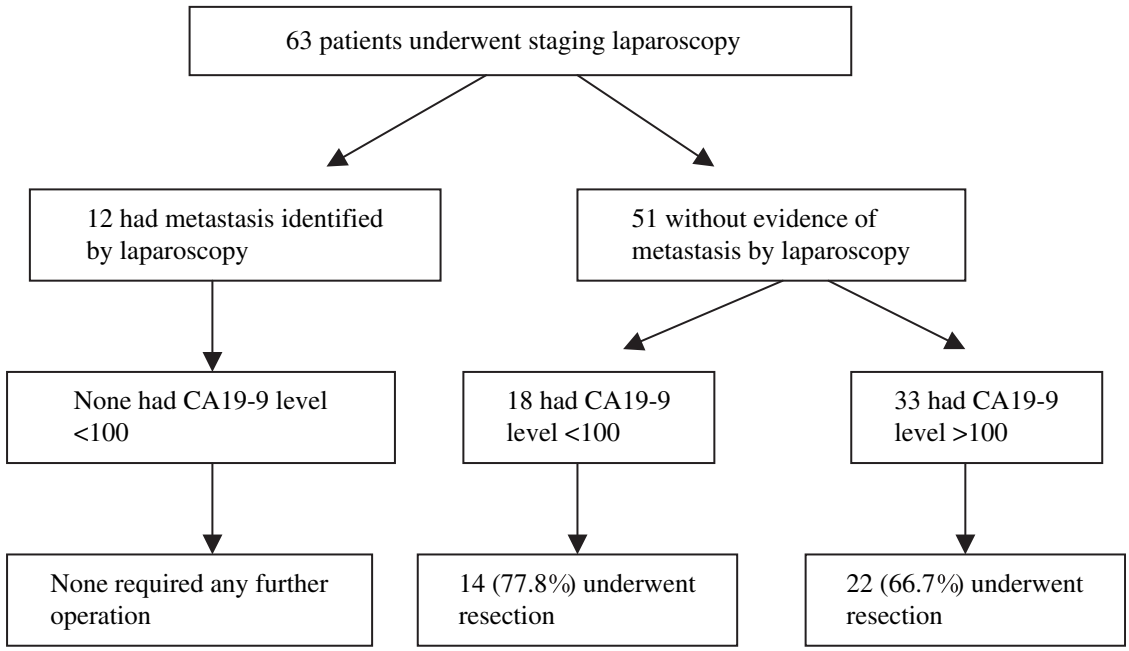

Fig. 2. Resection rates according to laparoscopic findings and CA 19-9 levels.

well as evaluation of local invasiveness and lymph node sampling.[5] Using this methodology with 108 patients, these authors were able to identify 20 patients with liver metastasis, 14 patients with vascular encasement, 16 with extrapancreatic/peritoneal involvement, and 8 with portal lymphadenopathy. Laparoscopy failed to identify hepatic metastasis in 5 patients.

In our experience of 63 patients potentially resectable by CT, laparoscopy prevented unnecessary laparotomy in 12 (19%). Of these, 10 patients had liver and/or peritoneal spread, and in 2, metastatic nodules were detected on peritoneal surfaces only. One patient had a false-negative laparoscopy. After laparotomy, a small (<5 mm) hepatic metastasis was identified on the posterior surface of segment 8. We believe that the location and the size of the lesion obviated detection by SL. After SL, the overall resectability rate was 73.5%. Specifically, the resectability for patients with favorable lesions (group 1) was 83.3%, and in those with tumors in close proximity to portal or mesenteric vein, it was 67.7%. This difference is not significant, probably reflecting our low threshold of portal or mesenteric vein resection, in order to achieve negative margins. However, the resection rates are not directly comparable because the latter group underwent neoadjuvant chemoradiation.

The yield of SL for identifying metastasis in our study is higher than that in other reports, possibly because the number of our patients with small tumors (≤2 cm) is low (7.8%). However, tumor size was not associated with laparoscopic findings, possibly because 81% of our patients had tumors of 3 cm or greater. Patients with tumors located at the body or tail comprised only 19% of our study population, which might explain the lower rate of metastasis identified by laparoscopy. Several publications have demonstrated that patients with distal masses have higher rates of positive laparoscopy compared with those with proximal tumors.[3,4] In agreement with these studies, our results show that 25% of patients with distal tumors had metastasis revealed by laparoscopy, in contrast to 17.7% of those with proximal tumors. In the model constructed, patients with distal tumors had a significantly higher probability of having metastasis identified by SL. It has been suggested that tumors located at the distal part of the gland may harbor a more aggressive biology.

In our study, SL altered the treatment strategy in 12 patients (19%) with occult metastatic spread. Seven of these had resectable-by-CT tumors, and SL allowed immediate application of systemic chemotherapy. Five patients with cancers in unfavorable locations would have received locoregional neoadjuvant chemoradiation, treatment unsuitable for patients with metastatic disease. Recently, it has been shown that SL can improve staging in 34%–37% of patients with locally advanced, unresectable disease.[7,8] Diagnosis of metastatic disease in those patients has important implications because it allows appropriate therapeutic plans and improves monitoring of therapeutic

outcomes. None of these 12 patients required further surgery. Application of expandable metallic stents for palliation of obstructive jaundice and gastric outlet obstruction makes palliative surgery seldom necessary after laparoscopic staging.[25]

Despite the obvious advantage of SL in potentially resectable pancreatic cancer, its yield in patients with favorable tumors located at the head or the uncinate process is approximately 10%–14% when high-quality contrast-enhanced spiral CT is used.[9,10,23] We examined whether the use of CA 19-9 could increase the diagnostic ability of laparoscopy. Safi et al[13] ascertained a sensitivity and specificity of CA 19-9 of 92% and 85%, respectively, in the diagnosis of pancreatic cancer. In that study, 92% of patients with adenocarcinoma of the pancreas had CA 19-9 levels above 37 U/ml and 77% had levels above 120 U/ml. In comparison, 92% of patients with healthy pancreas had levels below 37 U/ml. In a study of 31 patients with pancreatic cancer, patients with tumors less than 5 cm had median levels of CA 19-9 of 120 U/ml, in contrast to 750 U/ml for those with tumors greater than 5 cm ($P < .01$). Furthermore, in 7 patients whose CA 19-9 levels returned to normal after resection, the average survival time was longer than 21 months, whereas for 4 patients with persistently elevated postoperative levels, the average survival was only 7.8 months.[26] Similarly, in another report, patients whose CA 19-9 levels normalized postoperatively had longer disease-free survival (24 versus 10 months) and median survival (34 versus 13 months) from those with elevated postoperative levels.[19] Additional studies demonstrate that serial CA 19-9 measurements can predict response to chemotherapy or chemoradiation.[16,17] These studies reveal that CA 19-9 is a significant prognostic variable in patients with pancreatic cancer. Furthermore, CA 19-9 was recently shown to predict resectability in pancreatic cancer.[18] According to these authors, almost 90% of patients with CA 19-9 of 150 U/ml or greater will probably be unresectable. Indeed, 48% of these patients had metastatic disease. Our study, to the best of our knowledge, is the first to evaluate CA 19-9 as an adjunct in patient selection for SL. None of the patients with CA 19-9 levels below 100 U/ml had metastasis revealed by laparoscopy. The single patient whose SL failed to visualize the metastatic focus had a small hepatic deposit in a location difficult to be seen by the limited laparoscopic exploration we undertake. CA 19-9 levels independently predicted negative laparoscopy ($P = .04$) over and above age, gender, bilirubin, tumor location, and size. Eighteen of 63 (28.6%) patients had serum CA 19-9 levels below this cutoff point, and in those, SL could have been spared, increasing its diagnostic yield from 19% to 26.7%. According to our results, high levels of CA 19-9 significantly increase the likelihood of detecting metastatic disease by limited laparoscopy. This can have important implications. Serum CA 19-9 is an inexpensive test that is generally measured in every patient with pancreatic adenocarcinoma. Stratifying patients eligible for SL according to CA 19-9 levels may spare a significant number of patients from laparoscopies with low diagnostic yield. Consequently, SL will be used more appropriately, saving time and costs. The proposed CA 19-9 cutoff point failed to predict resectability in our study, although more than half of our patients underwent neoadjuvant chemoradiation that might have altered the course of the disease.

In the present study, significantly more patients with evidence of malignant cells in peritoneal washings had metastasis demonstrated by laparoscopy, and positive peritoneal washings were inversely related with resectability. In the subgroup of patients without identifiable metastasis, 11.6% had positive peritoneal cytology for malignant cells and a small proportion of these (2 of 5) ultimately underwent resection. In our practice, we proceed to immediate exploration in resectable by CT patients with negative laparoscopy, and therefore the results of cytology are not known. We recently reviewed our experience of potentially resectable patients with positive peritoneal cytology and found that disease-free interval and median survival were comparable with patients with negative peritoneal washings (10 versus 12 months and 15 versus 19 months, respectively).[27] Others have suggested that positive peritoneal cytology in patients with pancreatic cancer, even in the absence of macroscopic metastasis, is an indicator of poor outcome and therefore the patients should not undergo resection.[28] However, these series did not examine outcomes of positive cytology in resected patients per se but instead addressed the prognostic significance of cytology in all patients with potentially resectable cancers. We believe knowledge of positive peritoneal cytology should prompt thorough evaluation for metastasis but that these patients should not be excluded from resection.

CONCLUSIONS

SL is particularly important in sparing potentially resectable patients with unsuspected metastatic disease from unnecessary laparotomy. Furthermore, SL guides appropriate therapy in patients with locally advanced tumors and patients entering

protocols of neoadjuvant chemoradiation. Modern imaging modalities significantly improve staging, and therefore the liberal use of laparoscopy is controversial, especially in low-risk patients, such as those with small tumors of the pancreatic head. CA 19-9 levels can predict patients with low probability of metastasis. As demonstrated by the present study, adding CA 19-9 level to the staging algorithm improves patient selection for SL and therefore increases its diagnostic yield. Tumors located at the body or tail of the pancreas have increased probability of metastatic spread. All potentially resectable patients with distal tumor locations should be staged with laparoscopy before exploration.

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Management of Delayed Visceral Arterial Bleeding After Pancreatic Head Resection

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Despite low mortality, postoperative complications are still relatively frequent after pancreatic head resection. The occurrence of delayed visceral arterial bleeding from erosions or pseudoaneurysms of branches of the celiac trunk or from the stump of the gastroduodenal artery is a rare but life-threatening complication and is probably underreported in the literature. During a 10-year period, we diagnosed and treated 12 patients (three referred from other hospitals) with severe visceral arterial bleeding, presenting 7 to 85 days after pancreatic head resection. Clinical presentation was gastrointestinal bleeding (seven patients) or abdominal bleeding (five patients). The bleeding source was identified by angiography in 10 of the 12 cases. Definitive bleeding control was achieved by angiography in six of the 12 patients (stent 2, coiling 4), or by surgery in five patients. None of the six patients with successful angiographic intervention required further surgery for bleeding control. One patient died due to hemorrhage before bleeding was controlled. Median transfusion requirement was 12.5 (range 3–37) units. Of five patients with interventional or surgical occlusion of the common hepatic artery, three developed hepatic abscesses and two had complications of the hepaticojejunostomy. One of those five patients died four months after definitive bleeding control because of recurrent hepatic abscesses. All other patients eventually recovered completely. We conclude that delayed arterial bleeding from visceral arteries is a rare but life-threatening complication after pancreatic head resection. Angiographic stenting with preservation of hepatic blood flow, if technically possible, represents the best treatment option. (J GASTROINTEST SURG 2005;9:1293–1299)

KEY WORDS: Pancreatic resection, morbidity, late postoperative bleeding, arterial pseudoaneurysm, interventional radiology

Mortality rates, clearly below 5% in many experienced centers,[1–5] led to an overall increase of the numbers of pancreatic head resections. Morbidity, however, is still high. Pancreatic leakage, delayed gastric emptying, and infections are the most frequent complications after resection.

Delayed visceral arterial bleeding after pancreatic head resection is probably underreported in the literature. It occurs rather late after surgery and often after patients are already discharged from the hospital. Main reasons for the development of such arterial lesions are erosions by clinically apparent, or unapparent, pancreatic fistulas[6,7] or lesions after prior radiologic interventions.[1] In our 10-year experience with more than 450 pancreatic head resections, we have observed delayed visceral arterial bleeding in nine patients. Furthermore, three patients with such bleeding entities were referred to our institution after surgery in other hospitals.

The aim of our study was to analyze the frequency, presentation, management, and outcome of delayed visceral hemorrhage after pancreatic head resection in our institution. By regarding our experience and by reviewing the current literature management, strategies for this severe complication are discussed.

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MATERIAL AND METHODS

From July 1994 to August 2004, 464 pancreatic head resections (including eight primary total pancreatectomies) were performed. Indications for surgery and operations performed are shown in Table 1. The surgical techniques, extent of lymphadenectomy in malignant tumors, and perioperative management have been reported in detail.[5,8] Routine octreotide prophylaxis was abandoned in 2004. Before abdominal closure, flat silicon drains were routinely placed at the pancreatic and biliary anastomoses and taken out through the right abdominal wall. These drains were left in place for at least 5 days postoperatively.

Delayed visceral arterial hemorrhage was defined as angiographically and/or surgically proven bleeding from main branches of the celiac axis beyond the first postoperative week. The definitions of pancreatic leakage in our patients have been described before[5] and were (I) amylase-rich discharge from the abdominal drains beyond day six postoperatively, (II) the need for interventional drainage of an abdominal fluid collection with high amylase concentration, or (III) the documented insufficiency of the pancreatic anastomosis during reoperation. Perioperative mortality was defined as 30-day mortality after surgery; morbidity was defined as the occurrence of complications during the hospital stay after the operation.

The data of all patients were gained by analysis of our prospective pancreatic surgery database. In addition, the charts and radiological examinations of the patients with delayed visceral arterial bleeding were separately re-evaluated.

Table 1. Indications and type of surgery in 464 pancreatic head resections

Indication	n	%
Chronic pancreatitis	220	47
Pancreatic adenocarcinoma	127	27
Ampullary cancer	42	9
Distal bile duct cancer	32	7
Other	43	9
Surgery		
Pylorus-preserving PD	289	62
Classical Whipple	69	15
Duodenum-preserving head resection (Frey 53, Beger 45)	98	21
Total pancreatectomy	8	2
Portal vein resection	55	12
Preoperative biliary drainage	203	44

PD = pancreatoduodenectomy.

RESULTS

Overall Morbidity and Mortality, and Frequency of Delayed Visceral Arterial Bleeding in Our Patient Cohort Study

The overall morbidity and mortality after 464 pancreatic head resections are shown in Table 2. Delayed visceral arterial bleeding was observed after nine of 464 (1.9%) pancreatic head resections performed in our institution. It occurred more frequently after surgery for malignant tumors when lymphadenectomy had been performed (seven bleedings in 221 patients; 3.2%) and was found in only two of the 220 patients after surgery for chronic pancreatitis (0.9%).

Pancreatic Leakage and Delayed Visceral Arterial Bleeding

Of the nine patients (later) developing delayed visceral arterial bleeding, four had other clinically evident complications before bleeding occurred; there were pancreatic leakages in all four. In three of them, pancreatic leakage was sufficiently drained by the operative silicone drains (no other symptoms); the fourth patient had CT-guided drainage of an intra-abdominal fluid collection with high concentration of amylase in the aspirate. In one further patient who was reoperated for bleeding, an insufficiency of the pancreatic anastomosis was not evident preoperatively but was demonstrated during surgery.

Characterization of the 12 Patients With Delayed Visceral Arterial Bleeding

At the time of pancreatic head resection, the 12 patients (11 men, 1 woman) had a median age of 61 (range 47–75) years. Indications for surgery were pancreatic cancer (n = 4), chronic pancreatitis (n = 4), distal bile duct cancer (n = 3), and a benign

Table 2. Perioperative morbidity and mortality in 464 pancreatic head resections

Complication	n	%
Any complication	200	43
Surgical complication	141	30
Pancreatic leakage	58	12.5
Wound infection	49	10.5
Abdominal abscess	42	9
Bleeding	26	6
Delayed gastric emptying (day 14)	22	5
Reoperation for complications	37	8
Mortality (30 days)		
Death	12	2.6

duodenal tumor in one patient. The following resections were performed: pylorus-preserving pancreatoduodenectomies in eight patients, duodenum-preserving head resections according to Beger in two patients, and classical Whipple procedures in another two patients.

Time Point and Presentation of Delayed Visceral Arterial Bleeding

Median time bleeding definitively diagnosed was 24 (range 7–85) days after the initial operation. Seven of the twelve patients had already been discharged from the hospital at the time bleeding occurred.

Seven patients presented with gastrointestinal bleeding, five patients with abdominal bleeding. None of the patients had clinical signs of simultaneous abdominal and gastrointestinal bleeding. In all five patients with abdominal bleeding, blood was evident in the drain bags or at former drain sites.

Definitive bleeding locations were the common hepatic artery (n = 5), the stump of the gastroduodenal artery (n = 5), the splenic artery (n = 1), or the pancreatoduodenal artery (n = 1; after a duodenum-preserving pancreatic head resection).

Management of Delayed Visceral Arterial Bleeding

Twelve patients with delayed visceral arterial bleeding were diagnosed and treated (Fig. 1). After initial resuscitation, all seven patients with clinical signs of gastrointestinal bleeding underwent upper gastrointestinal endoscopy without demonstrable bleeding source. They subsequently underwent angiography. Of the five patients with abdominal bleeding, two underwent immediate laparotomy without other diagnostic measures, and three went to angiography. One patient with severe bleeding from the stump of the gastroduodenal artery, 17 days after

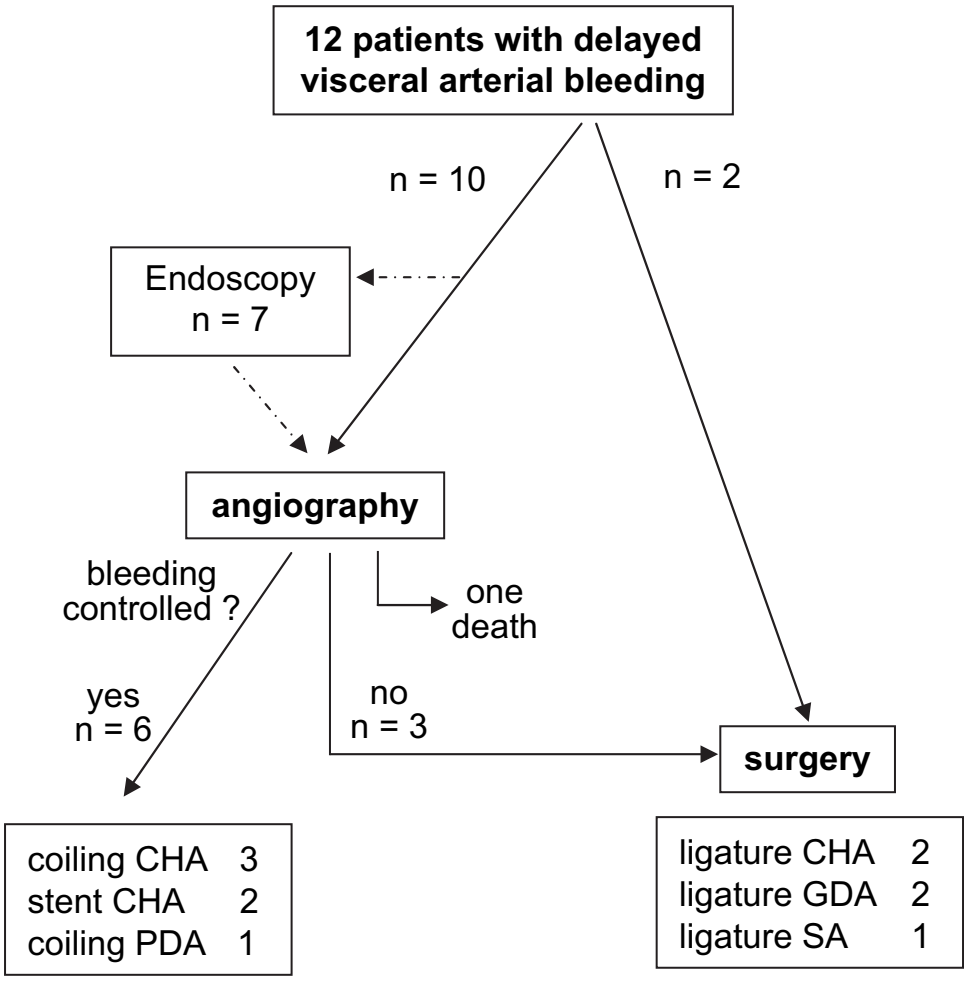

Fig. 1. Management of delayed visceral arterial bleeding in 12 patients. CHA = common hepatic artery; GDA = gastroduodenal artery; PDA = pancreaticoduodenal artery; SA = splenic artery.

surgery for distal bile duct cancer, died during angiography before bleeding could be controlled.

In the remaining nine patients who underwent angiography, the bleeding source could be detected radiologically in all cases. Bleeding was controlled definitively by angiography in six patients (three embolization coilings and two stentings of the common hepatic artery, one embolization coiling of the pancreatoduodenal artery; Figs. 2–4). Bleeding control was technically not possible by angiography in three patients; they underwent immediate laparotomy.

Reoperation

Surgical bleeding control was achieved definitively in all five patients undergoing relaparotomy (two primarily and three after angiography) and consisted of two ligations of the common hepatic artery, two suture ligations of the stump of the gastroduodenal artery, and one ligation of the splenic artery. Three of the five reoperated patients that had pancreatic leakage also demonstrated intraoperatively. Beyond bleeding control, resection of the pancreatic remnant was performed in two of the five patients. The pancreatojejunostomy was disconnected in one patient with blocking of the pancreatic duct. The anastomosis was successfully oversewn in one further case.

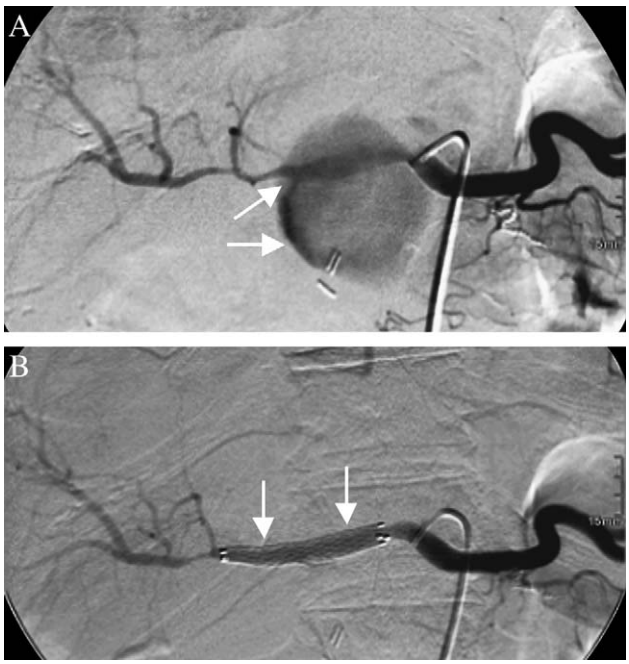

Fig. 2. Angiography of the common hepatic artery in a patient 36 days after classical Whipple operation for chronic pancreatitis. (**A**) Extravasation of contrast medium at the common hepatic artery (*arrows*) and (**B**) the result after insertion of an arterial stent (*arrows*) with preserved blood flow into the liver.

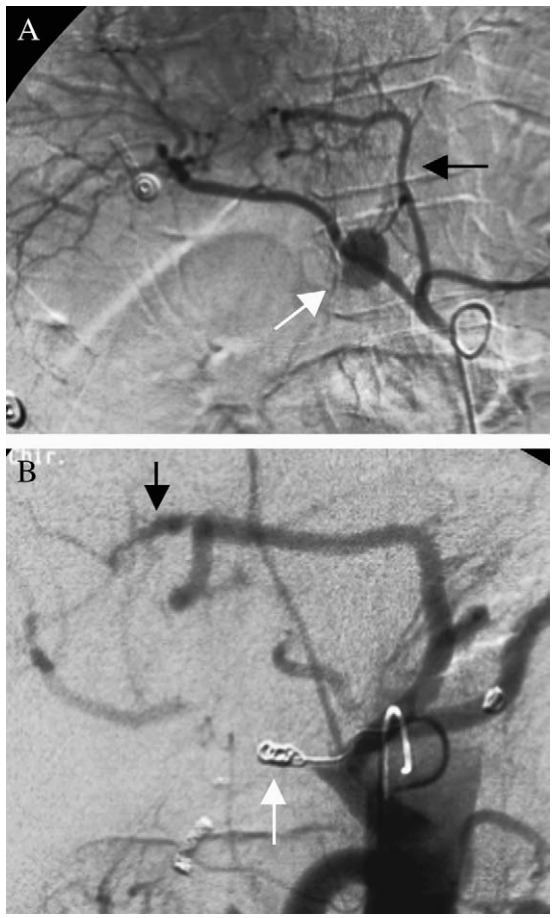

Fig. 3. (**A**) Angiography showing a bleeding pseudoaneurysm of the common hepatic artery (*white arrow*) 85 days after pylorus-preserving pancreatoduodenectomy for cancer of the pancreatic head. The patient has an accessory left hepatic artery (*black arrow*). (**B**) Imaging after coil embolization (*white arrow*) of the pseudoaneurysm, with successful bleeding control. The accessory left hepatic artery provides sufficient blood supply to the liver (*black arrow*). The patient recovered completely without further complications.

Outcome After Bleeding Control

Median transfusion requirement in the patients with delayed visceral arterial bleeding was 12.5 (range 3–37) units. All five pancreatic fistulas healed (three conservatively, one oversewing during reoperation, one resection of the pancreatic remnant). After definitive bleeding control, clinically relevant complications occurred only in those patients with interventional or surgical occlusion of the common hepatic artery. Of the five patients with occluded common hepatic artery, only one had an uneventful further course. He had an accessory left hepatic artery providing sufficient arterial blood supply to the liver (Fig. 3). Of the other four patients with occluded hepatic artery, three developed liver abscesses

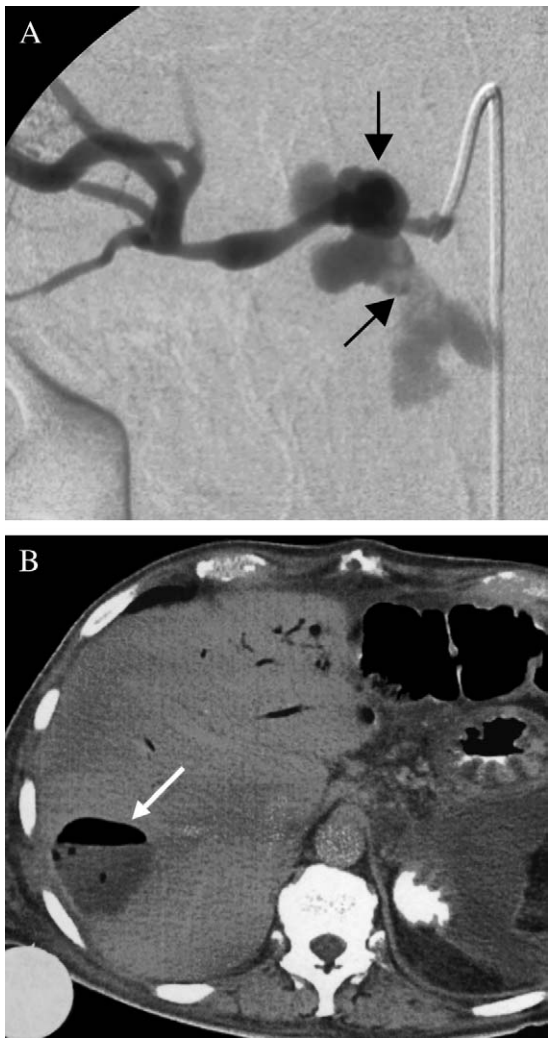

Fig. 4. (**A**) Bleeding originating from the common hepatic artery (*arrows*) 26 days after pylorus-preserving pancreatoduodenectomy for cancer of the pancreatic head. The bleeding was subsequently controlled by coil embolization of the common hepatic artery, but the patient had no arterial collaterals to the liver. (**B**) CT scan of the same patient three weeks after angiographic bleeding control showing an intrahepatic abscess (*arrow*) that was successfully treated by CT-guided drainage.

that were successfully drained interventionally. One patient had a recurrent liver abscess 14 months after initial liver abscess and was again treated by CT-guided drainage.

Biliary complications occurred in two patients with occluded hepatic artery after reoperation. One patient developed a bile leak from a 6-week-old hepaticojejunostomy that drained spontaneously through a deep wound infection (partially open abdomen) and healed without further interventions. The other patient who had a resection of the pancreatic remnant after an initial duodenum-preserving pancreatic head resection developed a leakage at the common bile duct, which was initially controlled by insertion of a T-tube followed by reconstruction in the form of a hepaticojejunostomy 10 months later.

DISCUSSION

Delayed visceral arterial bleeding after pancreatic head resection is rarely reported in the literature. There are only a few papers focusing directly on this complication.[1,6,7,9-13] In the papers giving overall numbers of operations, the frequency of postoperative arterial bleeding was in the range of 2 to 4%.[6,7,10,11,13] However, visceral arterial bleeding has also been reported after 1,061 pancreatoduodenectomies performed at the Johns Hopkins University between 1995 and 2001.[1] In this very large series, 16 patients (1.5%) had arterial bleeding of branches of the celiac trunk requiring angiographic intervention and/or surgical control. In our series of 464 pancreatic head resections, delayed visceral arterial bleeding was observed in 1.9%. However, when regarding surgery for malignancies the frequency was 3.2%.

When analyzing the literature, there seem to be at least two different entities of the vascular lesions leading to delayed visceral arterial bleeding. In the report from the Johns Hopkins group,[1] most lesions were, rather peripherally, not related to pancreatic leakage and probably a consequence of former percutaneous radiological interventions. Such vascular lesions, therefore, may represent injuries to the vessels and may not be related to the surgery. The other lesions, including pseudoaneurysms, more centrally (most of them at the common hepatic artery or the stump of the gastroduodenal artery), may be a consequence of clinically apparent or silent pancreatic fistulas and/or local infections. The aggressive pancreatic juice sorting from an insufficient anastomosis may directly erode the vessel wall. In the four studies reporting pancreatic leakage, including our data,[6,7,9] a pancreatic fistula was given in 24 of 34 patients. In one study where all six patients with bleeding had pancreatic leakage, those patients also had infection of the drain site.[7]

Beyond bleedings from the common hepatic artery, there seems to be a comparable number of bleedings from the stump of the gastroduodenal artery (Table 3). It cannot be clearly answered that this represents only erosion after pancreatic leakage or a problem of insufficient suture/ligation of the gastroduodenal artery. However, two of our five patients with bleeding from the gastroduodenal artery

Table 3. Presentation and management in reported series of delayed visceral arterial bleeding after pancreatic head resection

Author (reference)	n	Presentation	Bleeding Site	Pancreatic Leakage	Management
Sohn et al.[1]	16	16 UGI-B	10 branch CHA 4 GDA 1 celiac axis 1 other	N.a.	Embolization 16/16 Surgery 4/16
Reber et al.[10]	3	3 UGI-B	3 CHA	None	Embolization 3/3
Brodsky and Tumbull[9]	5	2 UGI-B 3 ABD-B	2 CHA 1 SMA 1 SA 1 RHA	2/5	Surgery 5/5 Embolization 1/5
Otah et al.[12]	5	1 UGI-B 5 ABD-B	2 RHA 2 LHA 1 GDA	N.a.	Embolization 5/5 Surgery 1/5
Rumstadt et al.[6]	11	N.a.	N.a.	All	Embolization 3/11 Surgery 8/11
Gebauer et al.[11]	5	5 ABD-B	5 GDA	N.a.	Stenting 3/5 Surgery 2/5
Yoshida et al.[7]	6	6 ABD-B	4 GDA 1 LGA 1 SMA	All	Embolization 3/6 Surgery 3/6
Own series	12	5 ABD-B 7 UGI-B	5 CHA 5 GDA 1 SA 1 other	5/12	Embolization 4/12 Stent 2/12 Surgery 5/12

N.a. = not available; ABD-B = abdominal bleeding; CHA = common hepatic artery; GDA = gastroduodenal artery; LGA = left gastric artery; LHA = left hepatic artery; RHA = right hepatic artery; SA = splenic artery; SMA = superior mesenteric artery; UGI-B = upper gastrointestinal bleeding.

stump also had documented pancreatic leakage. In the report of Yoshida et al.,[7] all four patients with bleeding from the gastroduodenal artery stump had prior pancreatic leakage.

It seems to be evident that lymphadenectomy with clearance of the hepatic artery may predispose to vessel erosion, because healthy tissue covering the vessels is removed during surgery, exposing the adventitia to pancreatic juice and/or inflammation. However, delayed visceral arterial hemorrhage has also been reported after surgery for chronic pancreatitis. In our series, 4 of 12 patients had pancreatic head resection for chronic pancreatitis, without systematic lymphadenectomy. In these patients, pancreatitis predisposed to arterial erosion must remain speculative, although acute or chronic pancreatitis is the main overall reason for pseudoaneurysms of branches of the celiac axis.

Abdominal drains have also been discussed as a potential risk factor for the development of vessel erosion by local mechanical irritation.[12] In our series, however, most patients developed bleeding rather late after drains were removed. In the study by Otah et al.,[12] all five patients developed bleeding with drains still in place, but these drains had all been shortened to the skin level to keep the drainage canal open. Therefore, the ends of the drains were at distance to the site of vessel erosion, again making an association between drains themselves and bleeding less probable.

In patients with late bleeding after pancreatic head resection, it is important to immediately recognize the entity of visceral arterial erosion. The fact that many patients have "sentinel bleeding" hours to days before a more severe bleeding episode occurs gives the possibility of performing visceral angiography early, and under optimal conditions, with definitive bleeding control. In the 12 patients from our study, four had sentinel bleeding hours to days before clinical evidence of major relevant bleeding. One of those patients referred to our hospital for severe bleeding six weeks after initial surgery even had an exploratory laparotomy three weeks after his initial operation for abdominal bleeding, but without demonstrable bleeding source.

In our series, two of the first patients with severe abdominal bleeding underwent immediate laparotomy. It is possible that, because of the dramatic clinical presentation, angiography was not considered in these cases.

In the management of patients with delayed visceral arterial bleeding, not only the hemorrhage itself but also other potential complications (e.g. septic or pancreatic leakage) have to be considered in the urgent diagnostic measures and treatment.[13] When regarding the literature and our experience, angiography with definitive bleeding control clearly represents the best management of delayed visceral arterial bleeding. When arterial bleeding can be controlled during angiography, subsequent emergency surgery can be avoided in most patients. In contrast to most other reports, however, De Castro et al.[13] recently proposed early surgery to control bleeding and sepsis in most patients, but in their series many patients had concomitantly severe septic complications requiring surgery; a relatively high mortality was experienced even after hemorrhage was controlled. In our study, none of the patients with successful interventional bleeding control required further operation.

Bleeding most frequently encounters the common hepatic or the stump of the gastroduodenal artery. Occlusion of the common hepatic artery by angiographic coil embolization or by surgical ligature, however, may be harmful to the liver. Although relatively infrequently reported in the mentioned series of delayed visceral arterial bleeding (Table 3), the lack of arterial blood supply to the liver postoperatively may lead to intrahepatic abscesses,[13,14] or to complications of the extrahepatic bile ducts. Both types of complications are well described after hepatic artery thrombosis in patients after liver transplantation.[15] In our study, four of five patients with interrupted hepatic artery flow developed such complications requiring further treatment. One patient eventually died due to recurrent liver abscesses months after surgery. The fifth patient with hepatic artery occlusion had an accessory left hepatic artery and recovered after bleeding control, without further complications (Fig. 3).

To avoid these potentially severe complications of arterial occlusion, bleeding control has been successfully performed by arterial stenting to maintain blood flow in two patients in our study. After immediate bleeding control, both patients recovered without further interventions. Comparable promising results have recently been reported from a German group in five patients.[11] It has to be mentioned, however, that this angiographic measure in branches of the celiac trunk is technically demanding, even in experienced centers, and is not always possible for anatomic reasons. In addition, personal and technical resources for such angiographic interventions may not always be provided in emergency situations, even in specialized centers,[13] leading to early surgery in some cases.

CONCLUSION

Delayed bleeding from visceral arteries is a rare but life-threatening complication after pancreatic head resection. When the hepatic artery has to be occluded to control bleeding, the risk of subsequent biliary complications or liver abscesses is high. Angiographic stenting with preservation of hepatic blood flow, if technically possible, may represent an improved treatment option as compared to arterial embolization or ligature.

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Local Resection of Ampullary Tumors

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There is no consensus on the appropriateness of local resection for ampullary tumors, because malignant recurrence of what were thought to be benign tumors has been reported. This study examined the role of local resection in the management of ampullary tumors. Thirty patients (mean age 66 years) had transduodenal local resections performed at UCSF-Moffitt Hospital or the San Francisco VA Medical Center (February, 1992 to March, 2004). Mean follow-up time was 5.8 years. Preoperative biopsies (obtained in all patients) showed 18 adenomas, four adenomas with dysplasia, five adenomas with atypia, one adenoma with dysplasia and focal adenocarcinoma, and two tumors seen on endoscopy, whose biopsies showed only duodenal mucosa. In comparison with the final pathology findings, the results of frozen section examinations for malignancy in 20 patients, during the operation, were false-negative in three cases. The final pathologic diagnosis was 23 villous adenomas, six adenocarcinomas, and one paraganglioma. On preoperative biopsies, all patients who had high-grade dysplasia and one of five patients with atypia turned out to have invasive adenocarcinoma when the entire specimen was examined postoperatively. Two (33%) adenocarcinomas recurred at a mean of 4 years; both had negative margins at the initial resection. Among the 23 adenomas, three (13%) recurred (all as adenomas) at a mean of 3.2 years; in only one of these cases was the margin positive at the time of resection. Tumor size did not influence recurrence rate. Ampullary tumors with high-grade dysplasia on preoperative biopsy should be treated by pancreaticoduodenectomy because they usually harbor malignancy. Recurrence is too common and unpredictable after local resection of malignant lesions for this to be considered an acceptable alternative to pancreaticoduodenectomy. Ampullary adenomas can be resected locally with good results, but the recurrence rate was 13%, so endoscopic surveillance is indicated postoperatively. Frozen sections were obtained during the operation, but they were less reliable than expected. No adenomas recurred as carcinomas, suggesting that local resection is appropriate for these tumors in the absence of dysplasia or atypia on preoperative biopsies. (J GASTROINTEST SURG 2005;9:1300–1306)

KEY WORDS: Ampullary tumors, duodenal adenoma, periampullary adenoma, ampullary adenoma, ampullary carcinoma, transduodenal resection, local resection

Neoplasms of the ampulla are uncommon, but they are being recognized more often due to improved imaging techniques and increased use of upper endoscopy. Endoscopic resection and ablative techniques are being employed for benign ampullary tumors.[1–6] Nevertheless, endoscopic snare polypectomy often fails, particularly for large tumors or those that extend up the bile duct.[3,5,6] Some authors believe that all ampullary tumors, even benign ones, should be treated by a Whipple resection because of the high risk of recurrence and the difficulty in excluding malignancy.[7,8] Thus, the role of transduodenal local resection for ampullary tumors deserves further inquiry. Four other groups have reported results on local resection of ampullary tumors in at least twenty-five patients.[9–12] The present study has the longest mean follow-up.

Our objective was to examine the role of local resection in the management of benign and malignant tumors of the ampulla. Preoperative evaluation, intraoperative management, and surgical outcomes are presented as well as long-term recurrence rates and compliance with recommendations for postoperative surveillance.

MATERIAL AND METHODS

We studied 30 patients who underwent local resections of ampullary tumors between February,

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1992 and March, 2004 at UCSF-Moffitt Hospital and the San Francisco Veterans Hospital. Data on demographics, tumor characteristics, clinical features, preoperative investigations, and hospital course were analyzed. Follow-up information was obtained through physician records, telephone interviews, and patient questionnaires.

Statistical Methods

Statistical analysis was performed using SPLUS 2000 statistical software (Insightful Corporation, Seattle, WA). Unless otherwise stated, all data are expressed as mean ± standard deviation. Logistic regression analysis was used to test for variables that might be significant for recurrence. Continuous variables were analyzed using Student's *t* test. Survival and recurrence curves were generated using the Kaplan-Meier method. A significance level of $P < 0.05$ was used for all tests.

Operative Technique

A Kocher maneuver was used to mobilize the duodenum and the head of the pancreas. A short transverse duodenotomy was made in the lateral aspect of the duodenum after the tumor had been located by palpation. The tumor was inspected to determine whether a local resection could be performed with adequate margins. In general, it had to be <3 cm in diameter and relatively pedunculated and mobile with respect to the medial wall of the duodenum to be considered a candidate for this operation. A 12 F catheter was passed into the common bile duct and advanced through the ampulla to retract (pedunculate) the tumor through the duodenotomy and to stent the common duct with nonconductive material. The catheter was inserted through a choledochotomy in the lower end of the common bile duct, or through the cystic duct if it would accommodate a catheter this big. A cholecystectomy was performed. The duodenal mucosa was cut through circumferentially about 5 mm from the edge of the tumor. The dissection was initially carried beneath the tumor at the bile duct (upstream) margin, and the bile duct wall was transected with the electrocautery. The nonconducting rubber tube, within the lumen of the duct, simplified identification of the duct and its transection without injuring other tissues. If gross tumor was seen at the cut bile duct margin, a complete resection will not be possible without resorting to a pancreaticoduodenectomy. If the gross bile duct margin was visibly uninvolved, we proceeded with the resection and separated the tumor from the underlying duodenal muscle and pancreatic duct. The specimen was removed and oriented for the pathologist, who was asked to perform frozen section examinations of the duodenal and bile duct margins and to determine whether the tumor was benign or malignant. Once the resection was complete, the common bile duct and pancreatic duct orifices were sutured to the edges of the duodenal mucosa. The duodenotomy and choledochotomy were closed, and a drain was left in the area.

RESULTS

Patient Demographics

There were 17 men and 13 women with a median age of 70 years (range 27 to 84; Table 1). Common early findings were abdominal pain (43%), elevated liver function tests (30%), and bile duct dilatation (30%). There was no case of a polyposis syndrome. Preoperative investigations included ultrasound, CT scan, endoscopic retrograde cholangiopancreatogram (ERCP), and endoscopic ultrasound (EUS; 3 patients). The bile or pancreatic ducts were dilated in 50% of patients whose final diagnosis was adenocarcinoma, and in 39% of patients whose final diagnosis was adenoma ($P = 0.63$).

Tumor Size and Histology

Preoperative biopsies were obtained in all patients (Table 2). The final pathologic diagnoses were 23 villous adenomas, 6 adenocarcinomas, and 1 paraganglioma. The mean tumor size was 2.4 cm (±1.0 cm). All adenomas that contained *high-grade dysplasia* on preoperative biopsy were found to contain invasive adenocarcinoma when the entire tumor had been examined. One of the five patients with adenomas that contained *atypia* on preoperative biopsy was found to contain adenocarcinoma on final examination.

Operative Morbidity and Mortality

Seven surgeons were involved; two of the seven performed 83% of the operations. The mean length

Table 1. Clinical presentation

Clinical/laboratory findings	No. of patients
Bile duct dilatation	9
Abdominal pain	13
Pancreatitis	4
Weight loss	1
Incidental	1
Anemia	4
↑ Liver enzymes	9
Pruritus	1

Table 2. Biopsy results

Preoperative biopsy	Final pathology
Adenoma (18)	Adenoma (18)
Adenoma with high-grade dysplasia (4)	Adenocarcinoma (4)
Adenoma with high-grade dysplasia and focal adenocarcinoma (1)	Adenocarcinoma (1)
Adenoma with atypia (5)	Adenocarcinoma (1) Adenoma (3) Paraganglioma (1)
Nonspecific (2)	Adenoma (2)

of stay was 7.9 days (±2.3 days). There were seven complications in 30 patients (morbidity rate, 23%; Table 3). There were no deaths in the hospital or within 60 days of the operation.

Frozen Section Analysis

Intraoperative frozen sections were performed in 20 patients (67%). The sensitivity and specificity of frozen section examination to detect adenocarcinoma were 25% and 100%, respectively. The positive predictive value and negative predictive value were 100% and 84%, respectively. The status of the margins was checked by frozen section in 17 of the 20 patients. In 11 of the 17 patients (65%), there was agreement between the frozen and permanent section margins. In one patient (6%), margins on permanent section were clear despite a questionably positive margin on frozen section. In five of the 17 patients (29%), frozen section margins were difficult to assess because of cautery artifact, but were felt to be clear. In two (12%) of these cases, margins that appeared clear on frozen section were positive on permanent section.

Frozen section examination was not performed on 10 small tumors (<2 cm) that were mobile, pedunculated, and thought to have ample margins. Two of these cases revealed adenocarcinoma on permanent section (Table 4).

Table 3. Postoperative course

Postoperative complications	No. of patients
Wound infection	2
Pneumonia	1
Urinary retention	1
C. difficile colitis	1
Chronic pancreatitis/stricture	1
Perinephric abscess	1

Six adenocarcinomas were treated by local excision (Table 4). In three, local excision was chosen because comorbid conditions precluded a pancreaticoduodenectomy (PD). Of these cases, one had been identified on the preoperative biopsy, one was identified by frozen section examination during the operation, and one was misdiagnosed as adenoma on frozen section but carcinoma was found later on the permanent sections. In the other three cases, frozen sections gave a diagnosis of benign adenoma in two cases, and a frozen section was not obtained in one patient with a small benign mobile tumor.

Two (33%) of the six adenocarcinomas recurred an average of four years after the operation. Both specimens had negative margins on permanent sections. At the time of recurrence, one patient had liver metastases; in the other patient, the tumor was unresectable due to local tumor invasion.

Follow-up and Tumor Recurrence

The mean follow-up was 5.8 years (±2.9 years). Of the 23 villous adenomas, three recurred (13%), all as benign adenomas. The average time to recurrence was 3.2 years. Two were treated with PD and one with endoscopic snare polypectomy. Six specimens (five benign and one malignant) had positive margins on permanent section, but the tumor recurred in only one.

Logistic regression analysis showed that tumor recurrence was independent of tumor size or the presence of a positive margin. The probability of tumor recurrence for patients with adenocarcinoma was 17% at 5 years and 58% at 10 years (Fig. 1). The probability of tumor recurrence for patients with benign adenomas was 9% at 5 years and 18% at 10 years (Fig. 2). Nine patients died during the follow-up period, two from recurrent adenocarcinoma. The 5-year and 10-year survival rates for patients with adenocarcinoma were 50% and 25%, respectively (Fig. 3).

Surveillance

The average time to recurrence for benign and malignant lesions was 3.5 years. Twenty-three patients were followed for at least 3.5 years, and of these, only 10 patients (43%) had undergone surveillance ERCP for more than 3.5 years after the initial operation.

DISCUSSION

The management of patients with ampullary tumors can be challenging to even the most experienced

Table 4. Patients with adenocarcinoma

Patient No.	Tumor size (cm)	Preop biopsy	Frozen section	Final pathology	T stage	Margin	Recurrence
1	1.8	High-grade dysplasia	Adenoma	Adenocarcinoma	1	Positive	No
2	1.6	High-grade dysplasia + focal area of carcinoma	—	Adenocarcinoma	1	Clear	Yes
3	5.0	High-grade dysplasia	Adenoma	Adenocarcinoma	1	Clear	No
4	1.2	High-grade dysplasia	—	Adenocarcinoma	1	Clear	No
5	3.0	High-grade dysplasia	Epithelial dysplasia	Adenocarcinoma	1	Clear	No
6	0.8	Marked atypia	Infiltrating adenocarcinoma	Adenocarcinoma	2	Clear	Yes

surgeon. In this study, thirty patients who underwent transduodenal local resection of benign and malignant tumors of the ampulla were analyzed. Twenty percent of the tumors were malignant, which is consistent with the experience of others,[13–16] and one of the malignancies was a T2 lesion. In 50% of the patients with malignant disease, bile duct dilatation was identified on preoperative imaging, a finding consistent with other studies that have found bile duct dilatation and obstructive jaundice to be more common in patients with invasive cancer than in those with benign ampullary tumors.[11,17] Nevertheless, obstructive jaundice may also be present in patients with benign tumors, and in this study, there were no clinical or imaging findings that predicted malignancy.

Preoperative biopsies were obtained in all patients, and of six patients who were ultimately found to have adenocarcinoma, the diagnosis had been made in only one (17%). Biopsies of ampullary tumors obtained via ERCP are prone to sampling errors and are inaccurate in about 25% of cases.[18–20] We found that all patients with high-grade dysplasia on preoperative biopsy had adenocarcinoma on the final pathologic examination. Heidecke and colleagues[21] found that 44% of adenomas containing areas of primary high-grade dysplasia had invasive carcinoma. We believe that patients with high-grade dysplasia should have a Whipple procedure rather than attempting a local resection in hopes that the lesion will be benign and curable by the lesser procedure.

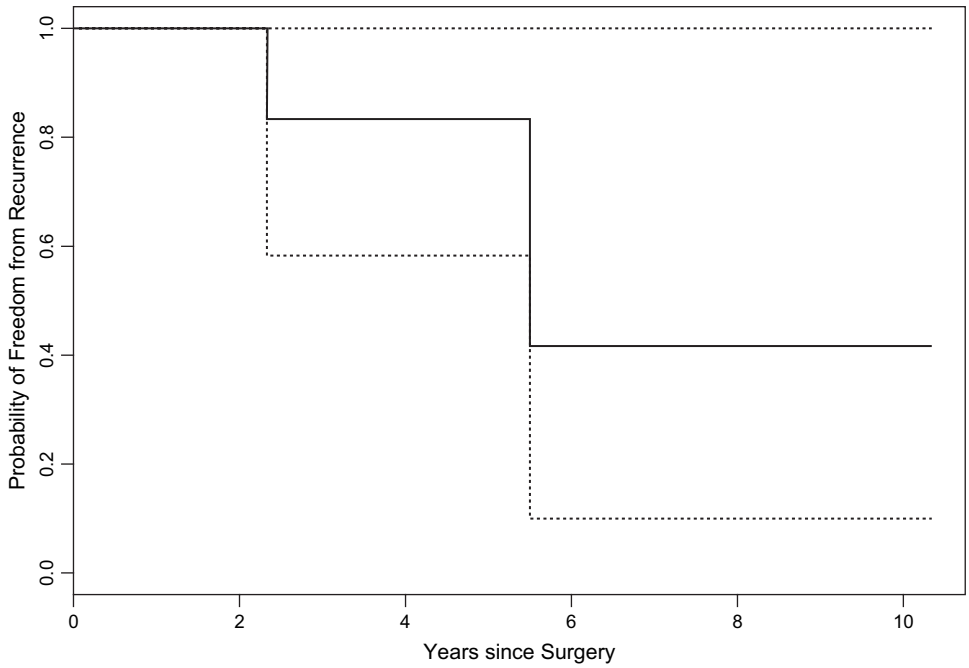

Fig. 1. Probability of freedom from recurrence (Kaplan-Meier) in six patients with adenocarcinoma after transduodenal local resection. Probability of recurrence at 5 years and 10 years was 17% and 58%, respectively. Dotted lines represent 95% confidence intervals.

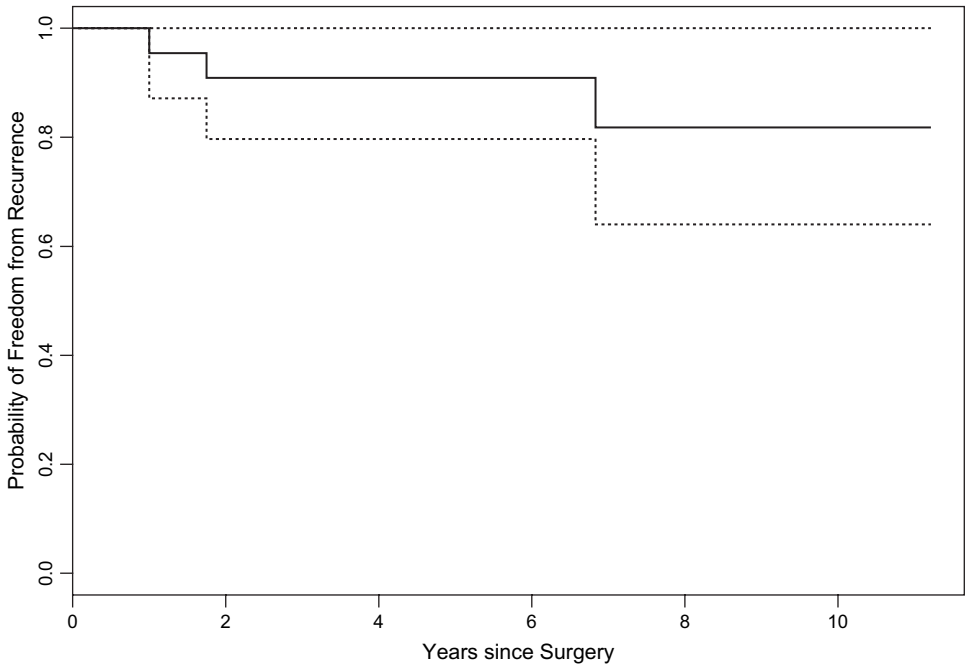

Fig. 2. Probability of freedom from recurrence (Kaplan-Meier) in 24 patients with benign adenomas after transduodenal local resection. Probability of recurrence at 5 years and 10 years was 9% and 18%, respectively. Dotted lines represent 95% confidence intervals.

Five patients demonstrated atypia on preoperative biopsy, one of whom had adenocarcinoma. The significance of atypia as a clue to adenocarcinoma elsewhere in the specimen is not as clearly defined as it is for high-grade dysplasia. Clary et al.[17] regarded severe atypia on preoperative biopsy as malignant and treated these patients with PD. Farnell et al.,[10] on the other hand, considered villous adenomas with atypia as a benign disease, and the majority of their 48 patients were treated with local resection. All that can be said is that atypia, particularly marked or severe atypia, should raise suspicions of an underlying malignancy and prompt further investigation such as frozen section analysis.

Intraoperative frozen section is useful in deciding whether to perform a local resection. Frozen sections were performed in 20 of our patients, and margins were reported in 17 patients. The positive and negative predictive values in detecting adenocarcinoma were 100% and 84%, respectively, which is comparable to the results of others.[22,23] All three cases with false-negative readings had high-grade dysplasia on the preoperative biopsy, and if this finding had been considered a signpost for adenocarcinoma, a more appropriate procedure (PD) would have been performed. The importance of negative margins for ampullary carcinomas has been stressed previously.[24–26] Negative margins were the most important determinant of survival. It is often difficult for pathologists to assess frozen section margins because the specimen is small and shapeless, and artifacts have been created by electrocautery. In this study, two false-positive margins were obtained on frozen section because of cautery changes to the margins. One patient with adenocarcinoma had a positive margin on permanent section. The tumor did not recur, but the patient died from unrelated causes, 2.4 years postoperatively, that limited the period of observation.

There were five recurrences. Neither tumor size nor positive margins correlated with recurrence of benign adenomas. Furthermore, tumor size was not a predictor of the presence of malignancy, which is in agreement with other studies.[10,22] Logistic regression analysis showed that recurrence was more likely in patients with malignant tumors as opposed to benign villous adenomas. One third of the malignant tumors in this study recurred, and in both cases was fatal. We believe that a Whipple procedure is preferable for cancers unless the patient is too ill to undergo this operation. Because the recurrence rate for benign tumors was only 13%, and none recurred as a malignant tumor, our study supports the idea that local resection, when technically feasible, is preferable for benign tumors.[9,11,22]

Endoscopic snare polypectomy is occasionally used as an alternative to surgery for benign ampullary tumors. Catalano and colleagues[1] performed this procedure in 103 patients with benign

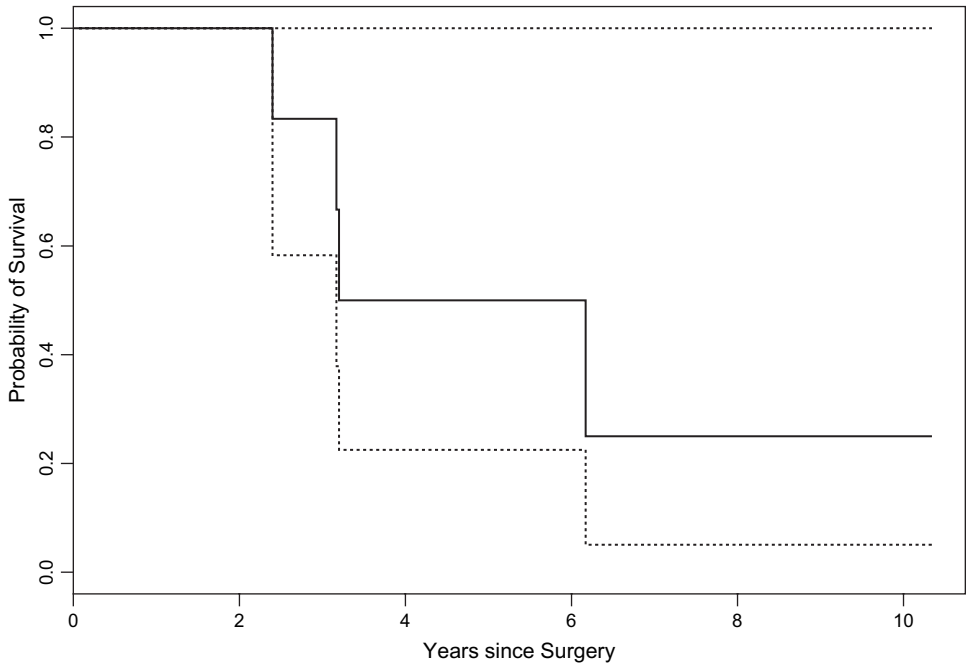

Fig. 3. Survival curve for six patients with adenocarcinoma after transduodenal local resection. Probability of survival at 5 years and 10 years was 50% and 25%, respectively. Dotted lines represent 95% confidence intervals.

adenomas. At a mean follow-up of 3 years, the recurrence rate was 20% and the complication rate was 10%. Small tumors (less than 2 cm) and older age (mean 55 years) were associated with a lower recurrence rate. Large tumors required piecemeal excision or thermal ablation for complete removal of tumor, and two or more endoscopic sessions were required in most cases. Other studies report initial success rates as low as 39%, recurrence rates between 10% and 40%, and complication rates around 25%.[2–6] Endoscopic polypectomy seems to be reasonable for small (less than 2 cm) benign adenomas, but more follow-up data are needed to be certain. We recently performed a Whipple procedure on a patient who had 15 endoscopic snare piecemeal excisions of a benign adenoma. After the final treatment session 3 years later, the lesion was considered eradicated, but it reappeared 1 year later and a PD was performed. When the specimen was examined, the tumor was noted to extend 2 cm up the common bile duct, where it was inaccessible to the endoscopist. The costs and risks of snare endoscopy, when pursued so doggedly, are extreme.

The overall average time to recurrence for both benign and malignant tumors was 3.5 years, and the range was 1–6.8 years. Thus, long-term endoscopic surveillance must be considered essential. Nevertheless, few of our patients had systematic follow-up inspections for more than 3.5 years. Follow-up surveillance should probably be carried out for 10 years, but the exact duration will have to be determined from further experience.

CONCLUSION

Transduodenal local resection is a good treatment for benign ampullary tumors, and it can be accomplished with little morbidity and a low recurrence rate (13%). Malignant ampullary tumors should be treated with PD, unless contraindicated, because of the high risk of recurrence (33%) and the unlikelihood that recurrent cancer can be cured. Preoperative biopsies have high false-negative rates for detecting adenocarcinoma and should be combined with intraoperative frozen section to improve diagnostic accuracy and to ensure negative margins. Endoscopic polypectomy is a reasonable alternative in patients with small, benign tumors, but the long-term recurrence rate is yet to be determined. Prolonged surveillance is indicated for local resections whether performed endoscopically or surgically.

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Predictors of Intensive Care Unit Admission and Related Outcome for Patients After Pancreaticoduodenectomy

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High-volume centers have low morbidity and mortality after pancreaticoduodenectomy (PD). Less is known about treatment pathways and their influence on intensive care unit (ICU) utilization. Patients who underwent PD at a tertiary cancer center during the five-year period between January 1998 and December 2003 were identified from a prospective database. Preoperative and intraoperative factors relating to ICU admission and outcome were analyzed. Five hundred ninety-one pancreaticoduodenectomies were performed during the study period. Of these, 536 patients had complete records for analysis. Of the 536 patients, 51 (10%) were admitted to the ICU after surgery. Admission to the ICU was associated with decreased overall survival ($P < .0001$). Of the preoperative predictors of ICU admission, serum creatinine, albumin, and increased body mass index (BMI) were associated with ICU admission ($P = .02$, .05, and .002, respectively). Age, blood glucose, diagnosis of diabetes mellitus, and chronic obstructive pulmonary disease were not predictive of ICU admission on univariate analysis. Of the intraoperative factors, longer operative time and estimated blood loss (EBL) correlated with ICU admission ($P = .003$ and .0001, respectively). On multivariate analysis, only preoperative BMI and intraoperative EBL were independent predictors of ICU admission ($P = .03$ and .003, respectively). Patients with a preoperative BMI greater than 30 had a substantially higher risk of ICU admission (relative risk 2.4). The majority of patients who undergo PD do not require admission to the ICU. Factors most associated with ICU admission after PD are increased preoperative BMI and intraoperative blood loss. (J GASTROINTEST SURG 2005;9:1307–1312)

KEY WORDS: Pancreas neoplasm, perioperative

Commonly performed elective surgical procedures on the alimentary tract are carried out with low morbidity and mortality in most hospitals in the United States. Certain procedures such as pancreaticoduodenectomy (PD) are performed with a relatively low frequency and have been associated with higher mortality at low-volume centers.[1] Several experienced high-volume centers have reported operative mortality rates of less than 5% for PD or total pancreatectomy performed for cancer,[1–7] a difference that influences overall survival 10 years after operation.[8] Little is known about what produces the observed associations between volume and outcome. High-volume centers have a broad range of specialist services and more consistent processes for postoperative care,[9] including intensive care unit (ICU) services that may not be available or cost-effective at low-volume centers.

The common practice of admitting all patients to an ICU after a complex operation is based upon concern for timely identification of adverse events, yet may not be necessary as general policy.[10] The specific aims of this study were to determine the prevalence and outcome of ICU admission among patients undergoing PD. Additionally, we sought to determine the preoperative and perioperative risk factors associated with ICU admission after PD.

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METHODS

Patients who underwent PD at a tertiary cancer center during the five-year period between January 1998 and December 2003 were identified from a prospective database. Data collected included patient demographics and preoperative and intraoperative factors relating to ICU admission and outcome. Review of clinicopathologic features and follow-up of all patients was approved by the Institutional Review Board.

The surgical techniques have previously been described.[4] Reconstruction methods varied, particularly for the pancreaticojejunostomy, and were at the discretion of the individual surgeon. Percutaneous drains were not placed routinely at the end of the procedure.[11] Routine feeding jejunostomy was not used.

Patients were routinely extubated in the operating room unless they were judged to have poor cardiopulmonary reserve or if surgery had been complicated or prolonged. Patients generally spend one evening monitored in the recovery room and are then transferred to the surgical ward. Patients who were subsequently admitted to the ICU were evaluated and compared to those patients who did not require ICU admission. Among patients admitted to the ICU after PD, the subset admitted immediately from the recovery room was compared to those transferred from the surgical ward. The rate of ICU admission was analyzed according to several preoperative and intraoperative factors including age, gender, body mass index (BMI), history of diabetes mellitus, coronary artery disease or chronic obstructive pulmonary disease, pathology, intraoperative estimated blood loss (EBL), and length of operation. Complications were defined according to criteria set forth by surgical secondary event guidelines at Memorial Sloan-Kettering Cancer Center[12] and have been previously defined.[13,14] Complications were graded as follows: Grade I was minor, requiring oral medication or bedside care; Grade II required additional intravenous medicine/treatment; Grade III required additional, more invasive treatment such as interventional radiology or operative intervention; Grade IV was severe, resulting in chronic deficit/disability; and Grade V was death. Anastomotic leak was defined as clinical signs and symptoms or radiographic confirmation of biliary/pancreatic leak. If a drain was in place, greater than 50 ml per day of amylase-rich fluid beyond day 5 was considered a pancreatic leak. Categorical variables were compared using the χ^2 test. Multivariate analyses were carried out with logistic regression using forward stepwise selection algorithm. Statistical significance was taken at $P < 0.05$. All statistical analyses were performed using the SPSS software (version 8.0, SPSS Inc., Chicago, IL).

Table 1. Preoperative patient characteristics (n = 536 patients)

	N (%)
Age (median) (range)	68 yr (21–90 yr)
Follow-up (median) (range)	14 mo (1–61 mo)
Gender	
Females	266 (49.6%)
Males	270 (50.3%)
Pathology	
Adenocarcinoma	
Pancreas	295 (55%)
Duodenal	31 (6%)
Ampullary	76 (14%)
Distal bile duct	28 (5%)
Other	1 (0.2%)
Premalignant	36 (7%)
Neuroendocrine	38 (7%)
Benign	31 (6%)

Premalignant = IPMN, high-grade dysplasia, PanIn lesions.
IPMN = intraductal papillary mucinous neoplasms; PanIn = pancreatic intraepithelial neoplasia.

RESULTS

Five hundred ninety-one pancreaticoduodenectomies were performed during the study period. Five hundred thirty-six patients had complete follow-up. The median follow-up for the study population was 14 months (range, 1–61 months). The distribution of histologic diagnoses is shown in Table 1. The thirty-day mortality rate was 3%. The overall complication rate was 51% (Table 2). The

Table 2. Intraoperative and postoperative characteristics (n = 536)

EBL (median)	700 ml (100–9000 ml)
OR time (median)	299 min (111–640 min)
LOS (median)	10.3 days (6–83 days)
Complications	
Overall	274 (51%)
Grade I	84 (16%)
Grade II	78 (15%)
Grade III	104 (19%)
Grade IV	1 (0.4%)
Grade V	7 (1.3%)

EBL = estimated blood loss; LOS = length of stay; OR = operating room. Complications are graded as follows: I = minor requiring oral medication or bedside care; II = requiring additional intravenous medicine/treatment; III = requiring additional more invasive treatment such interventional radiology or operative intervention; IV = severe resulting in chronic deficit/disability; V = death.

Table 3. Reasons for admission to the ICU

	No. of patients
Immediate	
Intraoperative blood loss/fluid management	7
Monitoring	2
Cardiac	1
Pulmonary	1
Delayed	
Pancreatic/biliary fistula	19
Cardiac	7
Pulmonary	7
Blood loss	4
Other	3

ICU = intensive care unit.

anastomotic leak rate for the entire group was 12%. The overall median EBL for operations during the study period was 700 ml (range, 100–9000 ml), and the median operative duration was 299 minutes (range, 111–640 minutes). Ten percent (51/536) of patients were admitted to the ICU postoperatively. Eleven patients were admitted immediately to the ICU from the recovery room, and 40 patients were transferred to the ICU from the surgical wards. The median postoperative day of transfer for these patients was postoperative day 4 (range, 2–15). Reasons for transfer to the ICU are outlined in Table 3. The median length of ICU stay was 4 days. The median length of postoperative stay overall was 10.3 days (range, 6–83 days), which was greater when there was a transfer to intensive care (23.2 days vs. 10.1 days, $P = .0001$). Admission to the ICU was associated with decreased overall survival (median survival 16 months vs. 31 months, $P < .0001$) for all patients undergoing PD during the study period (Fig. 1). The subset of patients with periampullary adenocarcinoma who were admitted to the ICU also had a significant decrease in overall survival (median survival 11 months vs. 28 months, $P < .0001$) despite earlier stage disease than the group not admitted to the ICU (Table 4).

Of the preoperative predictors of ICU admission, serum creatinine, serum albumin, and preoperative BMI were associated with ICU admission ($P = .02$, .05, and .002, respectively). Age and diagnosis of diabetes mellitus, coronary artery disease, or chronic obstructive pulmonary disease were not predictive of ICU admission on univariate analysis (Table 5). Of the intraoperative factors, operative time and intraoperative blood loss correlated with ICU admission ($P = .003$ and .0001, respectively). Patients who had a postoperative complication, including pancreatic and biliary anastomotic leak or fistula, were also more likely to be admitted to the ICU ($P = .01$).

On multivariate analysis, only preoperative BMI and intraoperative EBL were independent predictors of ICU admission ($P = .03$ and .003). Patients with a preoperative BMI greater than 30 had a substantially higher risk of ICU admission (relative risk 2.4). For the patients immediately transferred to the ICU from the recovery room, intraoperative EBL was the only independent predictor of admission to the ICU, whereas preoperative BMI greater than 30 was the only independent predictor of delayed ICU admission from the surgical ward.

DISCUSSION

The development of intensive patient care has increased our ability to monitor, diagnose, and treat

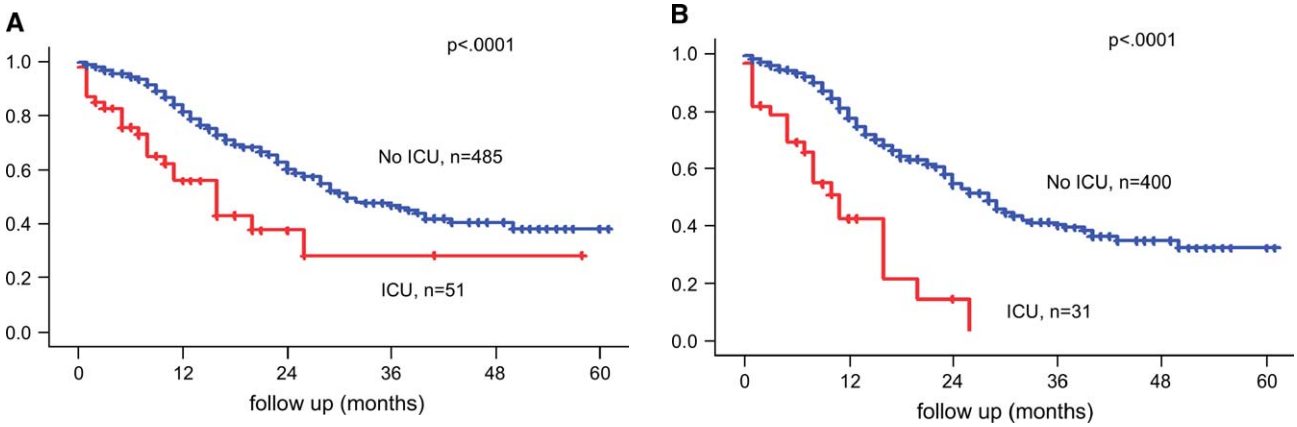

Fig. 1. (**A**) Overall survival of patients after pancreaticoduodenectomy. No ICU admission (n = 485); ICU admission (n = 51; $P = 0.02$). (**B**) Overall survival of patients with periampullary adenocarcinoma stratified by ICU admission (n = 400 without vs. n = 31 with, $P = <.0001$). ICU = intensive care unit.

Table 4. Pathologic staging of periampullary cancer

	Overall (n = 431)	No ICU (n = 400)	ICU (n = 31)	*P* value
T stage				.01
T1	40	35	5	
T2	54	53	1	
T3	301	283	18	
T4	36	29	7	
N stage				.001
N0	168	153	15	
N1	263	247	16	
AJCC stage				.03
I	46	40	6	
II	340	322	18	
III	45	38	7	

T stage = tumor size; N stage = lymph nodes; AJCC = The American Joint Committee on Cancer.

critically ill patients; consequently, critical care practice has grown substantially. However, ICU care is not only expensive but is limited in its capacity. As centers begin to increase capacity to perform complex oncologic procedures for an aging population, we wanted to define the need for intensive care for an index case pancreaticoduodenectomy at a high-volume cancer center. Many groups have examined ICU utilization after admission to the ICU in terms of length of stay and mortality rates.[15–17] We sought to evaluate ICU utilization for postoperative patients with pancreatic disease to optimize treatment pathways to facilitate the most efficient allocation of patient care resources. Treatment pathways have been used to lower ICU and laboratory utilization.[9,18] The current study shows that the incidence of ICU admission after PD is low at our institution.

The need for postoperative monitoring in high-risk patients is one of the most common reasons for ICU admission. An estimated 30% of admissions to the surgical ICU are for monitoring alone without need for any active therapeutic interventions.[19–21] Routine ICU admission after major operation has not been shown to improve outcome.[20,22] In fact, when comparing hospitals in the United States and Canada, the U.S. hospitals with the more liberal use of critical care after operation did not improve survival.[22] The influence of ICU admission on outcome has been evaluated after organ transplantation. Aggarwal et al.[23] found no influence of preoperative ICU admission for liver failure on survival after orthotopic liver transplantation. At our institution, patients are not routinely admitted to the ICU after PD. Our ICU admissions were for patients with either severe comorbities or for unexpected postoperative events, and so were associated with decreased overall survival.

Age was not a significant risk factor for ICU admission in this study, but the patients ⩾65 years had a higher rate of ICU admission than those <65 years (11% vs. 5%). Other groups have found varying influence of age on outcome. Su et al.[24] reported a 10-fold postoperative mortality rate for patients older than 75 years after PD. In contrast, Sohn et al.[25] reported a mortality rate of 4% for patients older than 80 years. Similarly, Yeo et al.[26] did not find any difference in outcome for patients who were older than 65 years.

Hyperglycemia associated with insulin resistance is common in the perioperative period in patients

Table 5. Predictors of ICU admission, univariate analysis

	No ICU (n = 485 patients)	ICU (n = 51 patients)	*P* value
Age (mean)	65	68.6	.07
DM	73 (15%)	11 (22%)	.09
CRI	38 (8%)	9 (18%)	.02
CAD	48 (10%)	7 (14%)	.24
COPD	15 (3%)	3 (5%)	.69
Albumin <3.5	68 (14%)	12 (24%)	.05
BMI >30 kg/m^2	31 (6%)	11 (22%)	.002
Gender			
Females	247 (51%)	19 (37%)	
Males	238 (49%)	32 (63%)	.04
EBL (median)	700 ml (100–4200 ml)	900 ml (100–9000 ml)	.0001
OR time (median)	295 min (111–530 min)	318 min (163–665 min)	.003
LOS (median)	10.1 days (6–59 days)	23 days (7–75 days)	.0001

BMI = body mass index; CAD = Coronary artery disease; COPD = chronic obstructive pulmonary disease; CRI = chronic renal insufficiency; DM = diabetes mellitus; EBL = estimated blood loss; ICU = intensive care unit; LOS = length of stay; OR= operative room.

undergoing PD.[27] It is well-known that hyperglycemia may contribute to complications in critically ill patients[28,29] and that glycemic control with intensive insulin therapy has been shown to reduce morbidity and mortality in these patients.[30–32] In our study, patients who had preoperative diabetes did not have a significantly higher rate of ICU admission, although there was a slight trend for higher admission rates for diabetics vs. nondiabetics (12% vs. 8%).

Other risk factors that we evaluated for adverse outcome after surgery included renal disease and elevated body weight. Previous groups[33,34] have identified even mild renal disease as a major risk factor for cardiovascular events and hospitalization. Go et al.[34] found an independent, graded association between mild renal dysfunction and the risk of death, cardiovascular events, and hospitalizations. In our study, preoperative renal insufficiency did not correlate with ICU admission after PD. Elevated body weight has also been shown to be a detrimental factor in long-term, cancer-related outcome. Enger et al.[35] found that women in the highest weight category experienced a 2.5-fold increased risk of dying of breast cancer. Hu et al.[36] reported that both excess weight and physical inactivity were significantly associated with increased mortality. For nonsmokers, overall mortality was twice as high among those who were obese (BMI $> 30 kg/m^2$), with mortality from cancer increased by 65%. Obesity has been described as a risk factor for anastomotic leakage and blood transfusion after rectal resection by laparotomy.[37–39] Sledzianowski et al.[40] showed on univariate analysis that obesity (BMI $> 25 kg/m^2$) is a risk factor for intraabdominal morbidity (abdominal collections and pancreatic fistula) after distal pancreatectomy. In the present study, we identified elevated BMI and intraoperative blood loss as independent risk factors for ICU admission. Excluding immediate transfers to the ICU from the recovery room, elevated BMI was the only patient factor identified to be independently associated with ICU admission.

Blood loss during operation is usually a marker for a more complex operation, a complication, or more extensive disease, and when elevated accompanies the need for blood transfusion. Blood loss during childbirth has been shown to be a risk factor for admission to the ICU.[41] Blood transfusion has been associated with adverse immunomodulatory effects and worse outcome, especially in cancer patients undergoing an operation.[42,43] Yeh et al.[44] evaluated the influence of blood transfusions on outcome in patients after PD for cancer and found that transfusion was predictive of worse outcome. We did not assess directly the influence of transfusion on outcome, only that elevated intraoperative EBL was associated with admission to the ICU.

In conclusion, the majority of patients who undergo PD do not require admission to the ICU. Factors most associated with ICU admission after pancreaticoduodenectomy are increased preoperative BMI and intraoperative blood loss.

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Esophageal Mucosal Damage May Promote Dysmotility and Worsen Esophageal Acid Exposure

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This study determines the relationship among esophageal dysmotility, esophageal acid exposure, and esophageal mucosal injury in patients with gastroesophageal reflux disease (GERD). A total of 827 patients with GERD (confirmed by ambulatory pH monitoring) were divided into three groups based on the degree of mucosal injury: *group A*, no esophagitis, 493 patients; *group B*, esophagitis grades I to III, 273 patients; and *group C*, Barrett's esophagus, 61 patients. As mucosal damage progressed from no esophagitis to Barrett's esophagus, there was a significant decrease in lower esophageal sphincter pressure and amplitude of peristalsis in the distal esophagus, with a subsequent increase in the number of reflux episodes in 24 hours, the number of reflux episodes longer than 5 minutes, and the reflux score. These data suggest that in patients with GERD, worsening of esophageal mucosal injury may determine progressive deterioration of esophageal motor function with impairment of acid clearance and increase of esophageal acid exposure. These findings suggest that Barrett's esophagus is an end-stage form of gastroesophageal reflux, and that if surgical therapy is performed early in the course of the disease, this cascade of events might be blocked. (J GASTROINTEST SURG 2005;9:1313–1317)

KEY WORDS: Gastroesophageal reflux disease, esophageal peristalsis, esophageal acid clearance, lower esophageal sphincter, Barrett's esophagus

The pathogenesis of gastroesophageal reflux disease (GERD) is clearly multifactorial.[1] Although some of the factors such as the mechanical properties and behavior of the lower esophageal sphincter (LES) have been described, the relationship among GERD, esophageal peristalsis, and esophageal mucosal changes has not been fully elucidated. It is unclear whether esophageal mucosal injury secondary to persistent reflux promotes esophageal dysmotility and poor acid clearance or whether peristaltic dysfunction is a primary pathogenetic factor independent of mucosal changes.[2] The aim of this study was to elucidate the relationship between esophageal mucosal damage and esophageal motility. We hypothesize that as esophageal mucosal damage progresses from esophagitis to Barrett's esophagus, there is a subsequent impairment of esophageal motility and esophageal acid clearance.

PATIENTS AND METHODS

A total of 827 patients with GERD confirmed by 24-hour pH monitoring (defined by the percentage of time the pH was < 4.0 and by the composite reflux score) were divided into three groups based on the degree of mucosal injury: group A, no esophagitis; group B, grades I to III esophagitis; group C, Barrett's esophagus. Exclusion criteria included patients with underlying primary motility disorders, paraesophageal hernias, and previous esophageal or gastric surgery.

Endoscopy

Endoscopy was performed in all patients to assess the degree of esophageal mucosal injury. Barrett's esophagus was confirmed both endoscopically and

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histologically. The degree of mucosal injury was graded according to the Savary-Miller classification.[3]

Symptoms

Every patient was questioned regarding the presence and duration of symptoms (heartburn, regurgitation, dysphagia, and cough). Symptoms were scored using a 5-point scale, ranging from 0 (no symptom) to 4 (disabling symptom).

Esophageal Manometry

All patients were studied after an overnight fast using techniques previously described.[4] Medications that might interfere with esophageal motor function (i.e., nitrates, metoclopramide, and calcium-channel blocking agents) were discontinued at least 48 hours before the study. Length and pressure of the LES were calculated using the station pull-through technique, with 0.5-cm increments between stations. Esophageal body function was recorded 3, 8, 13, and 18 cm above the upper border of the LES using 10 swallows of 5 mL of water, given at 30-second intervals. Amplitude of the peristaltic waves was calculated independently for the distal (3 and 8 cm above the LES) and proximal esophagus (13 and 18 cm above the LES).[5] Ineffective esophageal motility (IEM) was defined manometrically as distal esophageal amplitudes measuring less than 30 mm Hg or more than 30% simultaneous contractions.[6] The data were analyzed using a commercial software program (Gastrosoft, Medtronic Functional Diagnostic, Shoreview, MN).

Ambulatory 24-Hour pH Monitoring

Acid-suppressing medications were discontinued 3 days (H_2 blocking agents) or 14 days (proton pump inhibitors) before the study. The pH catheter was calibrated in a standard buffer solution at pH 1 and 7 before and after monitoring, and positioned 5 cm above the upper border of the manometrically determined LES.[7] During the study, the patients consumed an unrestricted diet and took no medications that could interfere with the results. Esophageal acid exposure (percentage of time pH < 4) in the upright and supine positions and esophageal acid clearance (mean duration of a reflux episode) were calculated for the distal and proximal esophagus using a commercial software program (Gastrosoft, Medtronic Functional Diagnostic).

Statistical Analysis

Analysis of variance, Mann-Whitney rank-sum test, and chi-square test were used for statistical evaluation of the data. Unless otherwise stated, all data are expressed as the median and range. *P* values of less than .05 were considered statistically significant.

RESULTS

A total of 827 patients with GERD confirmed by pH monitoring were included in this study. As shown in Table 1, there was no statistical difference in age or sex between the three groups. A larger percentage of patients in groups A and B were taking proton pump inhibitors; however, the overall percentage of patients on acid suppression in each group was not statistically significant. Both typical and atypical symptoms were experienced by patients in all groups (Table 2). There were more patients in group B (esophagitis) with heartburn (85%) and dysphagia (44%) compared with group A (no esophagitis). There were more patients in group C (Barrett's) with heartburn (89%) compared with patients in group A, but there was no difference with respect to the other symptoms.

As demonstrated in Table 3, there was a significant decrease in the LES pressure between patients without esophagitis and patients with esophagitis (12 vs. 10 mm Hg, $P = .001$) and between patients without esophagitis and patients with Barrett's esophagus (12 vs. 8 mm Hg, $P = .02$). In addition, there was a significant decrease in the distal esophageal

Table 1. Demographic data

	Group A	Group B	Group C
Number of patients	493	273	61
Age (y)	51 (15–83)	50 (14–90)	54 (26–81)
Sex (F/M)	246/247	131/142	24/37
No. (%) of patients on acid suppression	468 (95)	249 (91)	56 (90)
No. (%) of patients on H_2 blockers	12 (3)	10 (4)	0 (0)
No. (%) of patients on PPI	319 (68)*	149 (60)†	27 (48)
No. (%) of patients on H_2 blockers + PPI	137 (29)	90 (36)†	29 (52)‡
No. (%) of patients relieved with medication	147 (31)*	109 (44)	18 (32)

PPI = proton pump inhibitor.
*Group A vs. B $P < .05$.
†Group B vs. C $P < .05$.
‡Group A vs. C $P < .05$.

Table 2. Symptoms

	Group A	Group B	Group C
Duration of symptoms (mo)	48 (1–600)	60 (1–480)	72 (1–480)†
Symptoms	No. (%) of patients		
Dysphagia	172 (35)*	119 (44)	24 (39)
Regurgitation	334 (68)	200 (73)	47 (76)
Heartburn	379 (76)*	233 (85)	55 (89)†
Chest pain	211 (43)	132 (48)	29 (47)
Cough	173 (35)	78 (29)	19 (31)
Aspiration	76 (15)*	25 (9)	6 (10)

*Group A vs. B $P < .05$.
†Group A vs. C $P < .05$.

amplitude (67 vs. 47 mm Hg, $P = .007$) between patients with Barrett's esophagus and patients without esophagitis. IEM was most frequent in patients in group C (43%), but the incidence decreased significantly as the amount of mucosal damage decreased in patients in group A (13%). Overall, 56% of patients without esophagitis had normal peristalsis compared with only 38% of patients with Barrett's esophagus.

There was a significant increase in the number of reflux episodes in 24 hours and the number of reflux episodes longer than 5 minutes when comparing all three groups (Table 4). Patients with Barrett's esophagus had more upright and supine reflux and overall had higher DeMeester scores (65) compared with patients in group B (49) and group A (39).

Table 3. Esophageal manometry

	Group A	Group B	Group C
LES pressure (mm Hg)	12 (2–57)*	10 (2–40)	8 (2–43)‡
UES pressure (mm Hg)	78 (16–237)	78 (8–212)	78 (4–164)
Distal esophageal amplitude (mm Hg)	67 (10–354)	64 (9–434)	47 (11–269)‡
Proximal esophageal amplitude (mm Hg)	56 (8–212)*	52 (9–147)	48 (3–134)
Body diagnoses	No. (%) of patients		
Hypertensive	21 (4)	6 (2)	2 (3)
Ineffective esophageal motility	65 (13)*	63 (23)†	28 (43)‡
Nonspecific esophageal motility disorder	133 (27)	76 (29)	10 (16)
Normal	274 (56)*	128 (47)	21 (38)‡

LES = lower esophageal sphincter; UES = upper esophageal sphincter.
*Group A vs. B $P < .05$.
†Group B vs. C $P < .05$.
‡Group A vs. C $P < .05$.

DISCUSSION

In this study, 827 patients with GERD were divided into three groups with various stages of mucosal damage, and the esophageal manometric and 24-hour pH profiles were then compared. All three groups demonstrated abnormalities in esophageal motility and acid clearance. However, as the amount of mucosal damage increased from group A to group C, both esophageal motility and acid clearance worsened, suggesting that esophageal mucosal damage may contribute to esophageal dysmotility and worsening of reflux. As esophageal mucosal damage increased, there was a significant increase in the number of reflux episodes in 24 hours and the duration of reflux episodes longer than 5 minutes. Furthermore, there was a progression of esophageal dysmotility as shown by the changes in LES pressure and the decrease in distal esophageal amplitude, which has been shown to be the main determinant of esophageal acid clearance.[8–11] These changes were greatest between patients without esophagitis and patients with Barrett's esophagus (Table 3). Our

Table 4. Ambulatory 24-hour pH monitoring

Distal probe	Group A	Group B	Group C
No. of reflux episodes in 24 h	133 (6–537)*	147 (14–773)†	224 (33–659)‡
No. of reflux episodes > 5 min	4 (0–113)*	5 (0–36)†	11 (0–42)‡
% time pH <4			
Total	9 (2–57)*	11 (3–78)†	16 (3–80)‡
Upright position	10 (1–55)*	12 (0–80)†	20 (1–79)‡
Supine position	4 (0–92)*	7 (0–90)†	20 (0–92)‡
DeMeester score	39 (15–228)*	49 (15–261)†	65 (15–261)‡

*Group A vs. B $P < .05$.
†Group B vs. C $P < .05$.
‡Group A vs. C $P < .05$.

findings are in agreement with Chrysos et al.,[2] who found that esophageal motility deteriorated and the presence of a mechanically defective LES increased as mucosal injury increased in 147 patients with documented GERD.

Mucosal damage alone, however, is not the initiating factor, nor is it the only factor that may contribute to worsening of GERD. The patients in group A showed no evidence of esophagitis on endoscopy, yet 13% demonstrated IEM. In addition, it has been shown that healing of esophagitis with medical therapy does not appreciably improve peristaltic function.[12] This suggests that reflux-induced mucosal injury extends beyond the mucosa into the muscular wall of the esophagus, leading to inflammation, scarring, and peristaltic dysfunction.[13]

Despite numerous studies investigating GERD, the natural history of GERD has yet to be fully explained. Some authors believe that GERD should be categorized into three groups of patients, nonerosive GERD, erosive GERD, and Barrett's esophagus, and that these groups are distinct and noncommunicating.[14] On the basis of this concept, it would also follow that the duration of GERD symptoms would not be a risk factor for the development of Barrett's esophagus, because patients do not progress to Barrett's esophagus; they are either predisposed to its development or else they remain in the nonerosive GERD or GERD category. The retrospective analysis published by Shiino and colleagues[15] would support this conclusion; they found that the duration of GERD had little influence on esophageal function. Other authors provide evidence that the three categories described above are part of a spectrum of disease, and that GERD should be considered a continuum, in which it is possible for the severity of the disease to fluctuate over time.[16] A similar observation was also made by Campos and colleagues,[13] who found that duration of GERD symptoms longer than 5 years was a risk factor for the development of Barrett's esophagus.

CONCLUSIONS

In patients with GERD, our study shows that esophageal acid exposure increased progressively with the severity of mucosal injury, and that this increase was paralleled by an increase in the incidence of esophageal dysmotility. The data, however, do not definitively clarify whether the abnormal motility is a consequence of esophagitis or just another component of a complex foregut motor disorder. For instance, we did not assess the influence of hiatal hernia size in this large group of patients, which has been found to be an important determinant of esophagitis presence and severity in GERD.[17,18] In addition, patients were evaluated only one time so that the manometric findings are no more than a snapshot in time of an individual esophageal function.

Overall, even in the absence of a definitive answer, we advocate a laparoscopic fundoplication to be performed early in the course of the disease. The operation, in fact, corrects the abnormal reflux by eliminating the hiatal hernia and reestablishing the competence of the LES and might avoid the progression to more severe mucosal damage.

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Laparoscopic Nissen Fundoplication Decreases Gastroesophageal Junction Distensibility in Patients With Gastroesophageal Reflux Disease

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Laparoscopic Nissen fundoplication (LNF) is the surgical treatment of choice for gastroesophageal reflux disease (GERD). Post-LNF complications, such as gas bloat syndrome, inability to belch and vomit, and dysphagia, remain too common and prevent LNF from being more highly recommended. It remains controversial as to whether preoperative assessment can predict the development of post-LNF complications. Some authors have shown a correlation between pre-LNF manometry characteristics and post-LNF dysphagia, and others have not. We hypothesize that many post-LNF complications are caused by a decrease in the distensibility of the GEJ and that standard manometry is at best an indirect measure of this. The aim of this study is to directly measure the effect of LNF on gastroesophageal junction (GEJ) distensibility (GEJD).The lower esophageal sphincter (LES) of 15 patients undergoing LNF was characterized using standard manometry. The GEJD before and after a standardized LNF was measured using a specialized catheter, containing an infinitely compliant bag, placed within the LES. GEJD was measured, as dV/dP over volumes 5 to 25 mL distended at a rate of 20 mL/min. Mean dP ± standard error of the mean for each volume was calculated, and distensibility curves were generated and compared. Measurements were also taken after abolishing LES tone by mid-esophageal balloon distension. Patient symptoms were recorded before and after surgery. Statistical analysis was performed by two-way repeated-measures analysis of variance, paired *t* test, and the Tukey test. Laparoscopic Nissen fundoplication led to a statistically significant increase in Δpressure over each volume tested and therefore a significant decrease in the distensibility of the GEJ. Abolition of LES tone had no statistical effect on GEJD after fundoplication. There were no complications, and none of the patients developed the symptom of dysphagia postoperatively. These are the first direct measurements to show that LNF significantly reduces the distensibility of the GEJ. We hypothesize that the magnitude of this reduction may be the vital variable in the development of post-LNF complications and specifically post-LNF dysphagia. The intraoperative measurement of LES distensibility may provide a means for avoiding this feared and other post-LNF complications in the future. (J GASTROINTEST SURG 2005;9:1318–1325)

KEY WORDS: Fundoplication, gastroesophageal junction distensibility, intraoperative

Each year more than 30,000 laparoscopic fundoplications are performed in the United States for management of gastroesophageal reflux disease (GERD).[1] In the majority of instances these operations are free of significant short- and long-term complications. However, approximately 5% to 10% of patients experience dysphagia postoperatively,[2,3] and 1% to 3% of cases require surgical revision or dilatation.[3,4] This complication has been attributed to many factors.[5] However, some believe this complication is the result of an excessively restrictive fundoplication impeding the normal bolus flow.[2,6] Radiographically these patients may exhibit esophageal narrowing and postswallow residue.[7,8] Manometry may reveal an excessively high lower esophageal sphincter (LES) high pressure zone resting pressure

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or residual pressure within the fundoplication[9] and/or elevated esophageal intrabolus pressure above the fundoplication.[2,10] However, there are still many cases of persistent postoperative dysphagia without obvious anatomic, structural, and/or motility abnormalities.[11]

Anatomically, Nissen fundoplication reduces hiatal herniation if present and increases the intra-abdominal length of the LES. Manometrically, fundoplication increases the resting pressure of the LES, increases the length of the high pressure zone at the gastroesophageal junction (GEJ), increases the residual LES pressure with swallow-induced relaxation, increases the intra-bolus pressure of esophageal peristalsis, and decreases the episodes of transient LES relaxations.[10,12–18] Fundoplication also can normalize ineffective esophageal peristalsis or conversely impair esophageal peristalsis postoperatively.[17,19] Some recent studies, however, have shown no manometric changes after fundoplication.[20]

The differences in the manometric results after fundoplication between traditional longer fundoplication and short, "floppy" fundoplication, in one study, were not proven to be significant.[21] Whether these manometric changes contribute to the clinical outcomes postoperatively is still controversial.[16] DeMeester et al.[22] convincingly showed significant differences in the incidence of postoperative dysphagia between the traditional longer and shorter, "floppy" Nissen fundoplications.[22]

The causes of many postfundoplication complications are still not yet completely known. A recent study by Blom et al.[5] demonstrated a significant correlation between preoperative esophageal manometric characteristic and the risk of development of postoperative dysphagia. Others have failed to demonstrate such a correlation.[23]

Intraoperatively, manometry has been used to try and optimize creation of the fundoplication,[24–26] but to date these results have not been able to predict the outcomes of fundoplication, making its utility questionable. The relationship between postoperative esophageal manometric findings and the outcome of surgery is also very poor.[26] Therefore, currently there is no objective intraoperative technique that can quantify and guide the construction of the fundoplication.

With the recent introduction of a technique to define the pressure volume curve of the GEJ, it is now possible to study the distensibility of the GEJ under various clinical, physiologic, and experimental conditions.[27] We hypothesize that many post-LNF complications such as gas bloat syndrome, inability to belch or vomit, and particularly dysphagia are caused by a decrease in the distensibility of the GEJ and that standard esophageal manometry is at best an indirect measure of this. The aim of this study is to directly measure the effect of LNF on GEJ distensibility (GEJD).

MATERIAL AND METHODS

Subject Selection

Patients with GERD proven by symptomatology, endoscopic findings, esophageal manometry, and 24-hour ambulatory esophageal pH monitoring were investigated. Seventeen patients, nine women and eight men, participated in this study. Informed consent was obtained before surgery. Patients with hiatal hernia longer than 3 cm, severe complications of GERD such as peptic esophageal stricture or Barrett's metasplasia, or previous foregut surgery were excluded from this study. Each subject was compensated for his/her participation in the study. All research protocols were approved by the Human Research Review Committee and Institutional Review Board of the Medical College of Wisconsin before initiation of this study.

Recording Apparatus

Measurements of distensibility of the GEJ were obtained with a specially designed catheter. This catheter was built from a commercially available polyvinyl tube with eight radially oriented side-holes, 4.6 mm in diameter and 100 cm in length (Fig. 1). The second and third side-holes from the tip were enclosed into an infinitely compliant 11-cm polyethylene bag (maximal volume 45 mL, cylindrical length 8 cm, and 2.5 cm in diameter). The bag was designed so that it would be infinitely compliant up to its maximal distending volume, so that any pressure increase recorded within the bag at submaximal volumes would reflect the distensibility of the adjacent hollow viscus or anatomic structures, rather than the distensibility of the bag. Ex vivo continuous infusion of air at the rate of 20 mL/min into the infinitely compliant bag resulted in near zero intra-bag pressure up to 20 mL volume. Between 20 and 25 ml the intra-bag pressure increased to 3 ± 3 mm Hg. Beyond 25 mL the intra-bag pressure increased exponentially. Therefore, distension volumes of 0 to 25 ml were used in this analysis. The second side-hole (the distal port underneath the bag) was used to inject air continuously, and the third side-hole (the proximal port underneath the bag) was used to measure the pressure in the bag. A Havard pump was used for GEJD bag distension in the study (Compact Infusion Pump Model 975 Harvard Apparatus Co., Millis, MA). The catheter was also fitted with a 2.5-cm diameter

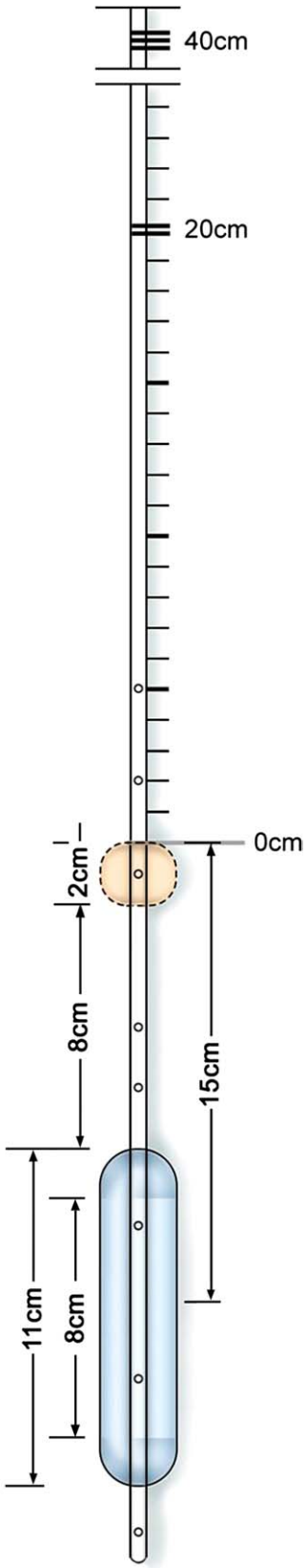

Fig. 1. Distensibility catheter.

balloon 8 cm above the distensibility bag. This balloon was used to distend the mid-esophagus to abolish LES basal tone (Fig. 1). The Medical Measurements System's manometry system (Enschede, The Netherlands) was used to measure the intraluminal pressure. The other five side-holes were infused with sterilized water 0.6 mL/min/sidehole. The system was calibrated before the study. The sampling rate was set at 4 Hz.

Study Protocol

Patients were placed under general anesthesia in a modified lithotomy position on the operating table. After equilibration for 5 minutes, the catheter was inserted transnasally into esophagus. The LES location was determined by station pull-through technique. The polyethylene bag was placed within the GEJ and was then distended to 30 mL by Harvard pump at the speed of 20 mL/min. The volume was marked every 5 mL. The procedure was repeated four times, and then the balloon in the middle esophagus was inflated to 2.5 cm in diameter and the GEJ distension measurements were repeated four additional times. The catheter was then pulled out. After the Nissen fundoplication was created, the procedure was repeated with the abdominal cavity insufflation pressure set at 5 mm Hg.

Laparoscopic Nissen Fundoplication

All laparoscopic fundoplications were performed by one of the authors (D.B.) using a previously described standard technique that included:[5]

1. Identification and complete dissection of the GEJ from the esophageal hiatus
2. Transhiatal mediastinal esophageal mobilization to allow for at least 2 cm of intra-abdominal tension free esophagus
3. Takedown of proximal short gastric vessels and all posterior lesser sac adhesions
4. Closure of the hiatus with nonabsorbable figure-of-eight sutures
5. Creation of a 1.5 to 2.0 cm "floppy" Nissen fundoplication over a 60F Bouge

Data Analysis

The pressure in the bag was measured at the mid-expiration point referring to the value at the beginning (i.e., at 0 mL) at 5-mL increments of bag distension, up to 25 mL. The first sequence after intubation before and after surgery was discarded because of possible initial stiffness of the bag and its surrounding tissues. The values of remaining three

sequences without mid-esophageal balloon distension and with mid-esophageal balloon distension before fundoplication and after fundoplication were used for analysis.

Pressure-volume curves for different distension protocols were generated. Differences in pressure for a given distension volume were compared for the different distension protocols. Distensibility was assessed by two different methods. First, the overall slope of the pressure-volume curve was taken as a measure of distensibility over the range of distension volume used, so that steeper slopes indicated less distensibility. Incremental distensibility was defined as the relationship between incremental changes in pressure relative to incremental changes in volume (i.e., 0–5 mL, 5–10 mL, 10–15 mL, 15–20 mL, and 20–25 mL).

Data were expressed as mean ± standard error of the mean unless otherwise stated. The effect of mid-esophageal balloon distension and fundoplication was analyzed using two-way repeated-measures analysis of variance with multiple pairwise comparisons (Tukey test). For the slope of pressure-volume curve, paired *t* test was used in the statistical analysis. *P* values less than .05 were considered to be significant. Statistical analysis was performed using Sigmastat for Windows 2.03 software (SPSS Inc., Chicago, IL).

RESULTS

Fifteen patients (gender: eight males and seven females; mean age = 46 years old [range 25–83 years]) with documented GERD were tested. Three patients, two females and one male, could not complete the study because of difficulty with proper catheter placement or mechanical problems. Each subject tolerated the procedure well. No morbidity or mortality was encountered in the study, and none of the patients participating experienced dysphagia in the postoperative period. The study procedure prolonged the duration of surgery approximately 30 to 45 minutes.

It was noted that for each subject studied, the pressure measured during the first sequence of bag distension was higher than the following, although the difference was not significant ($P > .05$). Therefore the first sequence of bag distention before and after surgery was ignored. The remaining three sequences were used for analysis (Fig. 2).

Before surgery, mid-esophageal balloon distension led to a significant decrease in the GEJD ($P = .03$) (Fig. 3). This effect of mid-esophageal balloon distention was lost postfundoplication ($P = .38$) (Fig. 4). There was no significant decrease in slope of pressure-volume curve with mid-esophageal balloon distension compared with no balloon distension after construction of the fundoplication.

After completion of the laparoscopic Nissen fundoplication, GEJD decreased significantly. The ΔP for each 5 mL incremental increase in volume increased significantly ($P = .007$) (Fig. 5). This was further illustrated by the significant increase in the slope of pressure-volume curve between volume 5 and 25 mL ($P = .004$) (Fig. 6).

DISCUSSION

Complications after laparoscopic Nissen fundoplication such as gas bloat syndrome, inability to belch or vomit, and dysphagia, especially those that

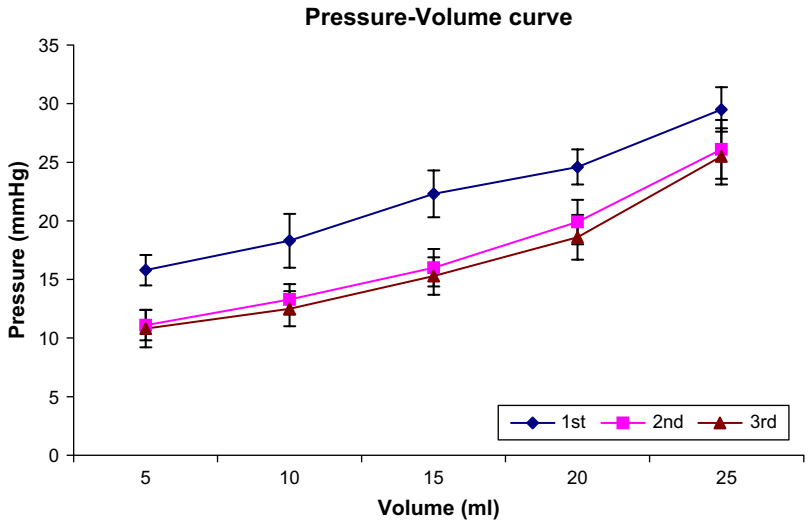

Fig. 2. Pressure-volume curves for each sequence before surgery. ($P = .06$ between sequences 1 and 2, $P = .307$ between sequences 2 and 3.)

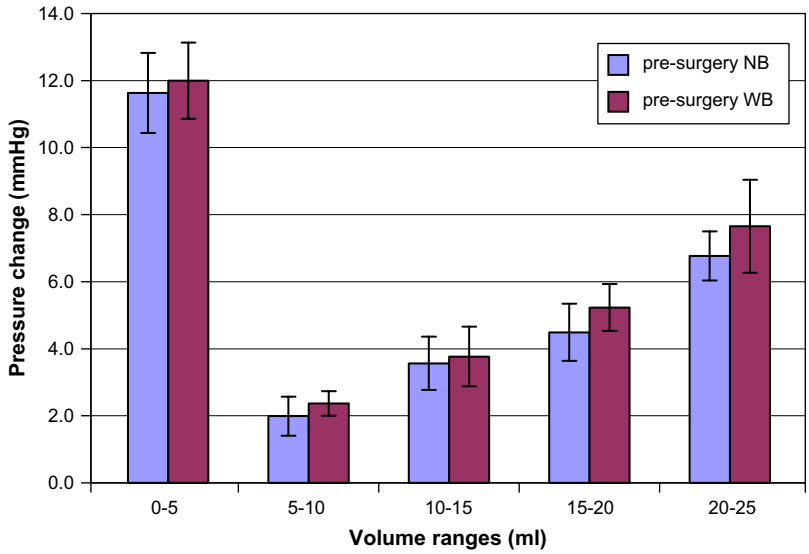

Fig. 3. Effect of mid-esophageal balloon distension on gastroesophageal junction distensibility (GEJD) before surgery (P = .03). NB = no balloon distention; WB = with balloon distention.

persist or develop de novo, have been important factors preventing many physicians from recommending this procedure for their patients with GERD.[28] Mild and/or short-term postfundoplication dysphagia (<3 months) is not uncommon and almost always resolves.[5,29] However, even a small percentage of patients with complications in the approximately 40,000 fundoplications performed per year in the United States is a significant clinical problem.

The cause of persistent or de novo postoperative dysphagia is not yet completely known. Possible causes include esophageal motility disorders, such as ineffective esophageal motility or achalasia missed preoperatively, or achalasia that develops postoperatively, GERD-related conditions such as peptic esophageal stricture or recurrent reflux esophagitis, and mechanical obstruction resulting from the fundoplication itself, such as a too long and/or too tight fundoplication, slipped/misplaced fundoplication, and/or paraesophageal hernia.[3,30] Although high resting LES and ramp pressures were revealed in some patients with postfundoplication dysphagia, they

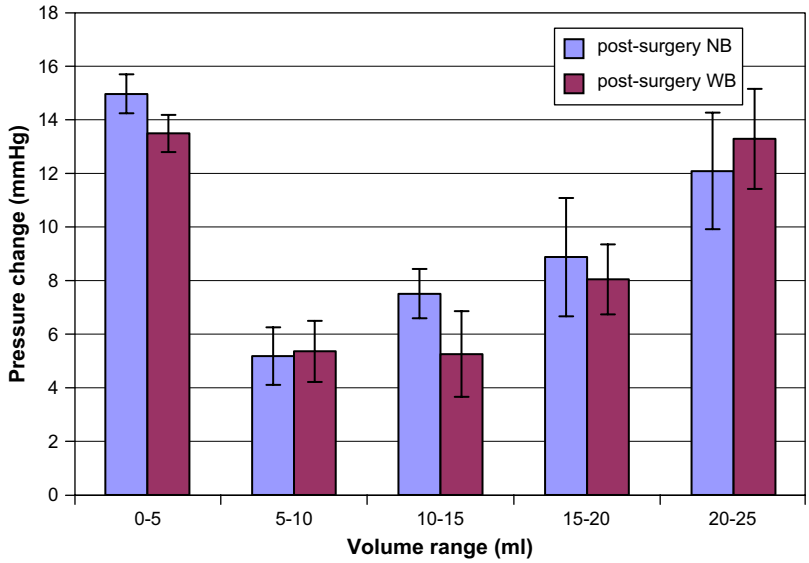

Fig 4. After fundoplication, the mid-esophageal balloon distension did not influence the ΔP in volume increments 0–25 mL (P = .38). NB =no balloon distention; WB = with balloon distention.

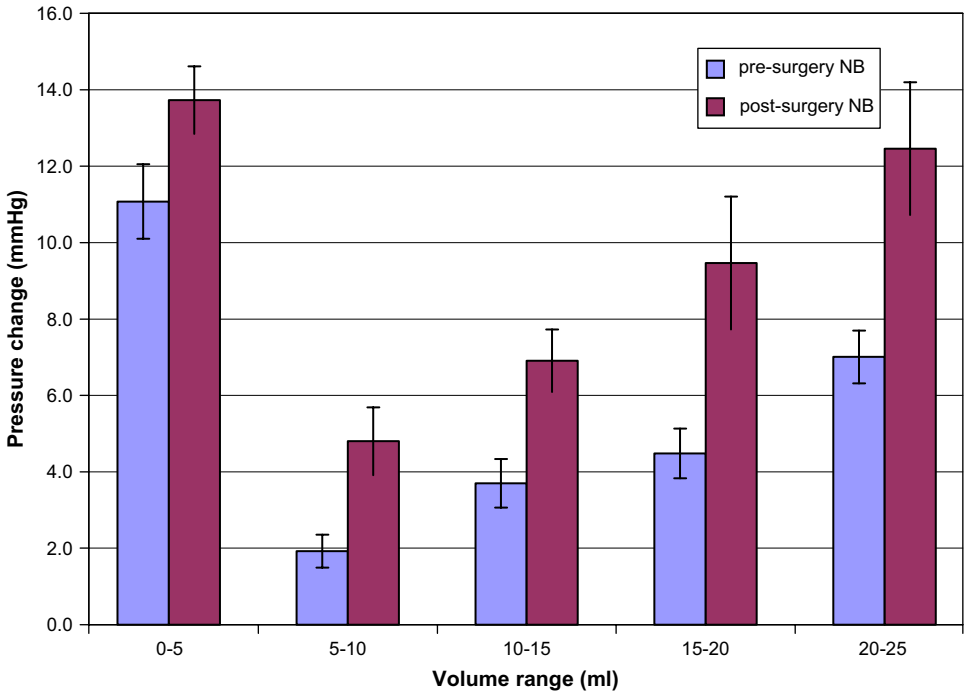

Fig 5. Fundoplication increased the ΔP for each 5 mL volume increment (0–25 mL) (P = .007) NB = no balloon distention.

correlated poorly to clinical outcome.[9] Blom et al.[5] demonstrated that normal LES characteristics measured preoperatively correlated with a significantly increased prevalence of postoperative dysphagia, and that there was a strong trend, although not statistically significant, between preoperative LES pressure and the degree of postoperative dysphagia. A subsequent study, however, failed to find a predictive correlation between preoperative LES pressure and postoperative dysphagia.[23]

We hypothesize that many of these post-LNF complications and particularly dysphagia may be caused by a decrease in the distensibility of the GEJ and that standard manometry is at best an indirect measure of this, therefore leading to the recent discordant conclusions about LES characteristics and dysphagia. To date, we are unaware of any reports measuring intraoperatively the effect of laparoscopic Nissen fundoplication on the distensibility of the gastroesophageal junction. This study was therefore undertaken to directly measure the effect of laparoscopic Nissen fundoplication on GEJD.

Mechanical properties of the lower esophageal high-pressure zone or LES are important to normal antegrade flow and to the development of pathologic retrograde flow across the gastroesophageal junction. As the GEJ opening becomes easier, as in the case of a hiatal hernia, the likelihood of retrograde

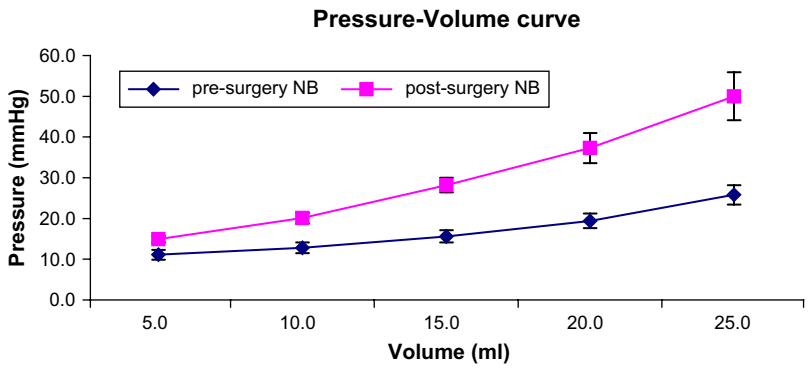

Fig. 6. Pressure-volume curves after fundoplication (P = .004). NB = no balloon distention.

flow increases and pathologic GERD develops.[7] As the GEJ opening becomes harder, eventually normal antegrade flow may become impeded and dysphagia develops.

In the unaltered state, the distensibility of GEJ depends on two components: the intrinsic component, which includes the active tone of LES; and the extrinsic component, including mainly the diaphragmatic crura.[7,31] Postoperative investigation has also shown that Nissen fundoplication increases the extrinsic component, and increases the intra-abdominal length of the GEJ.[31] The authors concluded that GEJ integrity post-LNF is independent of the intrinsic component (i.e., LES tone); however, in the normal condition the intrinsic and extrinsic components of the GEJ pressure should be additive, increasing resistance to bolus flow across the GEJ. Hiatal hernia leads to an obligate decrease of peak GEJ pressures by separating these two components and decreasing intra-abdominal length, therefore decreasing resistance to bolus flow. This helps explain the relationship between hiatal hernia and GERD.

Laparoscopic Nissen fundoplication prevents GERD by reducing hiatal herniation (therefore restoring the superimposition of the intrinsic and extrinsic components), increasing the intra-abdominal length of the GEJ, and augmenting the extrinsic component of the resting pressure of the GEJ, thereby increasing the resistance to flow, increasing the residual GEJ pressure with swallow-induced relaxation, increasing the intra-bolus pressure of esophageal peristalsis, and decreasing the episodes of transient LES relaxations.[10,12–18] This study illustrates that the distensibility of GEJ significantly decreases after fundoplication, revealing that it becomes significantly more difficult for the GEJ to be opened. After surgery, the ΔP in each 5-mL incremental volume increased significantly. This further demonstrates the mechanisms working to prevent GERD after LNF. It is not unreasonable to hypothesize that if the combined (intrinsic and extrinsic) components of the GEJ increase resistance too much, dysphagia can develop.

It is immediately intuitive and has been demonstrated that if the fundoplication (extrinsic component) is created incorrectly (too tight or to long or the crura closed too tightly), this will impede flow and lead to dysphagia.[22] We also submit that if the intrinsic component of the GEJ is too robust preoperatively, such as in patients with a competent LES by manometry, it would require lesser increases in the extrinsic component to lead to resistances to flow that might result in dysphagia.

How much the intrinsic and extrinsic components contributed to the decrease in GEJD could not be determined in this study because there was no significant effect from mid-esophageal balloon distension postfundoplication. Johnsson et al.[32] showed in subjects under general anesthesia that there was relaxation of LES before the construction of a fundoplication, which disappeared after the fundoplication, similar to our findings. We cannot explain the finding of a decreased GEJD with mid-esophageal balloon distention before surgery. This result is contrary to previous findings in healthy awake subjects.[27] It was interesting that both before and after surgery, with or without mid-esophageal balloon distension, ΔP in the interval of 0 to 5 mL was significantly higher than those of intervals 5 to 10 mL, 10 to 15 mL, 15 to 20 mL and 20 to 25mL ($P < .001$) (Figs. 3–5). This would indicate that GEJ distention or opening was the most difficult during the first inflation of the balloon and that subsequent inflation was relatively easier. We then saw the expected increase in ΔP for each subsequent 5-mL increment. It is likely that this phenomenon can be explained by strain softening.[33] Strain softening is a passive biomechanical property, which explains lower pressures for the same volumes subsequent to the first sequence. Studies in animals have shown that strain softening occurs in the gastrointestinal tract.[34]

CONCLUSION

This study quantifies for the first time the intraoperative distensibility of the GEJ before and after laparoscopic Nissen fundoplication and found that GEJD significantly decreases after laparoscopic Nissen fundoplication. We hypothesize that the magnitude of decrease of GEJD may be the vital variable in the development of postfundoplication complications, especially dysphagia, and that measurement of GEJD intraoperatively might evolve into a novel tool to optimize the creation of a Nissen fundoplication and to decrease these complications in the future.

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Myotomy: Follow-Up Study of 50 Patients

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Laparoscopic myotomy has become the standard treatment for definitive management of achalasia. This study was undertaken to assess the long-term results of the procedure. Perioperative data, including a symptom score questionnaire, were collected prospectively on all patients undergoing laparoscopic myotomy. The same questionnaire was readministered by phone to patients with follow-up greater than 3.75 years. The long-term success of myotomy was defined as a 50% or greater decrease in the dysphagia score and absence of further therapy (responders). Fifty of 95 patients (age = 57 years, 23 females) were successfully contacted. Average follow-up was 6.2 years. The overall long-term success rate was 64% (responders). Forty-two patients (84%) were able to gain or maintain their weight after the procedure. Five patients (10%) required one or more endoscopic dilations after the myotomy. The mean change in dysphagia score was higher in the responder group (7.8 ± 1.9 vs. 1.9 ± 2.1; $P = 0.001$). The two groups were similar in terms of age, gender distribution, and follow-up interval ($P > .05$). Dor fundoplication was performed in six patients (12%), and the outcome comparisons of these patients showed no significant differences from those patients undergoing Heller alone. Overall satisfaction was achieved in 94% of contacted patients. These results confirm that laparoscopic myotomy is an effective procedure with excellent long-term symptom resolution and overall satisfaction in patients with achalasia.
(J GASTROINTEST SURG 2005;9:1326–1331)

KEY WORDS: Achalasia, esophagomyotomy, minimally invasive surgery, dysphagia, outcomes

Achalasia, a common motility disorder of the esophagus, occurs with an incidence of 0.5 to 1.0 per 100,000.[1–3] Heller described the successful surgical cardiomyotomy for the treatment of this chronic cardiospasm in 1914.[4] This abdominal approach with an anterior and posterior cardiomyotomy was modified by Zaaijer in 1923 to include a single anterior myotomy, as is still used today.[5] For many years, the thoracic approach through the left chest, as described by Ellis in 1958, was the most common procedure used for management of achalasia.[6] The cumbersome nature of this thoracic approach led to renewed interest in the abdominal approach over the past decade. The minimally invasive abdominal approach to the esophagus, as first reported by Cuschieri et al. in 1991, was found to be much simpler.[7] We began treating achalasia via a laparoscopic approach in 1992, and here we report the long-term outcomes in 50 of our initial patients.[8]

PATIENTS AND METHODS

Between November 1992 and February 2001, 95 laparoscopic and five thoracoscopic Heller myotomies were performed by three surgeons at Vanderbilt University Medical Center. Perioperative data were collected prospectively on all patients, including a structured symptom score questionnaire. The same questionnaire was readministered by phone to 50 of these 100 patients at follow-up of at least 3.75 years. The score was calculated by combining the frequency of dysphagia (0 = never, 1 = less than 1 day/week, 2 = 1 day/week, 3 = 2–3 days/week, 4 = 4–6 days/week, 5 = daily) with the severity (0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = moderately severe, 5 = severe). The highest possible score was 10. The long-term success of myotomy was defined as a greater than 50% decrease from the preoperative dysphagia score and absence of further therapy (repeat myotomy or endoscopic therapy).

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Our technique for laparoscopic Heller myotomy has been previously well described.[8] Briefly, after exposure of the anterior gastroesophageal (GE) junction, the myotomy is created by incising the distal 4–6 cm of esophageal musculature. The myotomy is extended 1–2 cm onto the gastric cardia using cautery scissors or an ultrasonic scalpel. Intraoperative endoscopy is performed simultaneously with the myotomy to assess the adequacy of the myotomy. An anterior 180° Dor fundoplication is performed in standard fashion in selected patients. We were proponents of laparoscopic Heller myotomy without antireflux procedure during the last decade, before our recent randomized trial demonstrating the effectiveness of the Dor anterior fundoplication to reduce acid exposure in the distal esophagus.[9] We now routinely perform a Dor fundoplication on all patients.

The SPSS statistical software (version 11.0, SPSS, Chicago, IL) was used to perform statistical analysis. Paired *t* tests were used where appropriate, and the chi-square analysis was used for nonpaired data. Linear regression was used to correlate success with the type of surgical procedure (presence or absence of dor fundoplication). Data are presented as mean ± standard deviation. $P < .05$ was used to determine statistical significance.

RESULTS

We attempted to contact the 95 consecutive patients who underwent a laparoscopic Heller myotomy procedure at our institution between November 1992 and February 2001, and we were able to contact 50 by telephone for symptom score questionnaire administration, evaluation of weight status, current medication use, and overall satisfaction. There were 27 males and 23 females, with a mean age of 57.2 ± 16.2. The average follow-up was 6.2 ± 2.0 years (range, 3.7–10.7 years; Table 1).

Table 1. Overview of present series

Patient characteristics	Value
Number (% male)	50 (54%)
Mean ± SD age (yr)	57.2 ± 16.2
Mean weight (lbs)	183 ± 5.3
Total with Dor fundoplication	6 (12%)
Mean ± SD follow-up (yr)	6.2 ± 2.0
Overall satisfaction (%)	47 (94%)

The overall cumulative long-term success rate, as defined by a decrease in dysphagia score greater than 50% and absence of further therapy, was 64% (responders; Fig. 1). The change in the dysphagia score for this group was 7.8 ± 1.9 versus 1.9 ± 2.1 for the nonresponders ($P = 0.001$). The two groups were similar in terms of age (57.7 ± 15.9 vs. 56.4 ± 17.0 years) and gender distribution (20 males:12 females in the responder group vs. 7 males:11 females in the nonresponder group). The mean length of follow-up was 5.9 ± 1.8 years in the responders and 6.7 ± 2.2 years in the nonresponders ($P = 0.18$; Fig. 2).

Other indicators of success were weight change and patient satisfaction. In this group, 42 of 50 (84%) patients were able to maintain or gain weight (Fig. 3). Overall patient satisfaction was achieved in 94% (47/50). Thus, there were patients (n = 15) that reported satisfaction, yet did not meet our criteria for the responder group. Twelve of these 15 did have symptom score improvement, but not to 50%. The

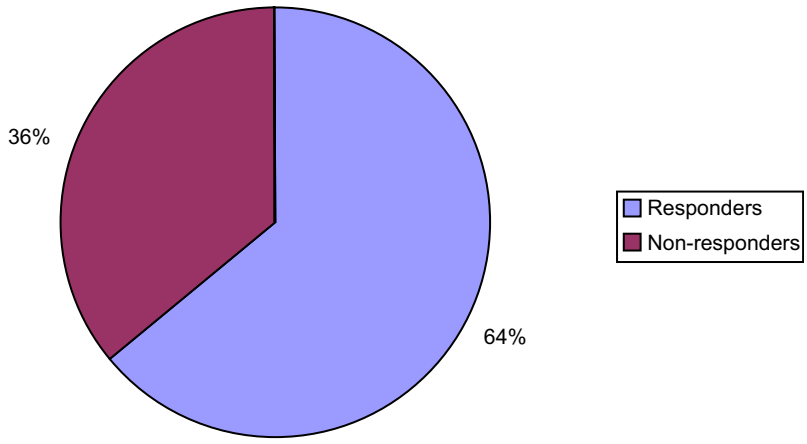

Fig 1. Responders were defined as greater than 50% decrease in dysphagia score and absence of further therapy (repeat myotomy/endoscopic therapy).

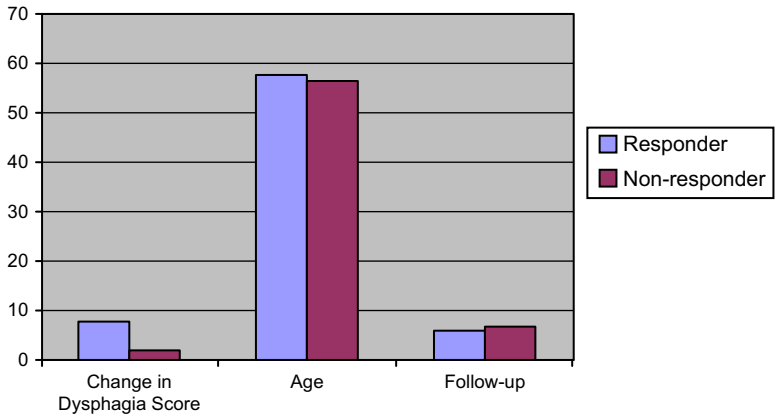

Fig 2. The change in dysphagia score was higher in the responder group ($P = 0.001$). The groups were similar in terms of age and follow-up intervals.

other three patients had no change in their score, but did report substantial weight increase. The three nonsatisfied patients reported worsening dysphagia scores and ongoing weight loss. In addition, they all required subsequent endoscopic therapy (dilation/botox injection) that was only partially effective. No patient in this study has required repeat myotomy or esophagectomy to this point.

Only six (12%) patients in this study underwent myotomy plus Dor fundoplication. Four of these patients had greater than 50% improvement in their dysphagia score, thus falling in the responder group. Nevertheless, the presence or absence of Dor did not prove to be significant in terms of dysphagia resolution ($P = 0.88$; Fig. 4). Ongoing use of antacid medication (proton pump inhibitors) was seen in 18 patients at long-term follow-up. Use of these medicines did not prove to be significant in terms of dysphagia outcome, nor did the use of these medicines correlate with the presence or absence of Dor fundoplication (Fig. 5). The indications for this antacid use in these patients were not based on objective evidence of reflux, to our knowledge.

DISCUSSION

The laparoscopic management of esophageal achalasia has achieved widespread acceptance and is now the treatment of choice in most centers. The satisfactory short-term results of this procedure are well documented in several large series (Table 2). We published the data for our first 100 patients in 2002, and our results were similar to others in that at about 12 months, 93% of patients had satisfactory relief of dysphagia.[8] However, in all these series, the average length of follow-up was from 12 to 24 months.

It has been reported that the results are less impressive when the patients are followed for several years. Torbey et al.[10] reported that only 33% of

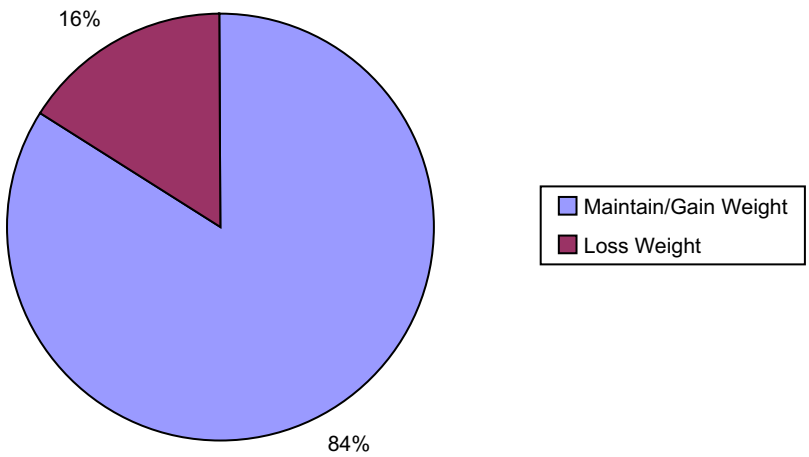

Fig 3. Weight gain or maintenance was seen in 42 (84%) patients at long-term follow-up.

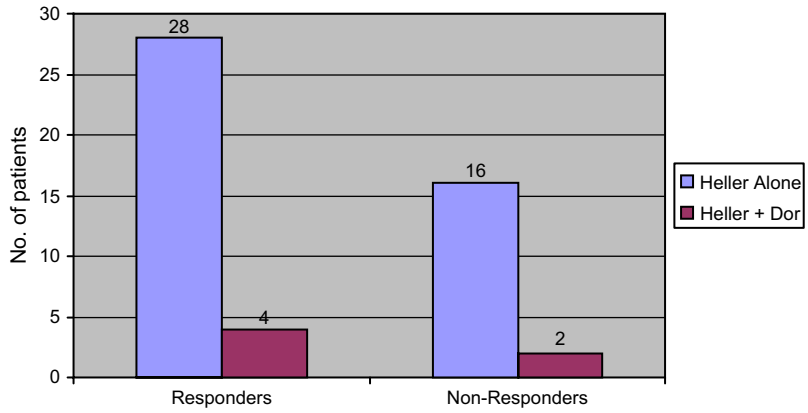

Fig 4. Presence or absence of Dor fundoplication was not significant in terms of response ($P = 0.88$).

patients undergoing thoracoscopic myotomy were satisfied at 4-year follow-up. This same result was seen in patients treated with pneumatic dilations (26% satisfaction at 4 years).

Nevertheless, several recent long-term follow-up series involving the laparoscopic approach clearly challenges these results. Bloomston et al.[11] recently compared the results in 87 patients undergoing laparoscopic Heller myotomy at 1-year and 3-year follow-up. They did not find a significant difference in the percentage of patients with good results (85% vs. 89% at early follow-up). In addition, Costantini et al.[12] recently reported comparable results at twice as long a follow-up. Their results in 71 patients followed up at several intervals—up to over 6years—showed that 85% were maintaining "good eating capabilities" with symptom scores below the tenth percentile of their preoperative score.

We were able to obtain data on 50 of our initial 95 patients, with an average follow-up of 6.2 years. Our definition of success, as in many studies, was arbitrarily defined as a greater than 50% improvement in the preoperative dysphagia score, as well as absence of further therapy. These rather strict, yet objective, criteria were met in 64% of these 50 patients. Nevertheless, 94% were satisfied with the result of their operation (i.e., able to eat normally without lifestyle-altering dysphagia). The 15 patients that were satisfied but considered nonresponders achieved either a less than 50% improvement in score (n = 14) and/or received further endoscopic therapy (n = 1). These patients were likely satisfied because they were able to eat with less dysphagia and gain weight.

Comparison of these long-term laparoscopic outcomes with the long-term effectiveness of endoscopic dilation reveals similar results. In a review by Vaezi and Richter,[13] the success rate of endoscopic dilation ranged from 59%–93%. Again, there are marked variations in the definitions of success, as well as the techniques being applied. A second study reported by this group[14] defined success using a

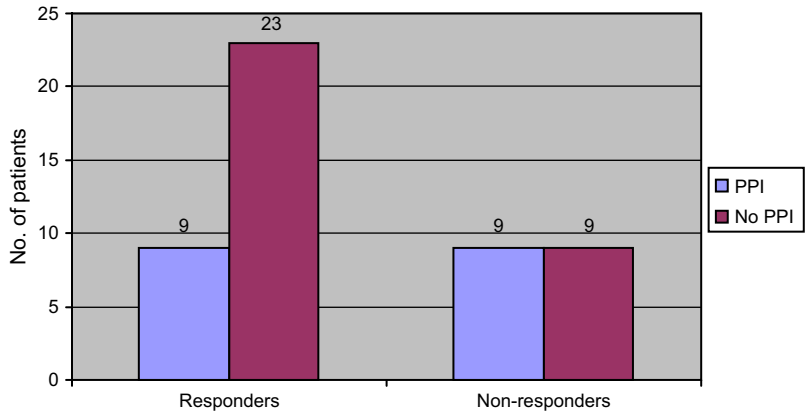

Fig 5. Proton pump inhibitor (PPI) use at long-term follow-up was not significant in terms of response ($P = .12$).

Table 2. Short-term results of laparoscopic treatment of esophageal achalasia

First author (yr)	No. of patients	Median/mean follow-up (mo)	Antireflux procedure	Good results (%)	GERD (%)
Raiser (1996)[19]	35	11–46	Dor/Toupet	97	Not reported
Boulez (1997)[20]	27	17	None	100	4
Graham (1997)[21]	26	4	Dor	90	11.1
Hunter (1997)[18]	40	12.5	Dor/Toupet	90	2.5
Wang (1998)[22]	27	18	None	89	11
Rosati (1998)[23]	61	12	Dor	98.2	7
Patti (1999)[24]	133	28	Dor	89	17
Hunt (2000)[25]	70	34	Nissen	81	4.5
Bloomston (2000)[26]	67	18	None	91	18
Zaninotto (2000)[27]	100	24	Dor	92	6.9
Sharp (2002)[8]	100	11	None/Dor	93	14
Frantzides (2004)[28]	53	36	Nissen	92	9

GERD = gastrointestinal reflux disease.

scoring system adapted from the one presented by Eckardt et al.[15] and reported a 70% success rate 1 year after dilation, whereas Katz et al.[16] defined success as being no need for additional therapy beyond two dilations and reported an 85% success rate within 6 years. However, Chan et al.[17] used both objective (less than 2 dilation procedures) and subjective assessment criteria (symptom score) in their study and reported a 5-year cumulative success rate of 74%. Despite this success reported in each of these studies, the perforation rate remains 2%–7% and a death rate of 1%–2%. In contrast, surgical myotomy has not been associated with any death in our series or in any others.[8,18] Our reported complication rate is very low at 2%, which is equal to or lower than any endoscopic treatment series.

In conclusion, laparoscopic Heller myotomy with or without an antireflux procedure is a safe and effective procedure with excellent long-term symptom resolution and overall satisfaction in patients with achalasia.

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Long-term Outcome of Laparoscopic Heller-Dor Surgery for Esophageal Achalasia: Possible Detrimental Role of Previous Endoscopic Treatment

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Laparoscopic Heller myotomy has recently emerged as the treatment of choice for esophageal achalasia. Previous unsuccessful treatments (pneumatic dilations or botulinum toxin [BT] injections) can make surgery more difficult, causing a higher risk of mucosal perforation and jeopardizing the outcome. The study goal was to evaluate the effects of prior endoscopic treatments on laparoscopic Heller myotomy. Between January 1992 and February 2005, 248 patients (130 males and 118 females; median age, 43 years) underwent a laparoscopic Heller-Dor operation for achalasia: 203 underwent primary surgery (group A), 19 had been previously treated with pneumatic dilations (group B), and 26 had BT injections (alone [22] or with dilations [4] (group C). Median duration of the operation and rate of intraoperative mucosal lesions were not different in the three groups. Median follow-up was 41 months. The 5-year actuarial of control of dysphagia was similar in groups A (86%) and B (94%), whereas only 75% of group C patients were symptom free at 5 years ($P = 0.02$). On logistic regression analysis, prior treatment with two BT injections or BT combined with dilation was associated with poor outcome of surgery. Further, dilations for surgical failure patients were effective in 80% of group A but in only 33% of group B or C patients. Heller-Dor surgery is safe and effective as a primary or a second-line treatment (after pneumatic dilations or BT injections) for achalasia. However, long-term results seem less satisfactory in patients previously treated with BT. (J GASTROINTEST SURG 2005;9:1332–1339)

KEY WORDS: Esophageal achalasia, laparoscopic surgery, Heller myotomy, pneumatic dilation, Botox injection

Esophageal achalasia is a primary motor disorder characterized by a virtually absent peristalsis of the esophageal body and incomplete relaxation of the lower esophageal sphincter (LES).[1] Although the primary pathophysiologic defect has been identified, that is, the loss of inhibitory ganglion cells and persistence of cholinergic stimuli, the etiology of achalasia is not entirely clear and its treatment remains controversial.[2] All therapies are palliative and designed to reduce the LES resting pressure by paralyzing the muscle with botulinum toxin (BT) injection or by stretching and/or disrupting the LES muscle fibers with endoscopic balloon dilations or surgical myotomy. In the past decade, the development of videoendoscopic techniques has rekindled interest in the surgical management of this disease. Laparoscopic myotomy of the distal esophagus and gastric cardia with a partial anterior fundoplication (the Heller-Dor procedure) is becoming the treatment of choice for patients with esophageal achalasia at most centers,[3,4] subject to the preferences of the physician or surgeon consulted and local availability of expertise. The small number of patients and limited advantage of myotomy over dilation make it very difficult to recruit enough patients to perform adequate randomized clinical trials to establish the optimal treatment,[5] so there are patients, still, referred for surgery only after unsuccessful endoscopic treatment with pneumatic dilations and/or BT injection(s). It has been suggested

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that preoperative treatments may induce histopathologic changes at the gastroesophageal junction, making surgery more difficult, with a higher risk of mucosal perforation and a less satisfactory outcome[6–8] The aim of this study was to evaluate the effects of prior endoscopic treatment on the outcome of laparoscopic Heller-Dor operation for achalasia.

MATERIAL AND METHODS

Patient Population

Between January 1992 and February 2005, 248 patients with a diagnosis of primary achalasia underwent laparoscopic Heller-Dor myotomy of the distal esophagus and gastric cardia at our department. They included 130 males and 118 females with a median age at diagnosis of 43 years (range, 11–80 years). Surgery was the primary treatment for 203 patients (group A), while 45 had already been treated endoscopically elsewhere, with one to four pneumatic dilations in 19 patients (group B) and BT injections in 26 patients (group C, BT alone in 22 cases, associated with dilations in 4).

Preoperative Work-up

The diagnosis of primary achalasia was based on clinical history, barium swallow, endoscopy, and esophageal manometry. Clinical data were prospectively collected by means of a symptom questionnaire and scored according to severity and frequency. The symptom score for dysphagia, regurgitation, and chest pain was calculated by combining the frequency (0–5) and severity (0–6) of each symptom; the highest possible score was 33. Surgery was considered as having failed when the patient's symptom score exceeded the tenth percentile of the pretreatment score.[9]

A barium swallow study was obtained in each patient before and 1 month after surgery (in more recent years, a "timed" barium swallow was used[10]). The maximum esophageal diameter was measured at the site of the barium air level in the standard anteroposterior image. Stationary esophageal manometry was performed before and 6 months after surgery, and whenever the patient had recurrent symptoms, using a pneumohydraulic perfusion system and standard techniques.[11] Twenty-four-hour pH monitoring was performed only after surgery to assess any abnormal gastroesophageal reflux, positioning a glass electrode 5 cm above the upper border of the LES, according to the standard procedure used at our laboratory and described elsewhere.[12] Traces from patients with abnormal reflux on computerized analysis were carefully reviewed to distinguish true episodes of gastroesophageal reflux from false reflux resulting from stasis.[13] Upper gastrointestinal endoscopy was used to rule out any malignancies before surgery and to evaluate any reflux esophagitis afterward.

Surgery

All patients had the same operation, introduced in 1992 and little changed since, described in detail elsewhere.[14] Briefly, only the anterior part of the esophagus was dissected, the anterior vagus nerve was identified, and a 6- to 8-cm-long myotomy was performed, extending 1–1.5 cm on the gastric side. A 30-mm Rigiflex balloon (Microvasive, Boston, MA) was positioned endoscopically at cardia level, gently inflated, and deflated with 40–60 ml of air during the myotomy. This facilitated the identification of the circular fibers, which were stretched and then cut or torn apart. Minimal bleeding from the submucosal vessels was easily controlled by inflating the balloon, thus reducing the need for cautery. An anterior partial fundoplication (180°) according to the Dor technique completed the procedure. Three stitches on each side were used to suture the gastric wall to the edges of the myotomy. All the operations were performed by four staff surgeons.

Postoperative Course

To rule out perforation, a swallow test with a water-soluble contrast (Gastrografin; Schering, Berlin, Germany) was obtained on postoperative day 1. The nasogastric tube was removed and patients were asked to drink for the next 12 hours, to remain on a soft diet for 10–15 days, and then to return to a normal diet. The hospital stay depended on the distance of the patient's home from the hospital: they were discharged on postoperative day 2 if they lived within 1 hour's drive from the hospital and on postoperative day 4 if they lived farther away.

Follow-up

Patients were followed by the operating surgeon. They were asked to come to the outpatient clinic 1, 6, and 12 months after surgery. A barium swallow was obtained at the first follow-up visit; manometry and pH-monitoring were performed immediately before the second checkup, when a second symptom assessment was obtained. Endoscopy was performed 12 months after surgery to check for complications (esophagitis) and then recommended every 24 months to rule out any neoplastic degeneration. If patients failed to show up for 12 months or longer,

they were interviewed by telephone. The median follow-up for the entire group was 41 months (range, 1–131 months).

Statistical Analysis

Data are expressed as medians with ranges in parentheses. Proportions were compared using the χ^2 or Fisher's exact test. Continuous variables were compared using the Wilcoxon, Mann-Whitney, and Kruskal-Wallis tests. Symptom-free survival estimates were calculated by the Kaplan-Meier method, and comparisons were made using the log-rank test. Logistic regression analysis was used to identify predictors of symptom recurrence. A P value <0.05 was considered significant.

RESULTS

Demographic information and preoperative data are summarized in Table 1. There was no significant difference between the three groups of patients in terms of duration of symptoms, symptom scores, and preoperative esophageal maximum diameters on barium swallow. The preoperative LES resting pressure was significantly lower in group B patients.

The surgical procedure was completed laparoscopically in 242 of 248 patients. Mortality due to the operation was zero. The reasons for conversion to open surgery were adhesions from previous upper abdominal surgery (two cases), mucosal perforations (two cases), spleen damage (one case), and finding an unexpected mass in the lower abdomen that could not be interpreted laparoscopically and that proved to be an ectopic kidney (one case). All six conversions occurred in the first 80 operations, in patients undergoing surgery as primary achalasia treatment.

The median operating time was much the same in the three groups (Table 2). It was slightly shorter in group B and C patients, probably because these patients underwent surgery in the latter period, by which time the technique had been well mastered.

The most common intraoperative complication was perforation of the esophageal mucosa during the myotomy. Two mucosal tears occurred at the beginning of our experience and required conversion to open surgery; six (five in group A and one in group C) were repaired laparoscopically with 4–0 reabsorbable stitches and healed with no further consequences. Two additional mucosal leaks (one in group A and one in group B) was revealed by the water-soluble contrast swallow performed routinely 24 hours after surgery. These two patients were maintained on parenteral nutrition, and the nasogastric tube was left in place for 7 days, leading to the healing of the leak. Postoperative complications were limited to trocar-site bleeding requiring laparotomy on postoperative day 2 (one case); pneumothorax (one case), which was drained with a chest tube; transient vocal cord palsy (one case); external sciatic popliteal nerve palsy (one case); and unexplained persistent fever (one case).

The postoperative clinical follow-up was completed by all 248 patients. Five patients died of unrelated causes 12, 13, 18, 33, and 69 months after surgery.

There was a significant decrease in symptom score after the operation in all patients, with no differences in the three groups (Fig. 1*A*). Thirty patients complaining of moderate to severe dysphagia (more than once a week or even daily) or severe chest pain on swallowing were considered surgical failures. In 23 of these patients (77%), symptoms recurred during the first year of follow-up (median, 5.5 months; range, 1–108 months).

The maximum esophageal diameter (measured by barium swallow) and the LES resting pressure were significantly reduced after surgery, with similar postoperative results in the three groups (Fig. 1*B* and 1*C*). Twenty-four-hour pH monitoring revealed a pathologic acid exposure in the distal esophagus

Table 1. Demographics and preoperative data

	Primary surgery (n = 203)	Preoperative dilations (n = 19)	Preoperative BT ± dilations (n = 26)	*P* value
Gender (M/F)	107/96	8/11	15/11	NS
Patient age (yr)	42 (11–80)	40 (22–73)	46 (21–73)	0.42
Duration of symptoms (mo)	24 (2–480)	30 (3–240)	25 (8–180)	0.08
Symptom score	20 (7–33)	21 (9–33)	20 (4–33)	0.91
Maximum esophageal diameter (cm)	3.8 (1.5–7.0)	3.5 (2.0–7.5)	4.0 (2.0–7.0)	0.55
LES resting pressure (mm Hg)	24 (6–71)	17 (6–35)	21 (7–53)	<0.01

Data are expressed as median (range).
BT = botulinum toxin; LES = lower esophageal sphincter.

Table 2. Postoperative results

	Primary surgery (n = 203)	Preoperative dilations (n = 19)	Preoperative BT ± dilations (n = 26)	*P* value
Duration of the operation (min)	150 (70–280)	132 (110–215)	130 (110–200)	0.08
Mucosal perforation*	8 (3.9)	1 (5.3)	1 (3.8)	NS
Postoperative hospital stay (days)	4 (3–11)	5 (3–11)	5 (3–11)	0.42
Postoperative gastroesophageal reflux*	6/107 (5.6)	2/12 (16)	1/11 (9)	NS
Follow-up (mo)	45 (1–131)	55.5 (1–131)	22.5 (1–89)	0.04

Data are expressed as median (range) or *no. (%).
BT = botulinum toxin.

(percentage of total time pH <4 greater than 4.2%, according to the upper limits of normal used at our laboratory) in 9 (6.9%) of the 130 patients who agreed to take the test 6 months after surgery (*P* = NS for groups A, B, and C).

There was no statistically significant difference in surgical outcome between patients treated primarily by surgery (group A) and those who had previously received unsuccessful endoscopic pneumatic dilations (group B), with a 5-year control of symptoms of 86% and 94%, respectively (Fig. 2). However, only 75% of group C patients were symptom-free at 5 years. This difference was even more evident when the four patients in group C who had had both BT injections and dilations were analyzed separately (only one had symptom control at 5 years). Logistic regression analysis showed that prior treatment with two BT injections or BT combined with dilation was significantly associated with an unsatisfactory outcome of surgery (Table 3)

Of the 30 patients with recurrent symptoms after laparoscopic myotomy, 29 were subsequently treated with pneumatic dilations, using 3.0, 3.5, or 4.0 Rigiflex balloons (median, two dilations; range, one to nine).[9] One patient (group A) was treated elsewhere with BT and, although still symptomatic, refused any further treatment. The additional pneumatic treatment was successful in 68% of the patients of group A and only 33% of those of groups B and C (*P* = NS).

DISCUSSION

The most appropriate initial treatment for esophageal achalasia has been a topic of discussion for many years and remains a matter of debate. The need to perform a laparotomy or thoracotomy for the surgical myotomy of the cardia in the 1980s explains why most patients were offered pneumatic dilation first, but the advent of laparoscopy in the early 1990s added a new dimension to the controversy. Several studies presented laparoscopic myotomy as a safe and effective treatment, with minimal discomfort for the patients and a short hospital stay. But the rarity of the disease has always prevented thorough randomized controlled trials between knife and balloon, giving rise to a customer (patient or physician)-based, rather than an evidence-based, choice of treatment. The availability of well-trained laparoscopic surgeons, the expertise of endoscopists in performing balloon dilations, and procedural costs have been the main factors influencing the choice of treatment for patients with achalasia. In the past decade, achalasia patients have been offered a new option—endoscopic injections of BT into the cardia.[15] BT blocks acetylcholine release from nerve endings, and, at LES level, this counterbalances the selective loss of inhibitory neurons and enables sphincter relaxation in achalasia patients. BT injection significantly improves dysphagia, but its effect is relatively short-lived (6 months to 1 year). The efficacy of surgery and dilation has been accurately assessed in prospective studies: mid and long-term results of surgery range from 80% to 94% at 10 years; dilation seems to be effective in 75–85% of patients at 5 years[16,17] and in up to 40% at 15 years.[18] The efficacy of BT was evaluated in three controlled randomized trials and compared with dilation (two trials[19–21]) and surgery[22] (one trial): the medium-term effect of BT is significantly less satisfactory than surgery or dilation (the chance of remaining symptom-free at 2 years ranges between 20% and 30%). This procedure is safe and easy, however, and the injection can be repeated, so it still has its appeal, especially in areas without laparoscopic surgeons or endoscopists with expertise in performing dilations.

One of the criteria to consider in choosing among these options is the influence they may have on the alternative treatment needed in the event of failure. Pneumatic dilation, forcefully disrupting the muscular layers of the esophagus, causes submucosal microhemorrhages that heal with a covering of fibrotic tissue, which might make it difficult to

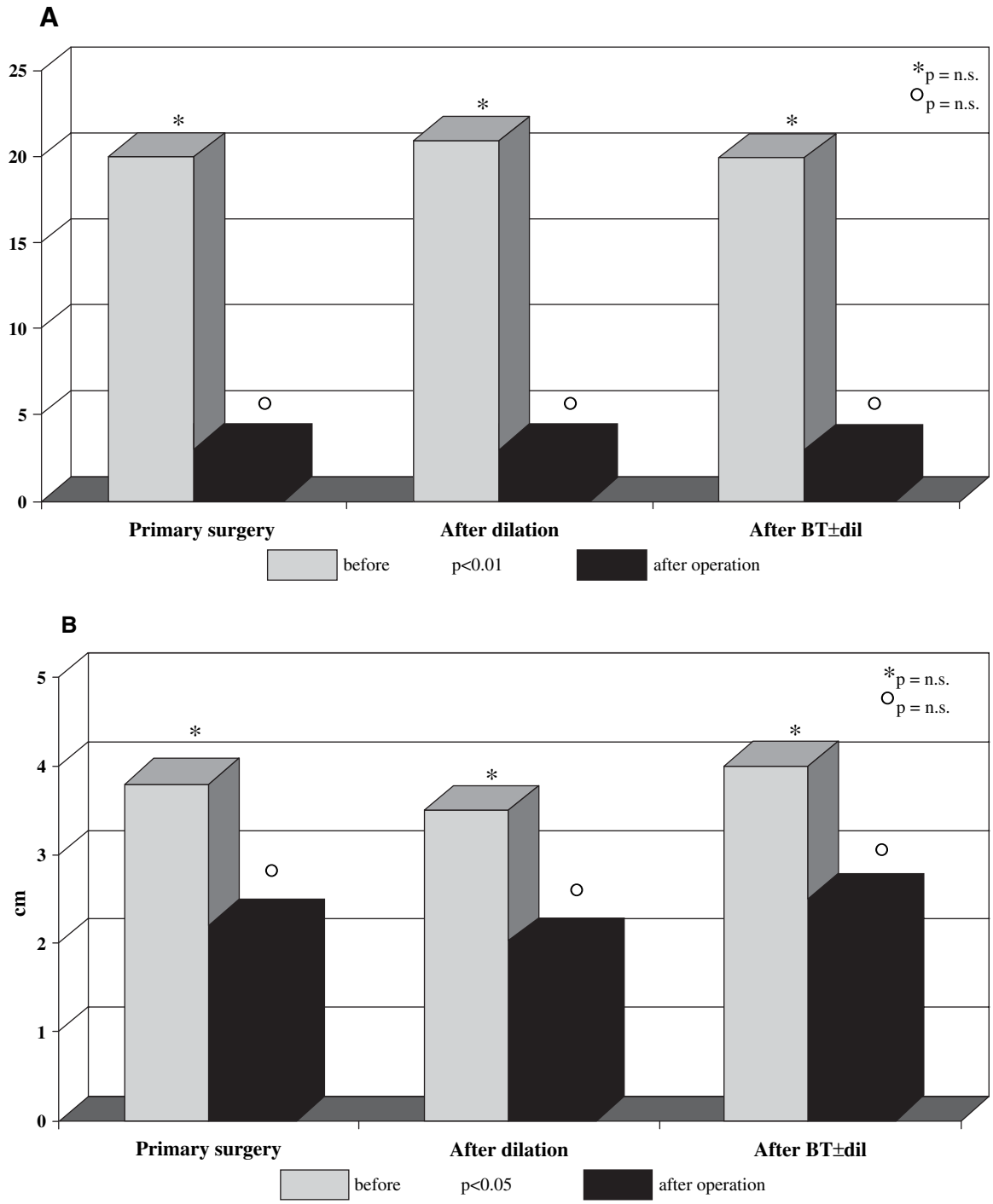

Fig. 1. Postoperative results: symptom score **(A)**, maximum esophageal diameter **(B)**, and LES resting pressure **(C)** significantly reduced after surgery with no differences in the three groups of patients. BT = botulinum toxin.

identify the plane between the muscle layers and the submucosa, increasing the risk of mucosal perforation.[8] Similarly, repeated BT injections and the consequent inflammation might conceal said plane, making the myotomy more difficult and hazardous. The study by Patti et al[7] was one of the first to report a higher rate of mucosal perforation at surgery attributable to fibrotic reactions at the cardia level in patients who had received numerous BT injections, especially in those responding (temporarily at least) to said treatment. A greater difficulty in performing the myotomy was also reported by Horgan et al,[6]

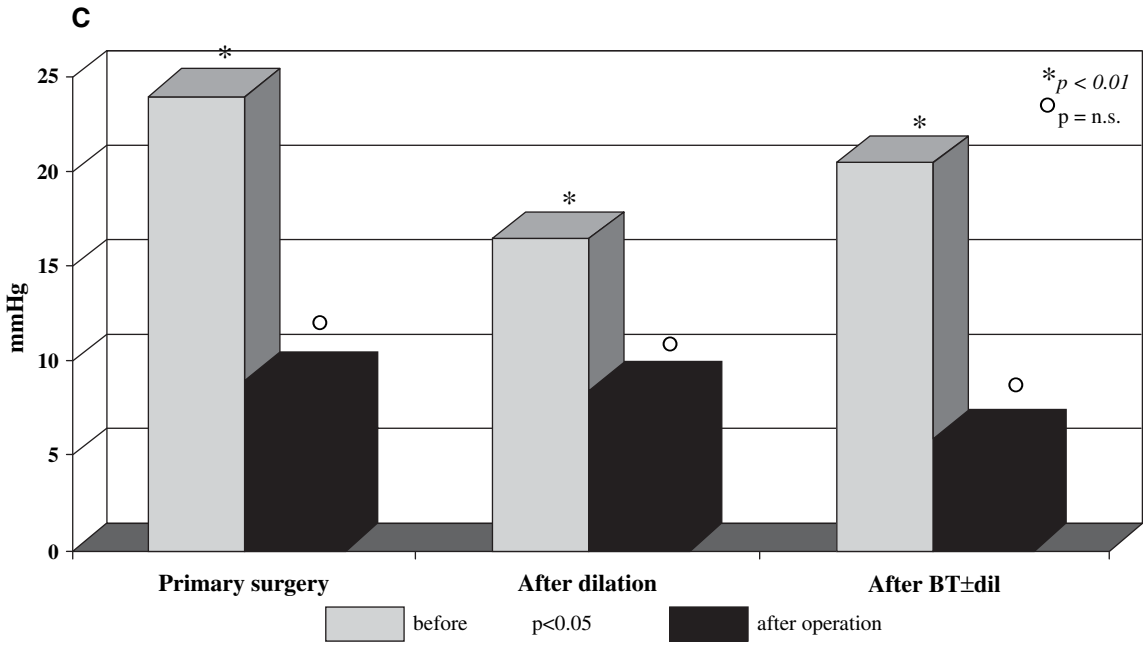

Fig. 1 (*continued*)

while other authors have denied any effect of previous treatments on the dissection and myotomy or on final outcome.[23–25]

In our study, despite the initial concern for an expected more challenging surgery in patients already treated endoscopically (especially if BT injections were involved), no special technique was required to complete a satisfactory myotomy in these cases. The mucosal perforation rate was much the same as in patients with no prior endoscopic treatment and, in the patients who had received BT injections, no significant correlation emerged between the number of injections and the perforation rate. In other words, laparoscopic Heller-Dor surgery

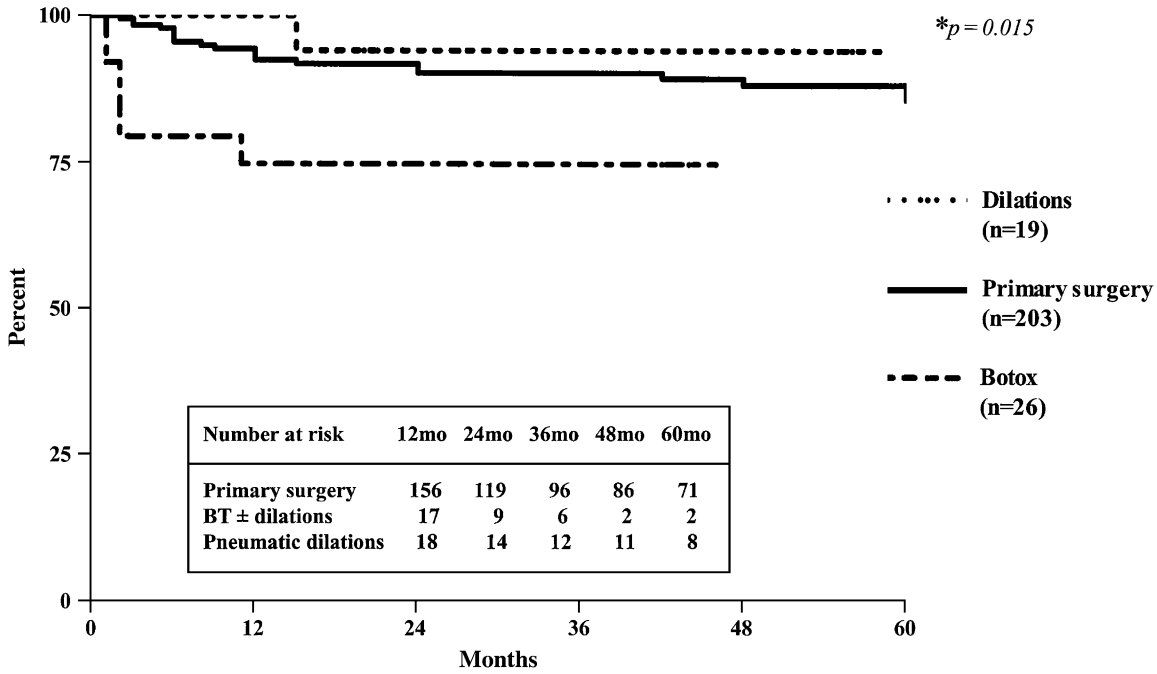

Fig. 2. The 5-year control of symptoms in the three study groups. BT = botulinum toxin.

Table 3. Logistic regression analysis: predictive factors of symptom recurrence

Group	Parameter	*P* value
B (n = 19)	No. of pneumatic dilations	0.721
	Dilations alone or combined with BT injections	0.023
C (n = 26)	No. of BT injections	0.002
	BT injections alone or combined with pneumatic dilations	0.021

BT = botulinum toxin.

appeared to be safe as both primary and second-line treatment.

When the final outcome of laparoscopic myotomy was analyzed, our patients who had already had BT injections were less likely to be asymptomatic than were patients who had surgery alone or surgery after dilation (who had similar chances of being symptom-free). It is worth noting that all 6 of 26 (23%) group C patients considered to be surgical failures had received two BT injections, and only two of four patients who had previously had both dilations and two BT injections were symptom-free at 5 years. Thus, as expected, the number of BT injections (n = 2) significantly predicted a poor outcome after surgical myotomy on logistic regression analysis.

As for the causes of failure, it is interesting that five of six (83%) failures in group C occurred within 2 months of surgery. In all except one of these patients with early symptom recurrence, barium swallow showed a short narrowing at the lower end of the myotomy (one patient had an associated esophageal motor disorder, diffuse esophageal spasm, and the obstruction was in the upper part of the myotomy). These patients were assumed to be cases of incomplete myotomy on the gastric side.

Extending the myotomy downward on the gastric side remains a critical aspect of the operation and essential to deal thoroughly with the unrelaxing sphincter problem.[26] This part of the operation is probably the most difficult, however, because it is not easy to identify a plane between the submucosa and the muscle layer, and bleeding from small vessels is frequent. These difficulties are more likely to occur in patients with a fibrotic reaction at the cardia level due to BT injection. It seems reasonable for surgeons to be concerned about the extension of the myotomy in the cardia region and farther down when they fear perforating a region that is difficult to dissect and has experienced the fibrotic effect of the BT.

Another finding of this study was that subsequent dilations (usually one to six) in patients considered surgical failures were less effective in patients who had received BT injections before surgery. Of the five patients with early symptom recurrence, two failed to respond to the dilation treatment (one was unavailable for further follow-up when dilation was offered after myotomy), possibly due to periesophageal fibrosis for the previous BT treatment (an assumption based on clinical and radiographic evidence of an esophageal obstruction and the absence of esophagitis and stricture).

Along with our objective findings, however, we acknowledge that patients receiving BT injections in this study formed a small group compared with those undergoing primary surgery. We also thought that patients refractory to multiple sessions of endoscopic treatments (several BT injections with/without pneumatic dilations), with a poor outcome of surgery and, further, minimal effect of postoperative ancillary dilations, might represent a subgroup with a more refractory disease. In these patients, then, the fibrotic reaction due to the treatment with BT would be only in part responsible for the poor outcome of subsequent surgery.

In conclusion, laparoscopic Heller myotomy is safe and effective as either primary or secondary treatment for esophageal achalasia. If an endoscopic treatment for the disease is preferred to surgery, we recommend pneumatic dilation. When BT has already been used, laparoscopic myotomy is equally safe, but patients are less likely to remain asymptomatic. We suggest the use of BT only in case of high-risk patients, unfit for surgery.

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Functional Comparison of Bone Marrow–Derived Liver Stem Cells: Selection Strategy for Cell-Based Therapy

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Several distinct subpopulations of bone marrow–derived liver progenitor cells were recently described. However, there is inadequate information comparing these subpopulations from a liver-function point of view. This study was undertaken to compare two subpopulations of liver progenitors: β_2-microglobulin (β_2m)–negative/Thy-1–positive cells, and liver progenitors obtained from the non-adherent cell fraction after a panning procedure. The cells were cultured under several conditions including high- and low-dose hepatocyte growth factor, various cellular densities, and different media. Growth characteristics, liver-specific metabolic capacity, and liver regeneration–associated gene expression were studied. Both isolation procedures yielded cells that produced albumin and metabolized ammonia into urea. The study demonstrated that the β_2m-negative/Thy-1–positive cell fraction metabolized ammonia into urea more efficiently and produced a superior amount of albumin compared with the panned cell fraction. The β_2m-negative/Thy-1–positive cell fraction could be optimal for the development of novel cell-based treatment strategies for congenital or acquired liver diseases. (J GASTROINTEST SURG 2005;9:1340–1345)

KEY WORDS: Hepatic stem cells, cell biology, metabolism, in vitro study, rodent

Bone marrow–derived cells can differentiate into hepatocytes, cholangiocytes, and hepatic endothelial cells under specific conditions.[1–11] Recently, several studies demonstrated that bone marrow–derived adult liver stem cells could be activated and mobilized from the bone marrow, on a specific demand, generated by a failing liver. After activation, these cells demonstrated exquisite ability to differentiate into several cell lineages and provide hepatic support.[5,8,12,13] Consequently, this plasticity of adult bone marrow cells elicited renewed enthusiasm in developing novel treatment strategies for the caring of congenital and acquired liver diseases.[14] One potential advantage of such an adult liver progenitor cell is the possibility of use in an autogenic manner, avoiding immunosuppression.

Currently, bone marrow–derived liver progenitors are thought to be a heterogeneous group of cells. This bone marrow fraction of cells is composed of several different subpopulations, as reflected by the diverse experimental strategies for their isolation, characterization, and culturing.[1,6,9,10,15–18] Adopting the concept of a single pluripotent adult stem cell, residing within the bone marrow, that carries a potential capability to generate progeny of several lineages with hepatocyte-associated function gave the impetus to the search for an optimal subpopulation of cells that should be used to develop novel cell therapy strategies.

As published recently by Oh et al.,[16] a subpopulation of putative liver progenitor cells can be isolated from the bone marrow by a panning procedure. When cultured for 21 days with initially high doses of hepatocyte growth factor (HGF), these bone marrow cells transformed into a hepatocyte lineage as shown by the expression of albumin mRNA. In a similar approach, Miyazaki et al.[6] were able to induce hepatocyte-specific mRNA expression in

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nonsorted bone marrow cells by the addition of HGF to the culture media.

A different approach to isolate adult liver progenitor cells from the bone marrow was developed by our group.[1,15] After a two-step magnetic cell sorting (MACS) immunoisolation procedure, β_2-microglobulin–negative, Thy-1 (CD-90)–positive (β_2m-negative/Thy-1–positive) cells (rodent and human) were shown to express hepatocyte-specific markers. Additionally, their hepatocyte-specific metabolic activity was detectable both in culture and in vivo.[1,5,11,15]

The aim of this study was to further characterize and functionally compare these two promising progenitor cell subpopulations. Prior to embarking on developing novel cell therapy strategies for the care of patients with failing livers, it is imperative to select the optimal cell source. We demonstrate here that the β_2m-negative/Thy-1–positive cell fraction is an attractive candidate to be used in such novel cell therapies.

MATERIAL AND METHODS

All animal experimentation was approved by the institutional committee for animal welfare in accordance with the European Convention on Animal Care. Twenty-eight male Sprague-Dawley rats (220–250 g; RCC Ltd., Füllinsdorf, Switzerland) were divided into seven experimental groups (Table 1). Cells from rat's bone marrow were isolated either by panning or by MACS, as described previously.[1,15,16] Cells were then either plated onto a low- or a high-density cellular culture on uncoated polystyrene dishes or onto a layer of Matrigel (Becton Dickinson, Bedfold, MA).[1,15,16] Culture medium was either DF medium, a 1:1 mixture of Ham's F12 (GIBCO, Invitrogen Corporation, Paisley, Scotland, UK) and Dulbecco's modified Eagle's medium (Sigma, Buchs, Switzerland) supplemented with 1000 ng/ml of HGF (R&D Systems, Minneapolis, MN) for 5 days, followed by 5 ng/ml of HGF or DF medium supplemented with 20 ng/ml of HGF for the entire culture period of 12 days. Alternatively cells were cultured in small hepatocyte media (SHM)[19] with 10% heat-inactivated fetal calf serum (Invitrogen, Basel, Switzerland) and supplemented with HGF (20 ng/ml) and epidermal growth factor (10 ng/ml; Biosource, Camarillo, CA). The cells were cultured in 500 µl of media for 12 days with a change of medium every third day.

The Panning Procedure

Femoral bone marrow was harvested by aspiration through an 18-gauge needle (Venflon; Becton Dickinson, Fraga, Spain) with a 1-ml syringe (Plastipak; Becton Dickinson, Madrid, Spain) and transferred into sterile phosphate-buffered saline. The bone marrow pellet was gently drawn into and expelled from the syringe 10 times to release the cells into suspension. After red cell lysis, the remaining cells were precultured in DF medium supplemented with 10% fetal bovine serum on a 60-mm polystyrene dish (Corning Costar Corporation, Bodenheim, Germany).[16] After 60 minutes of panning, the nonadherent cells were collected, washed with serum-free DF medium, and plated.

The MACS Procedure

For the isolation of β_2m-negative/Thy-1–positive cells, the recently developed MACS procedure was used as described recently by Avital et al.[15] and Inderbitzin et al.[1]

Table 1. Experimental Groups

Experimental Group	Cell Isolation Procedure	Cells Plated/cm^2	Coating of Culture Dishes	Culture Medium	Hepatocyte Growth Factor
PaL20	Panning	5000	None	DF medium	20 ng/ml
MaL20	MACS	5000	None	DF medium	20 ng/ml
PaL1000	Panning	5000	None	DF medium	1000 ng/ml; >day 6: 5 ng/ml
MaL1000	MACS	5000	None	DF medium	1000 ng/ml; >day 6: 5 ng/ml
PaH1000	Panning	50,000	None	DF medium	1000 ng/ml; >day 6: 5 ng/ml
PaHSHM	Panning	50,000	Matrigel	SHM	20 ng/ml
MaHSHM	MACS	50,000	Matrigel	SHM	20 ng/ml

Subpopulations of bone marrow cells were isolated by two different procedures: panning (Pa) and magnetic cell sorting of β_2-microglobulin negative/Thy-1–positive cells (Ma). The cells were plated using two different cell densities: L = 5000 cells/cm^2, H = 50,000 cells/cm^2, on uncoated polystyrene dishes or on a layer of Matrigel, in small hepatocyte medium (SHM) or a mixure of Dulbecco's modified Eagle medium and Ham's-F12 medium (DF medium) containing 1000 ng/ml (1000) of hepatocyte growth factor for 5 days and 5 ng/ml thereafter, or 20 ng/ml (20) of HGF for the entire culture period.

Matrigel Coating of Polystyrene Culture Dishes

For all culture experiments, 24-well cell culture plates were used (Corning Costar Corporation). Where indicated, dishes were coated with a gel layer of Matrigel (25 μg/cm^2).[1,15]

Enzyme-Linked Immunosorbent Assay (Albumin)

Albumin secretion was measured at culture days 3, 6, 9, and 12 by sandwich enzyme-linked immunosorbent assay. Samples were analyzed in several dilutions (1:1, 1:2, 1:5, 1:10) and compared with a standard curve of rat albumin (RSA, Rat albumin fraction V; ICN Biomedicals GmbH, Eschwege, Germany).[20]

Determination of Urea Synthesis

Bone marrow derived liver progenitors were spiked with ammonia (Sigma A 4514, 2.5 mmol/l, pH 7.40) for 5 hours at 3, 6, 9, and 12 days of culturing. Ammonia and urea content were then immediately determined by the use of an enzymatic colorimetric method (Roche Diagnostics, Rotkreuz, Switzerland). The precision of the test in the described experimental setting is ±2.6% for ammonia and ±2.7% for urea.[1,21]

Total RNA Extraction

Cells were harvested by addition of 500 μl of TRIZOL (Invitrogen AG) to the culture dish after complete removal of the media immediately after determination of urea formation. Total RNA was extracted as described previously,[22] and cDNA then was synthesized with random primers, using the Promega Reverse Transcription System (Promega Corporation, Madison, WI).

Albumin, Multidrug Resistance Associated Protein-1 (mrp-1), Multidrug Resistance Associated Protein-2 (mrp-2) mRNA Expression

Quantitative mRNA expression was measured by TaqMan real-time polymerase chain reaction (PCR) using albumin, mrp-1, and mrp-2 primers and probes as described.[1,19] Standard TaqMan real-time PCR conditions (Applied Biosystems, Rotkreuz, Switzerland) were used. The cycler conditions were set to 50°C for 2 minutes, followed by 50 cycles of the amplification step (95°C for 15 seconds to activate the Taq DNA polymerase and 60°C for 15 seconds to anneal and extend the amplicon).

18S rRNA Content

The content of 18S rRNA in each individual culture dish was quantified by TaqMan real-time PCR (AB Applied Biosystems). Average threshold cycle values (CT values) from triplicate real-time PCRs were obtained. Standardization of the metabolic signal (e.g., urea formation or albumin secretion) for total cell number was achieved by the following formula: (Metabolic signal/hr)/($2^{\exp(50-\text{CT value of 18S rRNA})}$).[1,23] CT values of the gene of interest were related to 18S rRNA content: (ΔCT gene of interest=CT gene of interest−CT 18S rRNA).

Statistical Analysis

Results are expressed as mean ± SD. Paired *t* test was used to compare parallel cell cultures from the same donor animal. Student's *t* test was applied to compare groups with normally distributed data. For the correction of pairwise multiple comparisons, the Student-Newman-Keuls method was applied. The significance level was set at $P < .05$.

RESULTS

In the first experiment, we examined whether β_2m-negative/Thy-1–positive cells attach within 60 minutes to a polystyrene culture dish. Of the cells plated, 16.7% ± 5.5% (n = 5) were nonadherent and accordingly harvested in suspension, as described previously after the panning procedure. In a subsequent experiment, using the MACS procedure on the nonadherent cell fraction, 6.5% ± 1.5% of cells were identified as β_2m-negative/Thy-1–positive.

ATP Binding Cassette Transporter Gene Expression (mrp-1, mrp-2)

mrp-1 was expressed in both subpopulations (i.e., after panning and after the MACS procedure) immediately after isolation. The corresponding ΔCT values for mrp-1 were 13.7 ± 0.2 for panned cells and 12.9 ± 3.4 for β_2m-negative/Thy-1–positive cells. After culturing, mrp-1 expression was maintained in the PaHSHM group (see Table 1) for 12 days of culture (average ΔCT values, 11.5 ± 0.7). In contrast, in the MaHSHM group the mrp-1 signal was not detectable.

No expression of mrp-2 mRNA was found in real-time PCR in freshly isolated or cultured cells from both cell isolation methods.

Experimental Groups With Low Density of Plated Cells

Analyzing the total cell number of parallel cell cultures (i.e., PaL20 with PaL1000; MaL20 with MaL1000) in culture media containing different amounts of HGF revealed no significant difference in the panned groups, whereas cell number in the MACS groups was significantly decreased in the culture media with high HGF content (Table 2). Albumin secretion or urea genesis was not detectable in any of these four experimental conditions (Table 3). Of note, high doses of HGF significantly reduced the total amount of β_2m-negative/Thy-1–positive cells in culture, whereas no change in total cell number was detected in the cells after panning.

Experimental Groups With High Density of Plated Cells

Pairwise comparison of 18S rRNA content in the high-density cultures (PaHSHM, PaH, MaSHM) showed significant differences in the total number of cells between all groups (Table 2). The highest cell number was observed in panned cells cultured on Matrigel in SHM (PaHSHM). Cell number was stable over a culture period of 12 days (Table 2). Urea genesis was maintained for 12 days and albumin synthesis was detectable in the culture media until culture day 9 (Table 3). The metabolic capacity of the panned subpopulation on Matrigel in SHM contrasts with the absence of any albumin formation or urea genesis in the PaH1000 group.

Table 2. Average 18S rRNA Content in Each Individual Culture

Experimental group	Average CT values for 18S rRNA Content			
	3 days	6 days	9 days	12 days
PaL20	33 ± 1.9	31 ± 2.7	33 ± 1.7	33 ± 2.2
MaL20	32 ± 3.0	32 ± 2.2	33 ± 2.7	34 ± 4.7
PaL1000	32 ± 2.6	31 ± 3.4	33 ± 2.4	31 ± 2.5
MaL1000	37 ± 1.3	35 ± 6.3	36 ± 0.9	36 ± 1.8
PaH1000	28 ± 1.2	30 ± 5.0	29 ± 1.8	29 ± 0.5
PaHSHM	26 ± 0.7	25 ± 0.6	28 ± 0.6	25 ± 4.0
MaHSHM	34 ± 0.7	35 ± 0.6	37 ± 1.4	37 ± 1.8

For groups, see Table 1 footnotes.
No significant cell growth or cell loss was detected under any of the seven experimental conditions examined over a period of 12 days. In the low-density (L) cultures, high doses of hepatocyte growth factor significantly reduced the total amount of cells in the groups after MACS sorting (MaL20 versus MaL1000). Total cell numbers in all three high-density (H) cultures (PaH1000, PaHSHM, MaHSHM) were significantly different, indicating individual responses of the isolated subpopulations to the different culture conditions.

Table 3. Hepatocyte Specific Metabolic Activity

Experimental Group	Albumin Expression (mRNA)	Albumin Secretion: Detection Limit (DL): <1 ng/ml	Urea Genesis: Detection Limit (DL): <0.8 μg/ml
PaL20	ND	<DL	<DL
MaL20	ND	<DL	<DL
PaL1000	ND	<DL	<DL
MaL1000	ND	<DL	<DL
PaH1000	+	<DL	<DL
PaHSHM	+	+	+
MaHSHM	+	+	+

For groups, see Table 1 footnotes.
Albumin secretion and urea genesis were determined in the seven experimental groups. Only the high-density cultures on a layer of Matrigel in small hepatocyte media showed albumin secretion (+) and urea formation (+). High doses of hepatocyte growth factor induced albumin mRNA expression in bone marrow cells after panning as described, but no urea synthesis or albumin secretion was observed in this group (<DL, values below detection limit). Albumin mRNA was not determined (ND) in the metabolically quiescent experimental groups.

An intermediate cell number was seen in panned cells cultured in DF medium with an initial amount of 1000 ng/ml of HGF for 5 days (PaH1000). Cell number was maintained over a total of 12 days in culture (Table 2). Reduction of the HGF supplemented to 5 ng/ml did not influence the total amount of cells in culture. With real-time PCR analysis, albumin mRNA was detectable in minute amounts in the PaH1000 group over time, but no albumin secretion or urea formation was detectable in the metabolic assays used (Table 3).

The lowest cell number was determined in the cell cultures of β_2m-negative/Thy-1–positive cells in SHM (MaHSHM). Total cell number was maintained over time with a slight decrease after day 9 in culture (Table 2). Albumin synthesis and urea formation were evident over the entire culture period of 12 days (Table 3).

DISCUSSION

The β_2m-negative/Thy-1–positive bone marrow cells do not attach efficiently to a polystyrene dish. In fact, β_2m-negative/Thy-1–positive cells can be enriched by the panning procedure alone in the nonadherent cell fraction by an average of around 240%.[1]

mrp-1 expression in normal liver is low.[24] However, in regenerating livers[25] and in rodent livers after an oval cell induction protocol, mrp-1 specific staining was observed.[24] We therefore

studied mrp-1 expression by real-time PCR in the bone marrow subpopulations obtained by panning and after the MACS procedure. mrp-1 was expressed in both subpopulations immediately after isolation. The mrp-1 signal was then maintained only in the PaHSHM group for the entire culture period of 12 days. It is possible that the cells obtained after MACS have more "liver-like" gene expression and therefore express less mrp-1.

mrp-2 is expressed in the canalicular membrane of highly differentiated hepatocytes.[19] No expression of mrp-2 mRNA was found in real-time PCR in freshly isolated or cultured cells from both cell isolation methods. This is congruent with our assumption that both populations of cells are not well differentiated. Moreover, due to the lack of expression of mrp-1 and mrp-2 in the MaHSHM subpopulation, containing freshly isolated β_2m-negative/Thy-1–positive cells, we submit that this subpopulation of cells after culturing in SHM contain cells in a less-differentiated state.

Bone marrow subpopulations obtained by different cell isolation procedures [1,6–10,17,18,26] are likely to overlap and the amount of liver progenitor cells contained within these individual cell isolations could therefore vary considerably. Careful surface marker and liver specific functional analysis of the various cell populations physiologically present in the bone marrow is urgently needed.

To compare the liver specific functional capacity and the individual response of the two subpopulations to HGF, a series of in vitro studies were performed (Table 1).

As described by Oh et al.,[16] the panning cell isolation procedure yields a subpopulation of cells from the bone marrow that expresses albumin mRNA when cultured with high doses of HGF. However, in our series the high content of HGF in the DF medium did not propagate hepatocyte specific metabolic activities as demonstrated by the lack of albumin synthesis or urea formation. In a direct comparison, β_2m-negative/Thy-1–positive cells (MaHSHM) outperformed the panned cells (PaHSHM) significantly (Table 4). Albumin expression on the mRNA level corresponded well with the albumin secretion determined in the culture medium. The culture medium described induces strong hepatocyte specific metabolic activity in a pure culture of β_2m-negative/Thy-1–positive bone marrow cells. A 1:20 co-culture of β_2m-negative/Thy-1–positive cells with uncharacterized bone marrow cells (PaHSHM) resulted in rapid loss of the liver specific metabolic capacity while total cell number was maintained.

High doses of HGF[6,16] significantly reduced the total amount of β_2m-negative/Thy-1–positive cells in culture while no change in total cell number was detected in the cells after panning. We concluded that high-dose HGF is potentially toxic to β_2m-negative/Thy-1–positive cells, whereas it has lesser effect on the panned cell population. One explanation could be based on the heterogeneity of the panned cells.

Characterizing hormonally defined culture media for unlimited cell expansion of β_2m-negative/Thy-1–positive cells represents the next crucial step for the development of future cell-based liver therapy.

Table 4. Direct Metabolic Comparison of Cultured Adult Progenitor Cells Isolated by Either panning or MACS

	Relation of the Hepatocyte Specific Metabolic Signal PaHSHM:MaHSHM		
Culture Day	Albumin Expression (mRNA)*	Albumin Secretion*	Urea Genesis
3	1:770	1:310	1:250
6	1:1200	1:1750	1:530
9	1:1200	1:1380	1:720
12	1:1600	>1:2000	1:1070

The direct comparison of the hepatocyte specific metabolic activity between cell populations obtained after panning (PaHSHM) and after immunoisolation by magnetic cell sorting (MaHSHM) at culture days 3, 6, 9, and 12 showed significantly higher metabolic capacity of the β_2-microglobulin–negative, Thy-1–positive bone marrow cells in culture (*$P < 0.05$). Albumin mRNA expression and albumin secretion paralleled in both experimental groups.

CONCLUSION

Both cell isolation procedures yielded a subpopulation of bone marrow–derived liver progenitors capable of hepatocyte specific metabolic activity. Pure cell preparation of β_2m-negative/Thy-1–positive cells showed significantly stronger albumin synthesis and urea formation than did cells after panning. Culture conditions to propagate the unlimited cell expansion of liver progenitor cells would make it possible to use this cell pool for the development of novel adult progenitor cell–based treatment strategies. This approach might prove to be clinically valuable for the cure of congenital or acquired liver diseases.

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Limited Survival in Patients With Carcinomatosis From Foregut Malignancies After Cytoreduction and Continuous Hyperthermic Peritoneal Perfusion

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Peritoneal carcinomatosis is a frequent mode of metastasis in patients with gastric, duodenal, or pancreatic cancer. Survival in this setting is short and therapeutic options are limited. This analysis examines the outcomes of 18 patients treated with operative cytoreduction and continuous hyperthermic peritoneal perfusion. Eighteen patients (6 males and 12 females) with gastric (n = 9), pancreatic (n = 7), or duodenal (n = 2) cancer were treated on protocol. Patients underwent optimal cytoreduction (complete gross resection, 11; minimal residual disease, 7) and a 90-minute perfusion with cisplatin. Clinical parameters and tumor and treatment characteristics were analyzed. Survival curves were estimated using the Kaplan-Meier method. Procedures included gastrectomy (n = 8), pancreaticoduodenectomy (n = 3), and hemicolectomy (n = 2). After cytoreduction, patients had no evidence of residual disease (n = 11), fewer than 100 implants less than 5 mm (n = 1), more than 100 implants between 5–10 mm (n = 3), or multiple implants with greater than 1 cm (n = 3). Five patients received a postoperative intraperitoneal dwell with 5-fluorouracil and paclitaxel. There was one perioperative mortality, and complications occurred in 10 patients. The median progression-free survival was 8 months (mean, 10 months; range, 1–47 months) with a median overall survival of 8 months (mean, 18 months; range, 1–74 months). In this cohort, peritoneal perfusion with cisplatin used to treat foregut malignancies has a high incidence of complications and does not significantly alter the natural history of the disease. Investigation of novel therapeutic approaches should be considered. (J GASTROINTEST SURG 2005;9:1346–1353)

KEY WORDS: Regional therapy, peritoneal carcinomatosis, peritoneal perfusion, gastric cancer, duodenal cancer, pancreatic cancer

Peritoneal dissemination from primary gastric, duodenal, and pancreatic cancer is frequently found on exploration for curative resection of these malignancies. In a subset of patients, disease is confined to the abdominal cavity and has not yet spread systemically. It has been shown, through recurrence patterns after curative resection, that the peritoneum is frequently one of the first sites of recurrence. In gastric cancer, peritoneal recurrence accounts for 50%–60% of deaths after surgical resection with curative intent.[1–3] Even with complete resection, microscopic disease remains, leading to this early recurrence pattern and eventual death. The median survival for these patients remains exceedingly low.

Over the past decade, little progress has been made to improve survival in these neoplasms. Systemic chemotherapy and radiation have thus far failed to meaningfully affect outcomes with these malignancies. Researchers have looked at novel therapies to provide better treatments over surgery alone, systemic chemotherapy, or radiation.[4,5] Regional therapy is a rational approach in a disease process confined to the abdominal cavity. One of

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these approaches uses continuous hyperthermic peritoneal perfusion (CHPP), either intraoperatively or in the perioperative period, as a method to prevent or inhibit peritoneal dissemination of disease. The synergism of hyperthermia and chemotherapy allows a significantly higher concentration of therapeutic agent to be administered while limiting systemic toxicities.[6,7] This approach has been developed with the hope of palliating peritoneal recurrence and is currently undergoing trials for multiple different peritoneal malignancies.[8]

Numerous reports exist studying the effects of CHPP using predominantly mitomycin-C in the treatment and palliation of these aggressive upper gastrointestinal malignancies, most concentrating on gastric carcinoma.[9–12] The methods of the procedures vary; nonetheless, some investigators have reported an increase in time to recurrence and overall survival in gastric cancer when combining surgical resection and perfusion.[13,14] Our study is composed of 18 patients enrolled in clinical trials with upper gastrointestinal malignancy; including gastric, duodenal, and pancreatic adenocarcinoma, to determine time to recurrence and overall survival after CHPP with cisplatin.

MATERIAL AND METHODS

Patient Eligibility and Enrollment

From September 1993 through April 2002, 18 patients with gastric, duodenal, or pancreatic adenocarcinoma were referred to the Surgery Branch of the National Cancer Institute. These patients were treated following one of four Surgery Branch protocols approved by the Institutional Review Board of the National Cancer Institute. Study patients were required to be over 18 years of age with histologically or cytologically proven metastatic carcinoma in the peritoneal cavity. Additional eligibility criteria included (1) Eastern Cooperative Oncology Group (ECOG) performance status of less than or equal to 2; (2) lack of a comorbid disease that prevents them from being an operative candidate; (3) a life expectancy greater than or equal to 8 weeks; (4) adequate renal function (serum creatinine <1.5 or creatinine clearance of <70 ml/min; (5) normal hepatic function (normal bilirubin, prothrombin time/partial thromboplastin time, enzymes <2 times the upper limit of normal; (6) adequate hematopoietic parameters (white blood cell count $>3000/\mu l$ and platelet count greater than 75,000); (7) absence of previous ineffective intraperitoneal platinum therapy; (8) patient's weight more than 30 kg; and (9) no chemotherapy, radiotherapy, or immunotherapy in the past 30 days. None of the peritoneal carcinoma patients had evidence of extra-abdominal metastases at the time of treatment.

Patients were treated with one of four related and similar protocols of cytoreduction and hyperthermic peritoneal perfusion, including (1) escalating dose of cisplatin administered via a 90-minute CHPP; (2) neoadjuvant and intraoperative adjuvant CHPP with cisplatin/mitomycin-C; (3) CHPP with cisplatin followed by early postoperative escalating-dose 5-fluorouracil (5-FU) and paclitaxel administered as a single intraperitoneal dwell (2–8 days postoperatively); or (4) CHPP with cisplatin followed by early postoperative 5-FU and paclitaxel administered as a single intraperitoneal dwell (2–8 days postoperatively). All patients underwent pretreatment counseling and gave written informed consent according to institutional and federal guidelines.

CHPP Technique

Patients underwent an exploratory laparotomy, lysis of adhesions, tumor cytoreduction, and CHPP as previously described.[5] Every attempt was made at cytoreduction to leave no gross evidence of disease. Major bowel or abdominal organ resections were performed when deemed necessary to attain adequate debulking. At the completion of cytoreduction, two large-bore catheters were inserted through the abdominal wall; the inflow catheter was placed over the right lobe of the liver with the outflow catheter in the pelvis. Temperature probes were placed beneath the peritoneal lining on each side of the abdomen and in the pelvis. The abdominal fascia was temporarily closed. The catheters were connected to a closed circuit consisting of a roller pump, heat exchanger, and a reservoir (Fig. 1). Chemotherapy containing the perfusate was heated and recirculated for 90 minutes through the peritoneal cavity. The perfusion flow rate was maintained at 1.5 L/min with a perfusate volume that varied from 3 to 7 L depending on the size of the potential space of the peritoneal cavity (sufficient to moderately expand the peritoneum correlating with intra-abdominal pressures between 5 mm Hg and 15 mm Hg). After stable perfusion parameters were obtained and the peritoneal cavity was warmed to a median temperature of 41.4°C, cisplatin was added to the perfusate. Perfusion was continued for 90 minutes, during which there was constant, manual agitation of the abdomen to minimize streaming and ensure optimal and even distribution of perfusate throughout the abdominal cavity. Sodium thiosulfate was given via a loading dose of 7.5 g/m^2 intravenously over 20 minutes before the addition of cisplatin, followed by

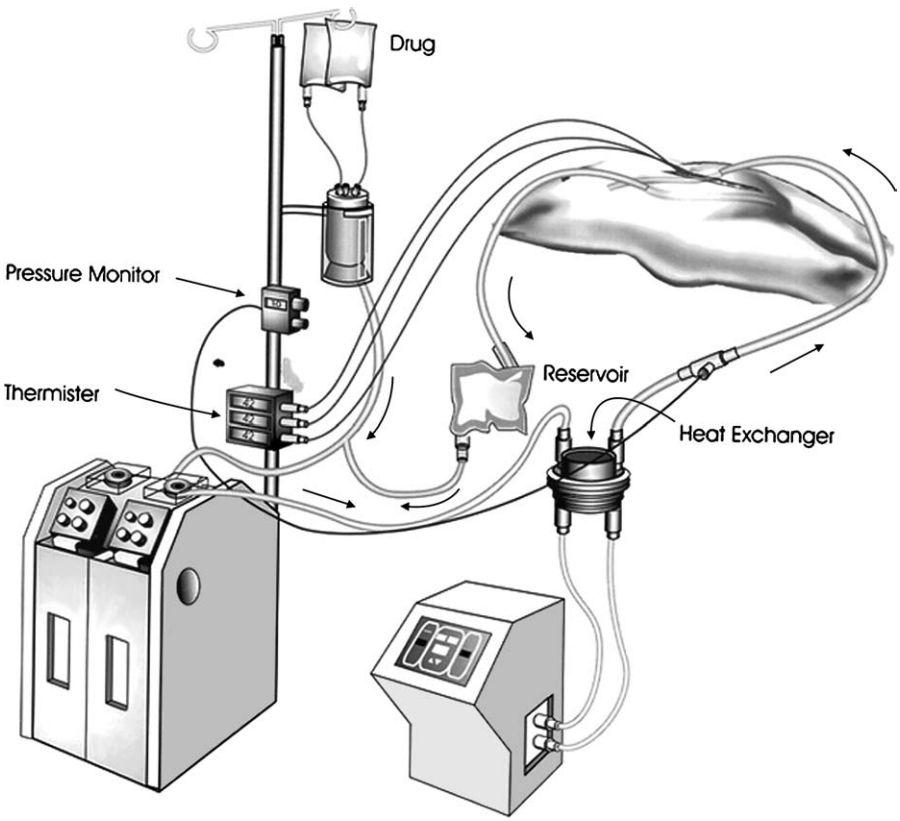

Fig. 1. Continuous hyperthermic peritoneal perfusion perfusion circuit. After aggressive optimal cytoreduction, two large-bore catheters were then inserted through the abdominal wall. Temperature probes were placed beneath the peritoneal lining. The catheters were connected to a closed circuit consisting of a roller pump, heat exchanger, and a reservoir. Chemotherapy containing the perfusate was heated and recirculated for 90 minutes throughout the peritoneal cavity.

a continuous infusion at 2.13 g/m^2/hr for 12 hours as described.[15] Sodium thiosulfate has been shown to bind cisplatin in the serum and decrease systemic toxicity from intraperitoneal administration of cisplatin.[16] Urine output was maximized through aggressive hydration (central venous pressure 12 mm Hg 30 minutes before CHPP) and diuretics to maintain urine output at greater than 200 ml/hr during the perfusion; this was continued for 12 hours postoperatively. Urine output was maintained at 100 ml/hr for an additional 12 hours.

The patient's mean core temperature was measured with an esophageal probe and maintained at a median of 38.5°C (range, 37.2°–39.8°) using a cooling blanket and topical ice packs. At the completion of the perfusion, the fascia was opened, the temperature probes and catheters were removed, the residual perfusate was evacuated, and the peritoneal cavity was irrigated with warm saline. A Tenckhoff catheter was inserted through the abdominal wall if postoperative dwell therapy was planned as deemed by the protocol.

Pretreatment and Follow-up Evaluation

Before treatment, each patient underwent a full history, physical examination, routine laboratory studies, and a computed tomography (CT) scan of the chest, abdomen, and pelvis. Intraoperatively, the residual disease after cytoreduction was assessed as B-0, no residual disease; B-1, fewer than 100 lesions and all smaller than 5 mm; B-2, more than 100 total lesions with some being greater than 5 mm but less than 1 cm; and B-3, residual tumors greater than 1 cm.

Patients were monitored in the intensive care unit for at least 24 hours. Routine laboratory screening was performed daily for the first 5 postoperative days and then twice weekly until the patient was discharged. All complications were recorded. The patients were seen in follow-up at 6 weeks from the time of discharge. At this visit, physical examination and routine laboratory screening were performed. The patients were then followed every 3 months for 1 year and then every 6 months for laboratory screening, physical examination, and CT

scans of the chest, abdomen, and pelvis. Special attention was paid to accumulation of ascites, new fluid collections, or soft tissue masses suspicious for recurrence.

Statistics

Progression-free and overall survival curves were estimated using the Kaplan-Meier method.

RESULTS

Patient Characteristics

Clinical features of the 18 patients are shown in Table 1. Eighteen patients were treated with CHPP for gastric, duodenal, or pancreatic carcinoma on one of four different protocols. The median age was 48 years (range, 32–72 years). Six males and twelve females were treated. Nine of the patients had gastric carcinoma (50%), seven patients had pancreatic carcinoma (39%), and two patients had duodenal cancer (11%). Seven of the patients had received initial surgery before being enrolled on one of our protocols. One patient had a subtotal gastrectomy; two patients had a pancreaticoduodenectomy; one patient had a colectomy, distal pancreatectomy, and splenectomy; one patient had a gastric bypass; and one patient underwent a total abdominal hysterectomy, bilateral salpingo-oopherectomy, and omentectomy with a retroperitoneal lymph node dissection. All patients had pathologically confirmed intraperitoneal disease at the time of CHPP. Four of the patients had received prior systemic chemotherapy, and one patient received prior radiation therapy.

Table 1. Patient characteristics

No. of patients	18
Median age (range) (yr)	48 (32–72)
Male-to-female ratio	6:12
Histology	
Gastric carcinoma	9
Pancreatic carcinoma	7
Duodenal carcinoma	2
Prior systemic chemotherapy	4
Prior laparotomy	7
Bowel resection/anastomosis	12
Received cisplatin	18
Received 5-FU dwell	5
Received Taxol dwell	5

Values given as number of patients unless otherwise indicated.

Surgery and Hyperthermic Peritoneal Perfusion

Table 2 demonstrates the various surgical resections performed as cytoreductive procedures prior to peritoneal perfusion. Eight patients underwent either a subtotal or a total gastrectomy. Three patients underwent a pancreaticoduodenectomy; one was delayed 6 weeks until after recovering from a neoadjuvant peritoneal perfusion. Two patients underwent a right hemicolectomy. The majority of patients (n = 11) had successful cytoreduction to no residual evidence of disease (B-0). One patient had fewer than 100 lesions, all being less than 5 mm (B-1). Two patients had more than 100 lesions, greater than 5 mm and less than 1 cm (B-2). Three patients had lesions greater than 1 cm (B-3). The median total operative time was 7.3 hours (range, 4.3–14 hours). The median operative time was 7.3 hours for patients with gastric carcinoma, 5.4 hours for duodenal carcinoma, and 7.1 hours for pancreatic carcinoma.

All 18 patients received cisplatin in the perfusate for a full 90 minutes, and 5 patients received cisplatin followed by 5-FU and paclitaxel administered as a postoperative intraperitoneal dwell. The median dose of 5-FU was 1296 mg/m^2 (range, 858–1740 mg/m^2), and of paclitaxel, 191 mg/m^2 (range, 71–225 mg/m^2). Hyperthermic perfusion with cisplatin was given at doses ranging from 150 to 300 mg/m^2, with a median total dose of 453 mg (range, 186–676 mg). Total perfusate volume ranged from 3 to 7 L and varied depending on abdominal wall distention. Hyperthermia was obtained with perfusate inflow temperatures of 47°–48°C. Perfusate flow was maintained at 0.85–1.55 L/min. Systemic temperatures ranged from 37.2°–39.8°C. There were no intraoperative complications.

Perioperative mortality was 5.6%, and postoperative complications occurred in 10 (55.6%) patients (Table 2). There was one perioperative death in a patient with gastric carcinoma who had a postoperative cerebrovascular accident. Five patients became neutropenic. Three patients had postoperative transient renal insufficiency. Two patients developed wound infections. There was one postoperative leak at the gastrojejunostomy anastomosis. One patient each developed biliary sepsis, postoperative small bowel obstruction, intra-abdominal abscess, urinary tract infection, and enterocutaneous fistula.

Outcomes

Table 3 summarizes progression-free survival and overall survival in these 18 patients treated with CHPP. There were no operative deaths. Nine

Table 2. Tumor and treatment characteristics

Patient	Pathology	Cisplatin dose (mg/m^2)	5-FU IP dwell dose (mg/m^2)	Taxol IP dwell dose (mg/m^2)	Procedures	Complications
1	Gastric	186	—	—	Debulking	None
2	Pancreatic	676	—	—	Right hemicolectomy, gastrojejunostomy	Renal insufficiency, anastomotic leak, VDRF
3	Gastric	581	—	—	Subtotal gastrectomy	Renal insufficiency, pleural effusion stroke, death
4	Gastric	576	—	—	Subtotal gastrectomy, small bowel resection	None
5	Pancreatic	*	—	—	Debulking, lysis of adhesions	None
6	Pancreatic	500	—	—	Pancreaticoduodenectomy	Biliary sepsis
7	Pancreatic	470	—	—	Pancreaticoduodenectomy	Delayed gastric emptying, SBO, neutropenia
8	Pancreas	400	—	—	Debulking	None
9	Gastric	450	—	—	Total gastrectomy, splenectomy, distal pancreatectomy	Neutropenia
10	Pancreatic	400	—	—	Debulking, lysis of adhesions	None
11	Gastric	455	—	—	Gastrectomy	None
12	Gastric	350	858	71	Total gastrectomy	Intra-abdominal abscess, neutropenia
13	Pancreas	425	—	—	Debulking pancreaticoduodenectomy	Enterocutaneous fistula, VDRF
14	Gastric	434	1740	87	Subtotal gastrectomy	Wound infection
15	Duodenal	550	—	—	Debulking, omentectomy	Renal insufficiency, wound infection, UTI
16	Duodenal	450	1296	203	Debulking, omentectomy	None
17	Gastric	460	1440	225	Partial gastrectomy, omentectomy, debulking, revision Roux-en-Y	Neutropenia
18	Gastric	383	1224	191	Total gastrectomy, right hemicolectomy, debulking	Neutropenic fever

VDRF = ventilator-dependent respiratory failure; SBO = small bowel obstruction; UTI = urinary tract infection.
*Not available.

patients with gastric adenocarcinoma were treated. Of these patients, three underwent tumor cytoreduction to no residual disease, with an overall survival of 1, 7, and 74 months. The patient who had 1-month overall survival had a postoperative cerebrovascular accident, which led to her eventual death. One patient had tumor cytoreduction to B-1, with an overall survival of 12 months; two patients had tumor cytoreduction to B-2, with an overall survival of 8 and 10 months; and two patients had tumor cytoreduction to B-3, with an overall survival of 6 and 7 months.

Two patients with duodenal adenocarcinoma were treated. One of these patients had tumor cytoreduction to no residual disease with an overall survival of 46 months. The other patient had tumor cytoreduction to B-2 with an overall survival of 15 months.

Seven patients with pancreatic carcinoma were treated. Six of these had tumor cytoreduction to no residual disease, with an overall survival of 2, 8, 8, 16, 25, and 62 months. The patient who had the 2-month overall survival died from a traumatic accident unrelated to her disease. One patient had tumor cytoreduction to B-3, with an overall survival of 5 months.

Five patients were treated with a postoperative intraperitoneal infusion of 5-FU and paclitaxel. Four

Table 3. Treatment results after continuous hyperthermic peritoneal perfusion

Pathology	Residual disease*	Progression-free survival (mo)	Overall survival (mo)
Gastric	0	1	1
	0	5	7
	0	9	9
	0	—	74
	1	9	12
	2	8	8
	2	6	10
	3	2	6
	3	3	7
Duodenal	0	—	46
	2	3	15
Pancreas	0	†	2
	0	8	8
	0	8	8
	0	8	16
	0	8	25
	0	36	62
	3	2	5

*Residual disease as B-score as described in text.
†Mortality from other causes.

of these patients had gastric carcinoma, with an overall survival of 7, 8, 9, and 10 months. One patient had duodenal carcinoma and she is currently alive at 63 months.

Figure 2 demonstrates the Kaplan-Meier survival curve for progression-free and overall survival for the 18 patients treated. The median progression-free survival was 8 months. The median overall survival was 8 months. Only two patients are alive: one patient with gastric carcinoma at 74 months and one patient with duodenal carcinoma at 63 months.

DISCUSSION

Regional therapy to treat peritoneal carcinomatosis using intraoperative hyperthermic peritoneal perfusion has been associated with long-term survival in multiple different neoplasms, including peritoneal mesothelioma and colorectal disease. Feldman et al.[17] published results for 49 patients with peritoneal mesothelioma treated by CHPP with cisplatin and postoperative dwell therapy with 5-FU and paclitaxel. In this cohort, median actuarial progression-free survival was 17 months with a median actuarial overall survival of 92 months. Factors associated with improved survival were a history of previous debulking surgery, absence of deep tissue invasion, minimal residual disease after surgical resection (overall survival only), and age younger than 60 years (overall survival only).

In 2003, Verwaal et al.[18] published a prospective trial in which 105 patients with peritoneal carcinomatosis from colorectal cancer were randomized to receive either standard treatment consisting of systemic chemotherapy (5-FU/leucovorin) with or without palliative surgery, or aggressive cytoreduction surgery with hyperthermic intraperitoneal chemotherapy, followed by the same systemic chemotherapy regimen. Median survival of the standard therapy group and the treatment group was 12.6 and 22.3 months, respectively ($P = .032$).

Yonemura et al.[19] recently published their series of 107 patients with peritoneal dissemination from a primary gastric cancer treated with peritonectomy and intraoperative chemohyperthermic peritoneal perfusion with mitomycin-C, cisplatin, and etoposide. Complete cytoreduction was achieved in 47 (43.9%) of patients. Postoperative complications occurred in 23 (21.5%) patients treated. Completeness of cytoreduction and peritonectomy were significant prognostic factors in univariate and multivariate analyses. The median survival of all patients was 11.5 months, with a 5-year survival rate of 6.7%. The 5-year survival increased to 27% in patients with complete cytoreduction and peritonectomy combined with CHPP; however, this was demonstrated in only four patients.

Glehen et al.[20] prospectively looked at their series of 49 patients with peritoneal dissemination from primary gastric carcinomas that were treated with cytoreductive surgery and peritoneal perfusion with mitomycin-C. Similarly, they found preoperative ascites and completeness of cancer resection by cytoreductive surgery to be independent predictors of survival. Overall median survival was 10.3 months. However, median survival was 21.3 months in patients who had a macroscopic complete resection or resection to residual nodules less than 5 mm compared with a median survival of 6.1 months in patients who had a diameter of residual nodules of greater than 5 mm.

Few studies have been published of the use of CHPP for the treatment of peritoneal dissemination from pancreatic cancer. This is in part due to the fact that a large majority of these patients have hepatic metastases that are found at the time of exploration.

In our cohort of patients, CHPP with cisplatin was associated with a high complication rate with little, if any, apparent effect on the natural progression of the disease. Mitomycin-C may have improved efficacy in histology over cisplatin when used in the perfusion circuit and combined with optimal tumor

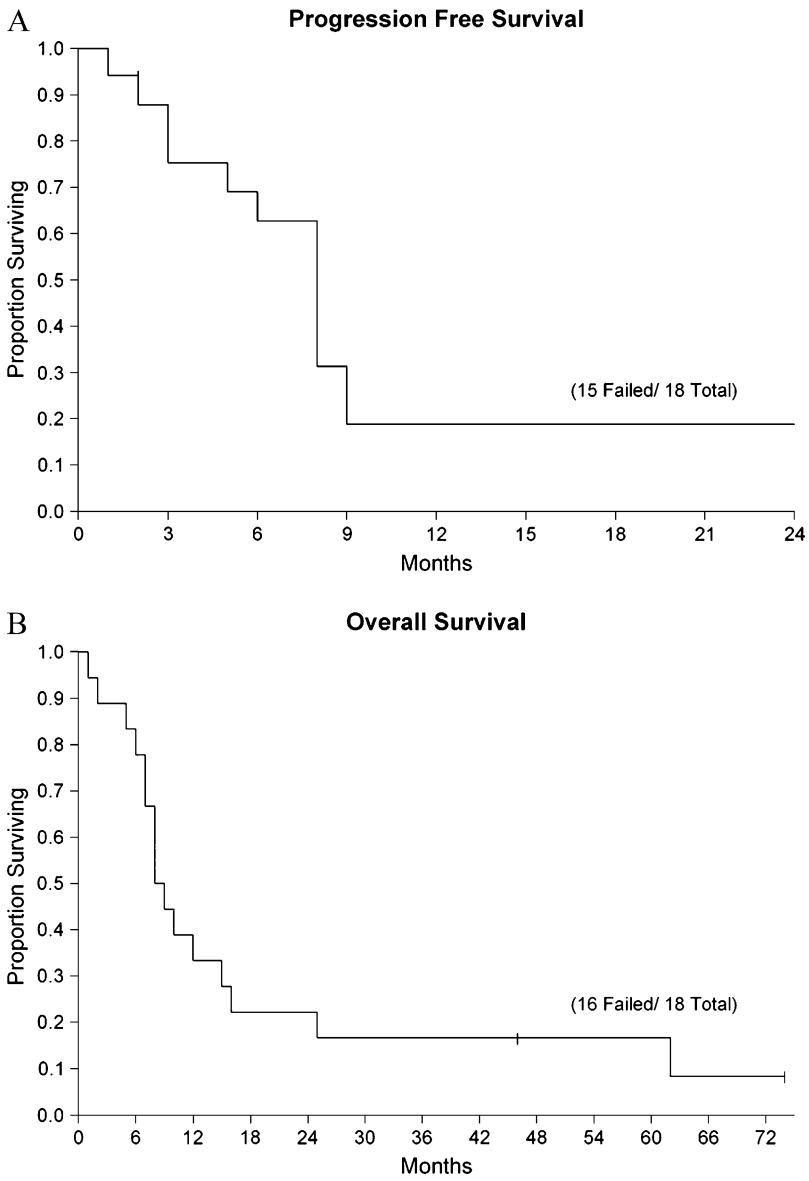

Fig. 2. Kaplan-Meier survival curves of 18 patients treated with continuous hyperthermic peritoneal perfusion with cisplatin and/or postoperative dwell therapy. (**A**) Progression-free survival. (**B**) Overall.

cytoreduction. Although this procedure may have some future role in the treatment of peritoneal dissemination in gastric, pancreatic, and duodenal cancers, it is clear that novel agents and/or approaches are necessary to combat these highly aggressive tumors.

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Microwave Ablation of Hepatic Tumors Using Dual-Loop Probes: Results of a Phase I Clinical Trial

Kenneth Meredith, M.D., Fred Lee, M.D., Mary Beth Henry, A.P.N.P., Thomas Warner, M.D., David Mahvi, M.D.

Hepatic tumors are a common cause of death worldwide. However, few patients are candidates for resection at the time of presentation. Microwave ablation is a viable alternative available for these patients. To date, only straight antennas are used for microwave ablation. Recently, a prototype loop-shaped microwave antenna was developed that, in animal studies, more effectively kills tumors. For this study, the dual-probe lesions were created by placing the probes in both tumors and normal livers. Lesions were created with 60 watts applied power for 5–7 minutes. The livers were sectioned and stained for viability. The average ablation volume was 63.9 ± 8.7 cm^3. Microwave ablation with the loop probes results in complete tumor kill at the ablation/tumor interface, and adjacent to surrounding blood vessels. In addition, vessels within the ablation/tumor interface failed to show viable cells. The shape of the lesions was not distorted by proximity to blood vessels. The advantages of this configuration over conventional straight probes include the ability to encircle a tumor, deliver large amounts of precisely targeted microwave energy to the tumor, and spare normal liver outside the loop. (J GASTROINTEST SURG 2005;9:1354–1360)

KEY WORDS: Hepatic carcinoma, microwave ablation, radiofrequency ablation, loop microwave probe

Primary and metastatic hepatic tumors are a common cause of death worldwide. Chemotherapy and radiation therapy are relatively ineffective against common tumors.[1] The only curative option currently available for patients with hepatic malignancies is surgical excision.[2]

A number of options are available to patients with hepatic tumors not amendable to a curative resection. Most promising are the local ablative techniques (microwave ablation, cryoablation, and radiofrequency ablation). Radiofrequency (RF) ablation remains the most popular minimally invasive thermal ablation technique worldwide. Despite its widespread popularity, the local recurrence rates after treatment remain high, particularly for hepatic colorectal metastases greater than 3.0 cm in diameter.[3] This has necessitated the development of more effective ablative techniques.

Microwave ablation results in the destruction of neoplastic cells by local thermal energy generated by a controlled microwave field.[4] Microwave ablation has several potential advantages when compared to radiofrequency ablation. Microwave ablation is not limited by tissue charring, or impedance to the same extent as RF ablation.[5] Rising tissue impedance results in substantially smaller ablated lesions. Thus, multiple overlapping ablations are needed to cover a large tumor. Microwave energy passed through an antenna heats a zone of tissue surrounding the antenna and does not rely on conduction of heat from a probe. This results in a larger zone of active heating, and thus, higher temperatures in the targeted tumor.

Thus far, only straight devices have been used for microwave ablation. These have resulted in elliptically shaped zones of necrosis that sometimes require overlapping ablations to cover a round tumor.[6] Additionally, straight microwave and RF probes require direct puncture of the tumor, potentially resulting in tract seeding the needle tract with tumor. Recently, a prototype loop-shaped microwave antenna has been developed. Use of the newly

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designed probe in a porcine model resulted in large ablation diameters, and tissue necrosis, with a single ablation. Theoretical advantages of the loop configuration include the ability to encircle a tumor, and to deliver large amounts of precisely targeted microwave energy to the tumor, with minimal collateral damage to normal structures. This technique may decrease the risk of tumor seeding because the tumor is never entered.

The purpose of this study was to determine if a loop microwave antenna is capable of heating tissues to consistently produce tissue/tumor necrosis. We evaluated this device in a group of patients already undergoing a curative resection for primary and metastatic hepatic tumors.

MATERIALS AND METHODS

This protocol was approved by the University Of Wisconsin Comprehensive Cancer Center Clinical Affairs Committee and the University of Wisconsin and Human Subjects Committee, Madison, Wisconsin. Specialized consent forms outlining the study were used to obtain informed consent by a study nurse prior to patient entry. All patients willfully participated in the trial. Five patients underwent ablation followed by resection. Only patients who were deemed candidates for a curative resection based on preoperative evaluation were included. Probe placement for all ablations was directed using intraoperative ultrasound and was performed in concert by a radiologist and surgeon.

All patients were explored via a right subcostal incision. Intraoperative ultrasound was used to evaluate the liver and to facilitate accurate probe placement. Probe placement was confirmed by a staff radiologist with ultrasound imaging in two views. Probe placement was such that only a portion of the tumor was ablated, leaving viable tumor for pathologic evaluation. Ablations were monitored by intraoperative ultrasound. After ablation, the hepatic resection was performed and the specimen evaluated.

MICROWAVE ABLATION

The microwave power unit used for all experiments described in this manuscript is a proprietary system capable of running up to eight probes simultaneously (Vivant Medical, Mountain View, CA). The microwave generator produced energy at a frequency of 2.45 GHz. The power could be continuously varied. Microwaves were transmitted along a coaxial cable to the dual-loop probe apparatus.

The dual-loop probe apparatus consists of two 13-gauge needles through which 24-gauge loop antennas (2.7 cm in diameter) are deployed. The loops are canted at 45-degree angles (Fig. 1). This orientation resulted in the most consistent lesions based on prior studies.[7] To assist in the deployment of the loop antennas, a conventional surgical electrocautery device (model E-8006; Valley Laboratories, Boulder, CO) was attached to the microwave probe. During deployment of the individual loops, 60–70 watts of continuous power was applied to assist the loop in "cutting" through hepatic tissue. As a result, loops could be placed using only minimal forward pressure, without distortion in the shape of the loop. For this study, the lesions were created by placing the probes in both the tumor and the normal liver, with ultrasound guidance. The loops were each deployed and lesions were ablated with 60 watts applied power for 5–7 minutes (Vivant Medical, Mountain View, CA).

After ablation, the lesions were resected and the specimens evaluated by a single pathologist. Tissue was frozen in liquid isopentane, and seven micron sections were cut in at -20° C. Sections were dried on Fischer Superfrost (Fisher Scientific, Pittsburgh, PA) coated slides for 90 minutes and stained by immersion in a solution of nitro blue tetrazolium (18.72 mg (NBT); Sigma-Aldrich, St. Louis, MO) and reduced dihydropyridine nucleotide (NADH: 15 mg; Sigma-Aldrich) in 18.72 ml Tris buffer, pH 7.4 for 30 minutes at 37° C. Sections were passed through a series of 30%, 60%, 90%, 60%, and 30% acetone and tap water before mounting Immunomount (Shandon, Pittsburgh, PA).

Staining of macroscopic sections, 4 mm in thickness, was performed on fresh tissue slices in a solution of 10 mgms of NBT (Sigma-Aldrich) in 100 ml of Sorensen's phosphate buffer, pH 7.4, at room temperature, until the nonablated tissue stained blue (10–20 minutes). NBT is an oxidant and is widely

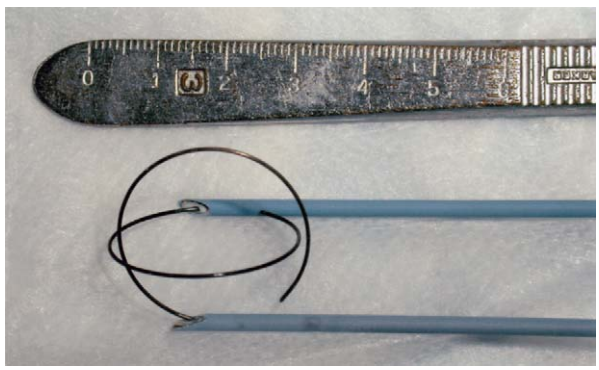

Fig. 1. Example of dual-loop microwave probe.

used to detect phosphatase activity; it is also a substrate for dehydrogenase and other oxidases. Thus, it is often used to assess cell viability, proliferation, and cytotoxicity. The colorless NBT is soluble in water, but forms a dark-blue, highly insoluble precipitate after being enzymatically reduced. Specimens were examined macroscopically, and microscopically on high power, after NBT staining. Cells in this study were designated "viable" if they stained positive (blue) for NBT.

The tumor and ablation mean maximum and mean minimum diameters were then measured. Each lesion was inspected for complete filling of ablation zone, vessel involvement in ablation zone, and extent of tumor ablation. Vessels were measured and considered small if less then 3 mm, moderate if between 3 and 5 mm, and large if greater than 5 mm in the zone of ablation or tumor ablation interface.

The volume of the zone of ablation and overall tumor volume was calculated by $4/3\ \pi\ R_1 \times R_2 \times T$, where R_1 is ½ the mean maximum diameter, R_2 is ½ the mean minimum diameter, and T is the total thickness of the ablated lesion or tumor.

RESULTS

Five patients underwent six microwave ablations and five hepatic resections. Three patients underwent left hepatic lobectomy for hepatocellular carcinoma or metastatic colorectal carcinoma, whereas two patients underwent right trisegmentectomy and right hepatic lobectomy for metastatic colorectal carcinoma. Two patients had low anterior resections performed concurrently for primary rectal adenocarcinoma. A single patient had two large tumors in the left lobe and subsequently underwent two separate ablations prior to resection (Table 1). There were no intraoperative or postoperative complications.

Ablation times and input power were monitored throughout the procedure. The first patient had a single superficial lesion ablated for 7 minutes at 60 watts; no change in ablation lesion was discernable after 5 minutes. Subsequent ablations were thus performed for 5 minutes at 60 watts applied power. Whereas the goal was to partially ablate all tumors, one tumor was completely ablated at 5 minutes.

Tumor volumes varied from 33 to 214.4 cm^3, (average 75.4 ± 69.8 cm^3). Ablation volumes varied 17.6 to 40.1 cm^3 (average 28.2 ± 8.4 cm^3; Table 2). There was no difference in the volume of ablated tissue at 7 minutes compared to those ablated for 5 minutes. The smaller ablation volumes were in tumors close to the liver surface.

All lesions had blood vessels within the zones of ablation (Fig. 2). The lesions were spherical in shape and no distortion resulted from the presence of the vessels (Fig. 3). All lesions filled completely between the loop antennas. There was complete tumor kill at the ablation/tumor interface as demonstrated by NTB staining. In addition, there were no viable cells surrounding the vessel within both the zone of ablation and the ablation/tumor interface (Figs. 4 and 5).

Table 1. Patient/procedure demographics

Patient	Procedure	Pathology	Additional procedures
1	Left hepatic lobectomy	Hepatocellular carcinoma	None
2*	Left hepatic lobectomy	Adenocarcinoma (rectum)	Low anterior resection multiple ablations
3	Right trisegmentectomy	Adenocarcinoma (colon)	None
4	Right hepatic lobectomy	Adenocarcinoma (colon)	None
5	Left hepatic lobectomy	Adenocarcinoma (rectum)	Low anterior resection

*Patient with two solitary tumors in left hepatic lobe underwent two separate ablations.

DISCUSSION

Historically, the only curative treatment option available for patients with tumors localized to the liver has been hepatic resection. Unfortunately, only 10%–20% of patients can undergo a curative resection.[8] A variety of factors have precluded surgical therapy, including poor hepatic reserve, multiple bilobar tumors, and unfavorable tumor locations. Local ablative therapies (cryoablation, microwave

Table 2. Ablation demographics

Ablation	Tumor volume cm^3	Time (min)	Ablation volume cm^3	Vessels present
1	214.4	7	30.7	Small
2a	68.0	5	17.6	Moderate
2b	38.8	5	40.1	Small
3	64.4	5	22.5	Small
4	33.0	5	34.6	Large
5	34.0	5	23.5	Moderate

Average ablation volume = 28.2 ± 8.4 $cm.^3$
Average ablation volume for 5 min ablations = 27.7 ± 9.3 $cm.^3$

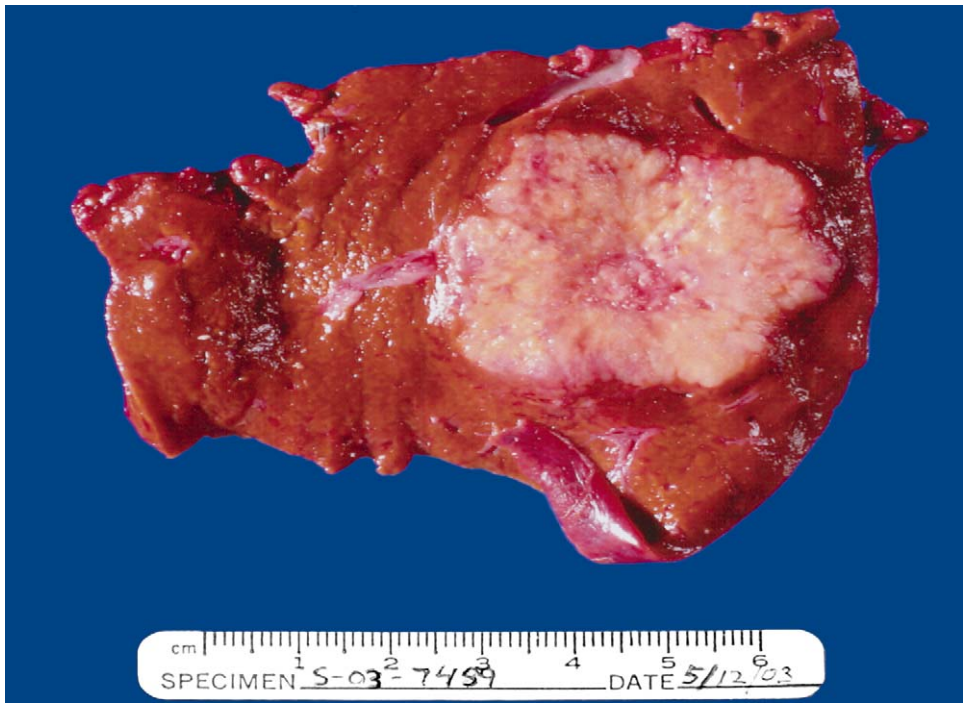

Fig. 2. Tumor demonstrating extent of vascular involvement.

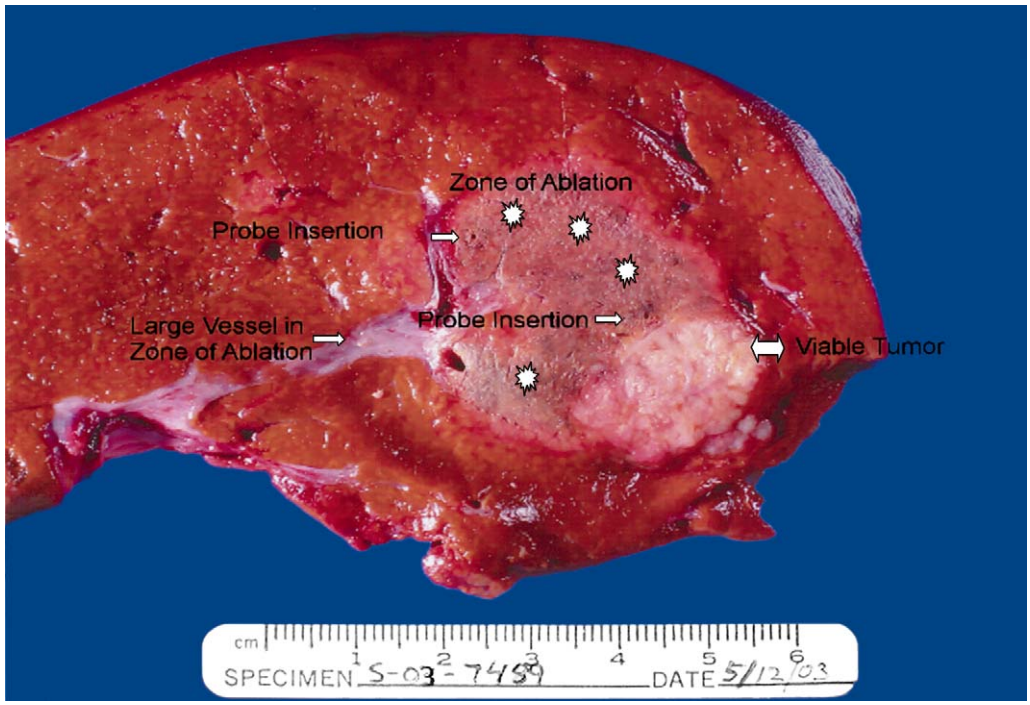

Fig. 3. Section of liver shows ablated focus of liver surrounded by a light 2 mm thick zone. The focus of ablation also includes a portion of adenocarcinoma.

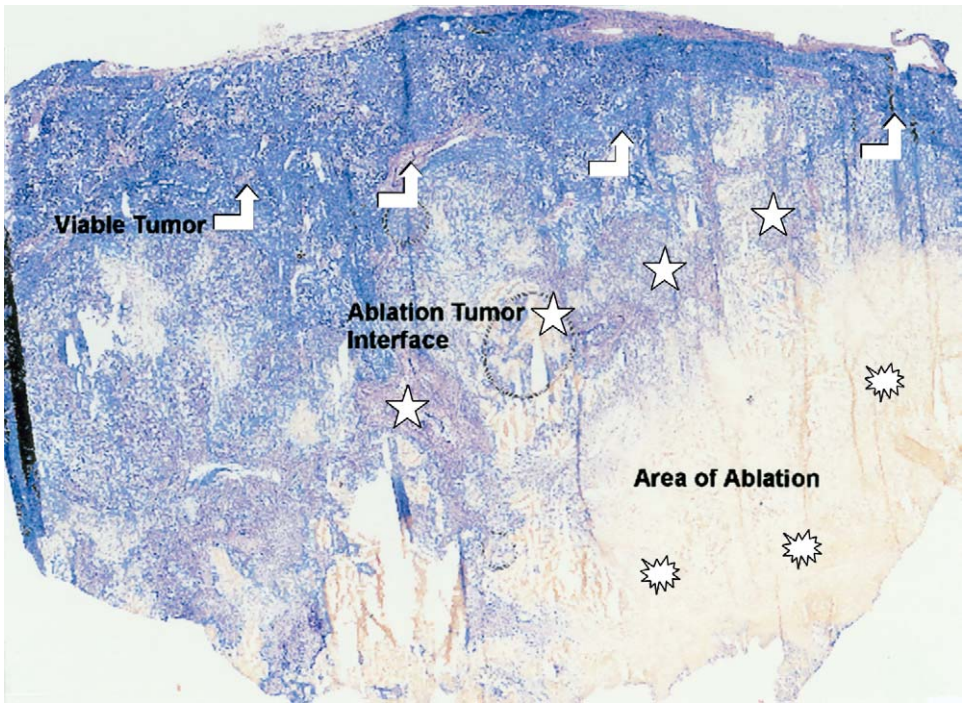

Fig. 4. Section of liver with adenocarcinoma showing NBT staining of normal liver, light staining of viable tumor, and nonstained area of ablated tumor, which also includes necrotic foci NBT (original magnification ×5).

ablation, and radiofrequency ablation) have been proposed as alternatives to resection in patients who are not candidates for a curative resection. Proper patient selection, precise diagnostic and procedural imaging, and thorough post ablation follow-up has led to long-term success, with 3 and 5 year survival rates approaching that of resection.[8]

Radiofrequency ablation remains the most common ablation technique currently employed. However, it is marked by sometimes-high local recurrence rates, the requirement for multiple overlapping ablations in larger tumors, and poor ablation response around blood vessels. The zone of active-tissue heating in RF is limited to a few millimeters

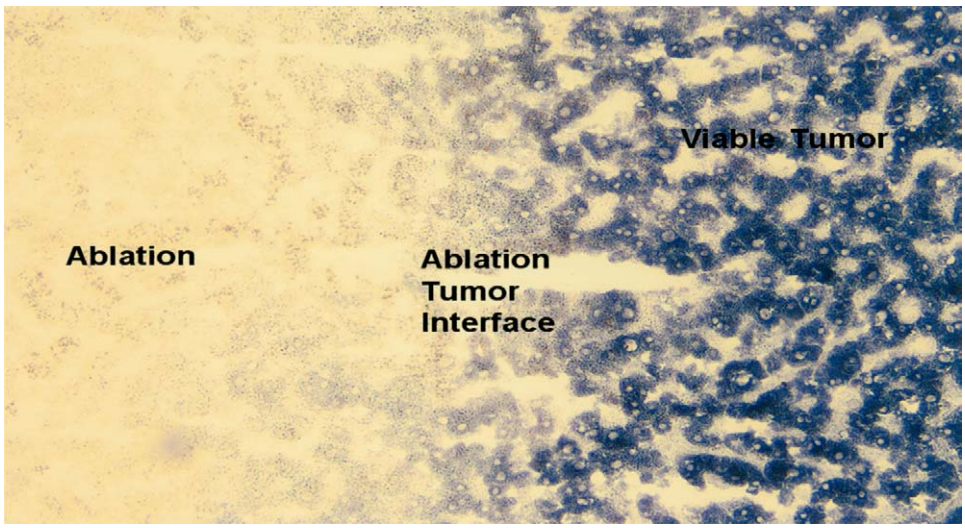

Fig. 5. Section of liver with adenocarcinoma showing dense staining of normal liver and light staining of viable tumor, NBT (original magnification ×100).

surrounding the probe.[9] Heat then radiates into surrounding tissue by thermal conduction. RF energy cannot consistently heat tissues above 100° C during ablation.[10] Temperature decreases rapidly with increasing distances from the RF probe ($1/r^4$).[9] In addition, heat is lost through low-resistance shunting through electrical paths such as blood vessels. Thus, lesions close to vessels offer the poorest response as a result of conduction and dissipation of heat by blood flow.[4] Several strategies have been developed to overcome this limitation, including multiple prong electrodes,[11] cooled-tip electrodes,[12,13] or combining RF and saline infusion.[14]

Microwave ablation has a zone of active heating up to 2.0 cm surrounding the antenna.[15] This theoretically allows for a more uniform tumor kill, both within a targeted area and next to the vessels. Unlike RF, microwave energy does not appear to be limited by charring and tissue desiccation, thus lesion temperature may be driven considerably higher than with RF systems.[5]

Microwave ablation with straight probes has been limited by the inability to treat large tumors without numerous sequential overlapping ablations. The utilization of multiple probes can be done simultaneously, unlike RF ablation, but the local recurrence rate for larger lesions will likely be higher, just as it is with RF.[16]

The difference between the loop antennas used in this study and other thermal ablation probes (multiple-prong RF electrodes) lies in the ability to completely encircle a tumor with the antennas, effectively creating a "cage" within which the tumor is trapped. This has the advantage of precisely targeting a specific area for thermal destruction while minimizing collateral damage. Once encircled, the tumor is heated from the outside in, rapidly truncating the blood supply to the tumor and minimizing the "heat sink" effect of tumor blood flow for the majority of the ablation time. This is evident in that all lesions with blood vessels present (irrespective of size) in the zone of ablation resulted in uniform ablations and complete tumor kill next to the vessels. In this study, all normal hepatic tissue and tumor contained within the loops were completely destroyed. Theoretically, loops can completely surround lesions and enough normal hepatic tissue to obtain adequate margins, while minimizing unnecessary ablation to surrounding structures or additional tissue. For RF devices, the precise destruction of targeted tissue without collateral damage remains tenuous, even with the advent of more powerful devices, particularly when combined with infusion of saline or other conductors.[17,18] The issue of precise control of the area of ablation is particularly important because of the lack of a highly accurate method to monitor the ablation while it is in progress.[3]

In the porcine model, high temperatures were recorded at a distance of 1.5 cm from any portion of the loop and may have been even higher closer to the loop.[7] These temperatures are much higher than those produced by RF ablation when measured 1.5 cm from the electrode.[10] Because of the rapid development of high temperatures produced by the loop antennas, it is likely that the time required to cause tumor necrosis is even less than the time used in this study. This should result in a decrease in overall treatment time (a major disadvantage in the application of RF ablation). In the porcine model, ablations were performed for 7 minutes. This resulted in an average ablation volume of 29.5 ± 8.1 cm^3. In comparison to the ablations performed for 5 minutes in this study (7 minute ablation excluded), the average volume of ablation was 27.7 ± 9.3 cm^3, which was not statistically significant ($P > 0.5$) when compared to the 7 minute ablations in the porcine model.

There are several disadvantages to the dual-loop microwave probes. The system employs the use of electrocautery for deployment of the probes. If not accurately placed, this could result in damage to adjacent tissue, vessels, or surrounding structures, leaving the lesion incompletely ablated. No complications in this study resulted from the use of the electrocautery device. Physicians using this system must be very confident that the loop entirely covers the targeted tumor, as the amount of ablation outside the loop is minimal (limited to approximately 0.4–0.8 cm outside the loop).[7] The precise placement of ablation probes, regardless of the technology (RF, cryoablation, or microwave ablation), requires unique skills and a clear understanding of differences in these devices.

CONCLUSIONS

Microwave ablation with a dual-loop probe system is a safe alternative for patients with unresectable hepatic tumors. Ablation resulted in complete tumor kill at the ablation/tumor interface as well as adjacent to surrounding blood vessels. No distortion of the lesions was created by proximity to blood vessels.

We demonstrate the ability to completely encircle a tumor and deliver large amounts of precisely targeted microwave energy to the tumor with minimal damage to tissue outside the loop. The future use of different diameter loops may eliminate the need for multiple ablations for larger tumors or excessive

ablation of normal liver tissue for smaller hepatic tumors.

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Hepatic Resection for Noncolorectal, Nonneuroendocrine Metastases

Fernando Cordera, M.D., David J. Rea, M.D., Manuel Rodriguez-Davalos, M.D., Tanya L. Hoskin, M.S., David M. Nagorney, M.D., Florencia G. Que, M.D.

Resection of certain hepatic metastases of noncolorectal, nonneuroendocrine (NCNNE) origin provides actual long-term (>5 years) survival. We conducted a retrospective outcome study at a single tertiary referral institution. Between January 1988 and October 1998, 64 consecutive patients underwent resection of hepatic metastases from NCNNE primary tumors. Overall and disease-free survival rates were correlated to clinicopathologic factors and operative morbidity and mortality. Thirteen patients underwent a right hepatectomy, 6 underwent a left hepatectomy, 3 had extended right and 2 extended left hepatectomy, 2 patients had segmentectomy, 24 underwent wedge resections, and 14 underwent a combination of these forms of resection. R0 resection was achieved in 56 patients (87.5%). The operative mortality was 1.5% (1 of 64). Actual 1-, 3-, and 5-year survivals were 81%, 43%, and 30%, respectively. The factor adversely associated with overall and disease-free survival was uniformly related to the interval between primary tumor resection and the development of hepatic metastases. A 1.5% operative mortality and an actual 5-year survival of 30% justifies hepatic resection, including major hepatic resection, for certain NCNNE metastases. The factor affecting prognosis in this highly select group of patients was the biological behavior of the tumor, with tumors that metastasize earlier having poorer survival rates. (J GASTROINTEST SURG 2005;9:1361–1370)

KEY WORDS: Liver resection, noncolorectal, nonneuroendocrine, hepatic metastases

Liver resection for metastatic cancer has become the standard of care for certain selected groups of patients. Refinements in the surgical technique have significantly improved the safety of liver resection, such that even the most extensive hepatic resections can now be performed with an operative mortality of less than 5%. The results of resection for metastatic colorectal cancer (the most common neoplasm to metastasize to the liver) have improved to the point that high volume centers have 5-year survival rates of 40–58%.[1,2] Although significant advances in chemotherapeutic agents have been made, hepatic resection remains the only curative option for colorectal cancer metastases. For symptomatic metastatic neuroendocrine tumors (carcinoid, islet tumors), hepatic resection offers long-term palliation due to the indolent nature of the disease.[3]

The role of hepatic resection for noncolorectal, nonneuroendocrine (NCNNE) metastases, however, is less well defined.[4] Many studies of hepatic resection for NCNNE metastases to the liver report small numbers of patients. In addition, several of these reports often include neuroendocrine tumors in the analysis, favorably biasing survival data. The present study examines a large, single-institution experience of hepatic resection of NCNNE metastases with a focus on long-term outcomes.

MATERIAL AND METHODS

This study was performed with approval by the Mayo Foundation Institutional Review Board. We reviewed the records of consecutive patients with NCNNE hepatic metastases who underwent hepatic resection at the Mayo Clinic, Rochester, from January 1988 to October 1998. Patients undergoing hepatic resection for direct tumor extension from the primary cancer were excluded. We limited our study through 1998 to obtain a minimum potential follow-up interval of 5 years. Data were abstracted from

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institutional medical, surgical, and pathologic records and from available extrainstitutional records. Some patients were unreachable for personal follow-up and their deaths were ascertained by search of the Accurint Database (Seisint, Inc., Boca Raton, FL). Records were reviewed and the following factors were analyzed for prognosis: tumor location, histologic type, synchronous versus metachronous presentation, unilobar versus bilobar involvement, disease free interval, and intraoperative factors including blood loss, transfusion requirements, and concomitant procedures. Because of the great variety of tumors encountered, patients were grouped in four categories according to the site of the primary tumor: gastrointestinal, genitourinary, soft tissue, and "other." Outcome measures included operative mortality, overall survival, and disease-free survival. The assessment of extent of the metastatic disease was estimated preoperatively with imaging studies, mainly computed tomography. Tumor grading and staging was according to the sixth edition of American Joint Committee on Cancer staging system. Major hepatic resection was defined as resection of three or more anatomic hepatic segments. Operative mortality was defined as death within 30 days of surgery.

Categorical variables were summarized with frequencies and percentages; continuous variables were summarized with mean, standard deviation, median, and range. Survival and disease-free (i.e., freedom from hepatic tumor) survival rates were estimated using the Kaplan-Meier method with 95% confidence intervals.[5] For the disease-free survival outcome, patients without known recurrence and alive at last follow-up were censored at the last date of adequate follow-up for disease recurrence even if longer follow-up was available for vital status determination.

Possible predictors of outcome were assessed with Cox proportional hazard models. Results were reported with the hazard ratio, 95% confidence interval, and *P* value. These possible predictors were then assessed in multivariable Cox proportional hazards models using the stepwise model selection procedure.[6] Bootstrap resampling with 500 iterations was used to validate the resulting multivariable models.[7] Variables were included in final models if they were significant in at least 50% of the 500 bootstrap samples. *P* values ≤ 0.05 were considered statistically significant for all analyses. Analyses were performed using SAS version 8.02 software (SAS Institute Inc., Cary, NC).

RESULTS

Sixty-four consecutive patients underwent resection of NCNNE hepatic metastases. Demographics and clinical findings of our patients are shown in Table 1. There were 21 men and 43 women. The primary tumor locations are as follows: 28 genitourinary (9 ovary, 9 kidney, 4 uterus, 4 endometrium, 2 cervix), 12 gastrointestinal (6 stomach, 3 esophagus, 2 pancreas, 1 small bowel), 15 skin and soft tissue (10 breast, 3 pelvic osteosarcomas, 2 retroperitoneum), and 9 other (3 lung, 1 pharynx, 1 brain, 1 thymus, and 3 unknown primary). Breast tumors were classified under the soft tissue group as seen in other studies[8] (Table 1). The mean age at the time of the primary tumor resection was 52.6 ± 15.0 years (range, 4.5–75.6 years) among 62 patients for whom this information was available; the date of primary tumor resection was unknown for two patients. The mean age at the time of the hepatic resection was 56.3 ± 14.5 years (range, 5.0–77.3 years). Six patients (9%) underwent more that one hepatic resection during the study interval. Five patients underwent a second hepatic resection for recurrent metastatic disease, and one patient underwent a total of three resections. The median time from the primary tumor resection to the first hepatic resection in all patients was 2.0 years (range, 0–13.7). In 69% of patients, the first hepatic resection was done

Table 1. Demographics and tumor types

Age at liver resection (yr) mean ± SD (range)	56.3 ± 14.5 (5.0–77.3)
Gender (M/F)	21:43
Primary tumor location, No. (%)	
Gastrointestinal	12 (18.8)
6 Stomach	
3 Esophagus	
2 Pancreas	
1 Small bowel	
Genitourinary	28 (43.8)
9 Ovary	
9 Kidney	
4 Uterus	
4 Endometrium	
2 Cervix	
Soft tissue	15 (23.4)
10 Breast	
3 Pelvic osteosarcomas	
2 Retroperitoneum	9 (14.1)
Other	
3 Lung	
1 Pharynx	
1 Brain	
1 Thymus	
3 Unknown primary	
Time from primary surgery to liver resection (yr)*, mean ± SD (range)	3.4 ± 3.5 (0–13.7)

*Date of primary surgery was available for only 62 of the 64 patients.

more than 1 year after the resection of the primary tumor.

In 40 patients (63%), resection of the primary tumor was performed at an outside institution before they were referred to the Mayo Clinic Rochester. The primary tumor resection (at outside institutions and at our institution) was complete (R0) in 88% of patients. We tabulated the original pathologic staging for all primary tumors, but because the diverse nature of these tumors we collapsed the staging data into a general four-stage scheme (stages I, II, III, and IV). For example, a stage IIA squamous cell cervical carcinoma, in our analysis, was grouped under stage II, and a stage IIIC ovarian adenocarcinoma was classified as stage III. At the time of primary tumor resection, 9 (14%) patients had stage I disease, 16 (25%) had stage II, 22 (34%) had stage III, and 17 (27%) had stage IV. Among stage IV patients, 10 (58%) had resection of the primary tumor and the hepatic metastases during the same operation.

Diagnostic imaging was not standardized over the study period because of the duration of the study period, the evolution of imaging modalities, and the sequence of imaging obtained. However, computed tomography (CT) was used in 98% of patients. Other imaging modalities used included ultrasonography in 14 patients (22%), magnetic resonance imaging in 4 (6%), and positron emission tomography (PET) scanning in 1 (2%).

At the time of the hepatic resection 41 (64%) patients had a single lesion, 13 (20%) had two lesions, 3 (5%) had three lesions, and 7 (11%) had four or more. The lesions involved one segment in 24 (38%) patients, two segments in 14 (22%) patients, three segments in 7 (11%) patients, and four or more segments in 19 (30%) patients. Twelve (18.8%) patients had bilobar disease. Thirteen patients underwent a right hepatectomy, six patients a left hepatectomy, two patients an extended right hepatectomy, and three patients an extended left hepatectomy. Two patients had a segmentectomy and 24 patients underwent wedge resections. Fourteen patients were classified as having some combination of the above procedures. Five patients (7%) had concomitant caudate lobe (segment 1) resection. Additional operative procedures included resection of the primary tumor in 10 (15%) patients, segmental small bowel resection in 3 (5%), and a transverse abdominal rectus muscle flap reconstruction in 2 (3%) (Table 2).

Fifty-six patients (88%) underwent a complete (R0) resection of the tumor. Four patients had complete gross resection with microscopic residual disease (R1): two patients in lymph nodes and two patients at the hepatic resection margin. Four additional patients had gross residual tumor: one in the liver and three in other locations. The hepatic margins of resection were negative in 61 patients (95.3%), microscopically positive in two patients (3.1%), and grossly positive in one patient (1.6%) (Table 3).

Seventeen (26.5%) patients required intraoperative red cell transfusions, with a mean transfusion requirement of 4 ± 5 units. One patient died within 30 days of the hepatic resection, giving an operative mortality of 1.6%. Serious operative morbidity occurred in five (7%) patients: intra-abdominal abscess

Table 2. Characteristics of the liver tumor(s) and hepatic resection

Primary surgery performed at our institution, No. (%)	24 (37.5)
Concomitant primary resection and hepatic resection, No. (%)	10 (15.6)
No. of liver lesions, median (range)	1 (1–50)
No. of segments involved, No. (%)	
1	24 (37.5)
2	14 (21.9)
3	7 (10.9)
⩾4	19 (29.7)
Bilobar involvement, No. (%)	12 (18.8)
Extrahepatic disease at hepatectomy, No. (%)*	
None	42 (65.6)
Lymph node(s)	9 (14.1)
Peritoneum	6 (9.4)
Visceral metastases	9 (14.1)
Visceral invasion	10 (15.6)
Hepatic resection, No. (%)	
Right hepatectomy	13 (20.3)
Left hepatectomy	6 (9.3)
Extended right hepatectomy	2 (3.1)
Extended left hepatectomy	3 (4.6)
Subsegmental/wedge	24 (37.5)
Segmentectomy	2 (3.1)
Combination	14 (21.7)

*Seven patients had multiple sites of extrahepatic disease.

Table 3. Outcomes of hepatic resection

Completeness of hepatic resection, No. (%)	
No disease (R0)	56 (87.5)
Microscopic disease (R1)	4 (6.3)
Gross disease (R2)	4 (6.3)
Operative mortality, No. (%)	1 (1.6)
Death during follow-up, No.	50
Time to death (yr), median (range)	1.9 (0.05–6.9)
Recurrence during follow-up, No.	44
Time to recurrence (yr), median (range)	1.1 (0–4.8)

(one), pneumothorax (one), deep vein thrombosis (one), and major hemorrhage (two patients, one of whom required reoperation).

Fifty-two (81%) patients received some form of chemotherapy (4 before excision of the primary tumor, 38 before the liver resection, and 45 after the resection) Most of the patients (75%) who received chemotherapy received it before and after the hepatic resection. Some form of radiation therapy was used in 35 (54.6%) patients (11 before excision of the primary tumor, 22 before the hepatic resection, and 11 after the operation). In addition, intraoperative radiation was used in eight patients at the time of the hepatic resection.

The overall median survival after the hepatic resection was 2.4 years and the actual 5-year survival was 30.2%, with 19 of 64 patients alive and still being followed. At last follow-up, 11 (17%) patients were alive with no evidence of disease, 3 (5%) patients were alive with disease, 34 (53%) patients were dead with disease, and 1 (2%) was dead with no evidence of disease. Fifteen additional deaths were noted without accurate follow-up related to disease recurrence from our use of the Accurint database. Therefore we did not perform statistical analysis of recurrence as a separate end point but used disease-free survival (death or recurrence) instead.

Tumor recurrence was documented in 37 of 56 patients who had an R0 resection. All 37 recurrences occurred within 5 years. The median time to recurrence among these 37 patients was 1.1 years (range, 0.2–4.8 years). The primary sites of recurrence were the liver in 13 (35%) of 37 patients, the peritoneum in 13 (35%), and lungs in 7 (18%). Eighteen (48%) patients had multiple sites of recurrence. Most recurrences in patients with incomplete resections (R1/R2) were in the liver and the peritoneum. Six patients (two soft tissue, two genitourinary, one gastrointestinal, one other) had repeated hepatic resections for liver-only recurrence including one patient who had a total of three hepatic resections for metastatic retroperitoneal sarcoma (survival 2.7 years from first hepatic resection).

The overall 1- and 5-year survival rates were 81.1% and 30.2%, respectively. The overall disease-free rates at 1 and 5 years were 64.7% and 15.8%, respectively. For the 56 patients who underwent a curative (R0) resection, the 1- and 5-year survival rates were 83.8% and 32.8%, respectively. The 1- and 5-year disease-free survival rates for this group were 68.5% and 16.2%, respectively. Of note, 10 patients were alive and still being followed 10 years after the hepatic resection, and 3 have been followed for over 14 years. For the patients with incomplete (R1 and R2) resections, the 1- and 5-year survival rates were 62.5% and 12.5%, respectively, and the disease-free survival for this group was 37.5% at 1 year and 12.5% at 5 years. This trend toward better prognosis after R0 resections can be observed in Figure 1; however, this difference was not statistically significant for survival ($P = 0.21$) or disease-free survival ($P = 0.22$).

The univariate analysis of variables associated with improved overall and disease-free survival is shown in Tables 4 and 5. The only variables that showed a statistically significant difference in overall survival were the histologic stage at the time of primary tumor resection ($P = 0.001$), time interval between resection of the primary tumor and the hepatic metastases ($P = 0.009$), and concurrent primary tumor and hepatic resection ($P = 0.003$) (Fig. 2). There was a trend toward decreased survival for gastrointestinal primary tumors with a hazard ratio of 1.7 compared with all other primary sites ($P = 0.12$) (Fig. 3). The variables associated with a worse outcome for the disease-free survival end point included the following: histologic stage at the time of primary tumor resection ($P = 0.002$), the time interval between resection of the primary tumor and the hepatic metastases ($P = 0.02$), concurrent primary tumor and hepatic resection ($P = 0.002$), and gastrointestinal primary tumors ($P = 0.02$). For both end points, males had a trend toward poorer prognosis with hazard ratios of 1.7 ($P = 0.08$) and 1.6 ($P = 0.10$) for overall survival and disease-free survival, respectively. In the univariate analysis, incomplete resection (R1 or R2) was not associated with a significantly poorer overall survival or disease-free survival ($P = 0.21$ and 0.22, respectively), although power was limited for these analyses because only 8 of 64 patients had incomplete resection.

The stepwise selection model for overall survival included only histologic stage at the time of primary tumor resection ($P = 0.001$) as shown in Table 6. For the composite end point of death or recurrence (disease-free survival), the variables that were statistically significant in the multivariable model were histologic stage at the time of primary tumor resection ($P = 0.001$) and a gastrointestinal primary tumor ($P = 0.01$). Using a bootstrap resampling model, only the histologic stage at the time of primary tumor resection was validated as a prognostic factor for worse overall and disease-free survival.

DISCUSSION

Hepatic resection continues to be the mainstay of therapy for most metastatic disease to the liver. For metastatic neuroendocrine tumors, hepatic resection is safe, achieves symptom control in most patients, and prolongs survival. A recent study showed that

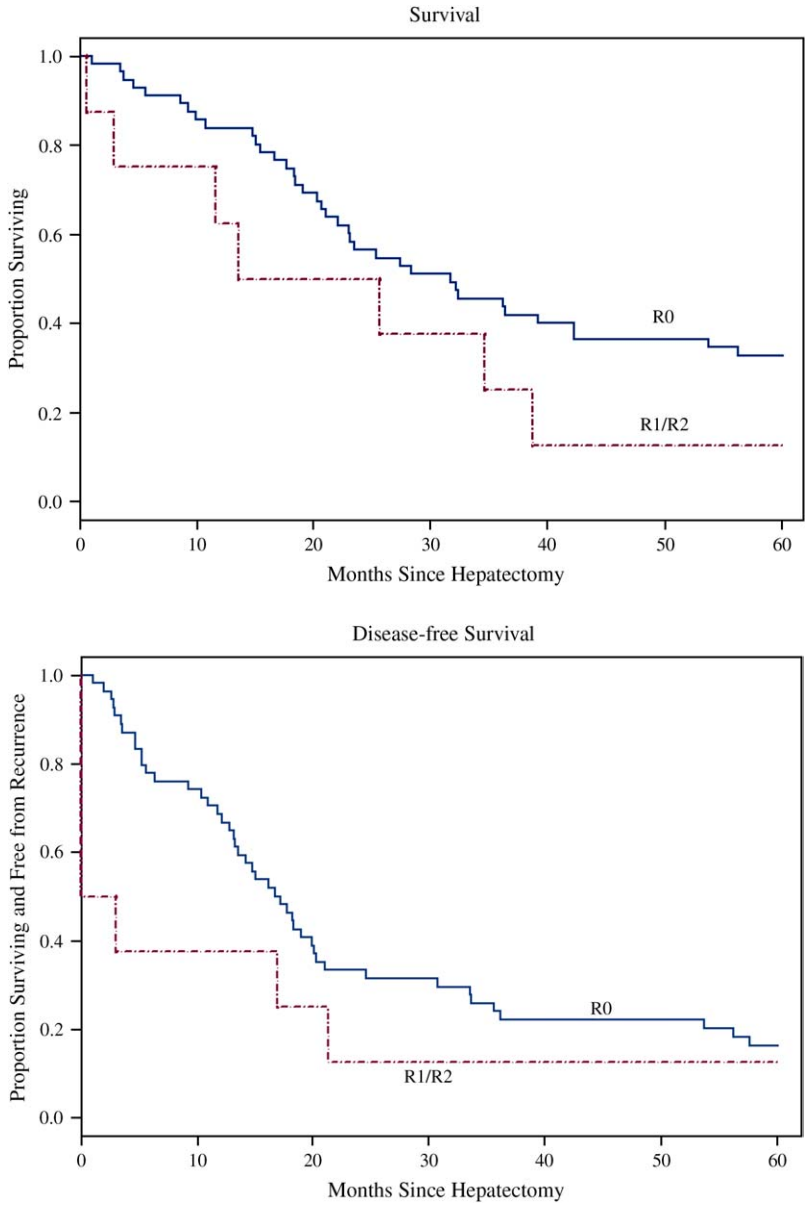

Fig. 1. Survival and disease-free survival by type of resection.

hepatic resection for metastatic neuroendocrine tumors controlled symptoms in 104 of 108 patients and provided an overall survival of 61% at 5 years.[3] For metastatic colorectal cancer, hepatic resection provides the only chance for cure and has been reported to have a 5-year survival ranging from 20% to 54%.[9] The resection of NCNNE hepatic metastases, however, remains controversial.

Recently, there have been several reports that suggest that some patients with NCNNE hepatic metastases may benefit from surgical resection. Table 7 summarizes the largest series that have been reported. The largest series to date of hepatic resection for NCNNE metastases is that published by Weitz et al.,[10] summarizing 141 patients operated on at Memorial Sloan-Kettering Cancer Center over a 21-year period. They report an actuarial 3-year cancer-specific survival rate of 57% similar to that observed in this study. The best results were obtained in patients who had resection of genitourinary tumors. This group also found that the interval between primary tumor resection and hepatic resection was a significant predictor of survival. Patients with a disease-free interval less than 24 months (median) had an actuarial 3-year survival rate of 36%, compared with 72% when the disease-free interval was

Table 4. Univariate predictors of overall survival

Variable (units)	Hazard ratio (95% confidence interval)	*P* value
Age at liver resection (per 10 years)	1.2 (0.97–1.4)	0.09
Male gender	1.7 (0.9–3.0)	0.08
Pathologic stage of primary tumor (per 1 stage)	1.6 (1.2–2.2)	0.001
Non-R0 resection	1.7 (0.7–3.7)	0.21
Lymph node involvement	2.5 (0.6–10.4)	0.22
Bilobar involvement	0.8 (0.4–1.7)	0.59
No. of liver segments involved (per segment)	1.0 (0.8–1.2)	0.72
Major hepatic resection (≥4 segments)	0.9 (0.5–1.7)	0.81
No. of lesions (per lesion)	1.0 (0.97–1.05)	0.54
Time between primary surgery and liver resection (per year)	0.9 (0.8–0.97)	0.009
Primary surgery and hepatic resection performed concurrently	3.0 (1.5–6.3)	0.003
Hepatic resection >1 year after primary surgery	0.7 (0.4–1.3)	0.22
Primary surgery done at Mayo Clinic	1.3 (0.7–2.3)	0.40
Gastrointestinal primary (versus all other sites)	1.7 (0.9–3.3)	0.12

Table 5. Univariate predictors of disease-free survival

Variable (units)	Hazard ratio (95% confidence interval)	*P* value
Age at liver resection (per 10 years)	1.0 (0.9–1.2)	0.81
Male gender	1.6 (0.9–2.8)	0.10
Pathologic stage of primary tumor (per 1 stage)	1.5 (1.2–2.0)	0.002
Non-R0 resection	1.6 (0.7–3.6)	0.22
Lymph node involvement	1.7 (0.4–6.9)	0.48
Bilobar involvement	1.0 (0.5–1.9)	0.89
No. of liver segments involved (per segment)	1.0 (0.8–1.2)	0.99
Major hepatic resection (≥4 segments)	1.1 (0.6–1.9)	0.78
No. of lesions (per lesion)	1.0 (0.97–1.04)	0.93
Time between primary surgery and liver resection (per year)	0.9 (0.8–1.0)	0.02
Primary surgery and hepatic resection performed concurrently	3.0 (1.5–6.1)	0.002
Hepatic resection >1 year after primary surgery	0.7 (0.4–1.3)	0.23
Primary surgery done at Mayo Clinic	1.4 (0.8–2.3)	0.27
Gastrointestinal primary (versus all other sites)	2.2 (1.1–4.4)	0.02

greater that 24 months. These findings are consistent with the findings of the present study, indicating that the biology of the primary tumor plays an important role in overall survival.

Patients with sarcomas metastatic to the liver can benefit hepatic resection provided that an R0 resection can be achieved. DeMatteo and colleagues[11] reported their experience with 56 hepatectomies for sarcomas involving the liver, the majority of which were either gastrointestinal stromal tumors or gastrointestinal leiomyosarcomas (61%). The resectability rate was 15% and the median survival for all patients was 39 months with a 5-year survival of 30%. Half of the patients in this study underwent at least a formal hepatic lobectomy, and this variable approached statistical significance in a multivariate analysis for improved survival. The variable most strongly associated with improved survival in their multivariate analysis was an interval from primary tumor resection to hepatectomy of greater than 2 years. This again emphasizes that the biology of the primary tumor is one of the most significant predictors of patient outcome.

Other studies have examined resection of gastric cancer metastatic to the liver, which is perhaps more controversial because of the aggressive nature of these tumors. Sakamoto et al.[12] reported on 22 patients who underwent hepatic resection of synchronous and metachronous liver metastases from gastric cancer, which occurred in 5% of their patient population. The overall resectability rate was only 9.6% but patients in that series had a 38% 5-year survival rate. The multivariate predictors of improved survival were a solitary metastatic lesion and tumor size less than 5 cm. From these data and others, a thorough evaluation for extrahepatic disease must be undertaken in order to provide these patients with better results from hepatic resection.

The decision to proceed with hepatic resection for NCNNE metastases must come after thorough evaluation of the patient. In order for a patient to be a candidate for this form of treatment, certain criteria must be met: (1) the primary tumor has been or can be completely excised, (2) the metastatic disease is confined to the liver, (3) the hepatic metastases can be completely excised, and (4) the patient can tolerate the procedures required to achieve complete excision of the tumors. Few studies comment on the modalities used to exclude patients from resection. Many of these studies have taken place in a retrospective fashion over a 10- to 20-year period. In such

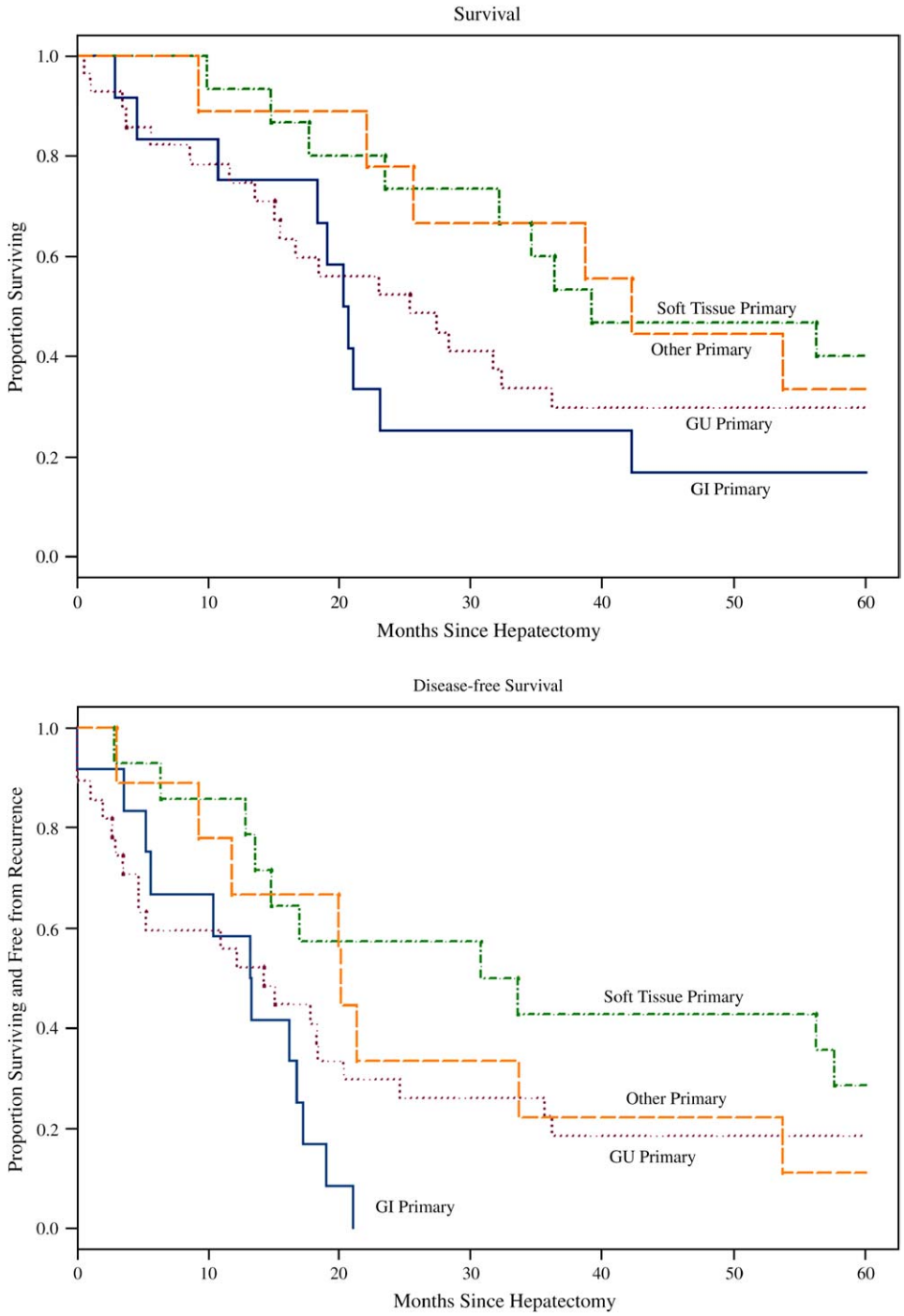

Fig. 2. Survival and disease-free survival by tumor type. GU = genitourinary; GI = gastrointestinal.

a long time frame, the resolution of many imaging modalities has improved considerably (e.g., CT) and new imaging modalities have arisen (e.g., PET). While the focus of this study was not the economics of evaluating these rare patients, an argument can be made that avoiding operation in those patients with occult extrahepatic disease offsets the cost of adding extra, complementary imaging tests. Recent work using PET scanning to detect occult extrahepatic metastases in candidates for liver resection for metastatic colorectal cancer has shown that approximately 25% of patients have changes in management with the use of this test.[13,14]

In the treatment of metastatic disease to the liver, radiofrequency ablation (RFA) is an emerging technology that has shown promising results. In this

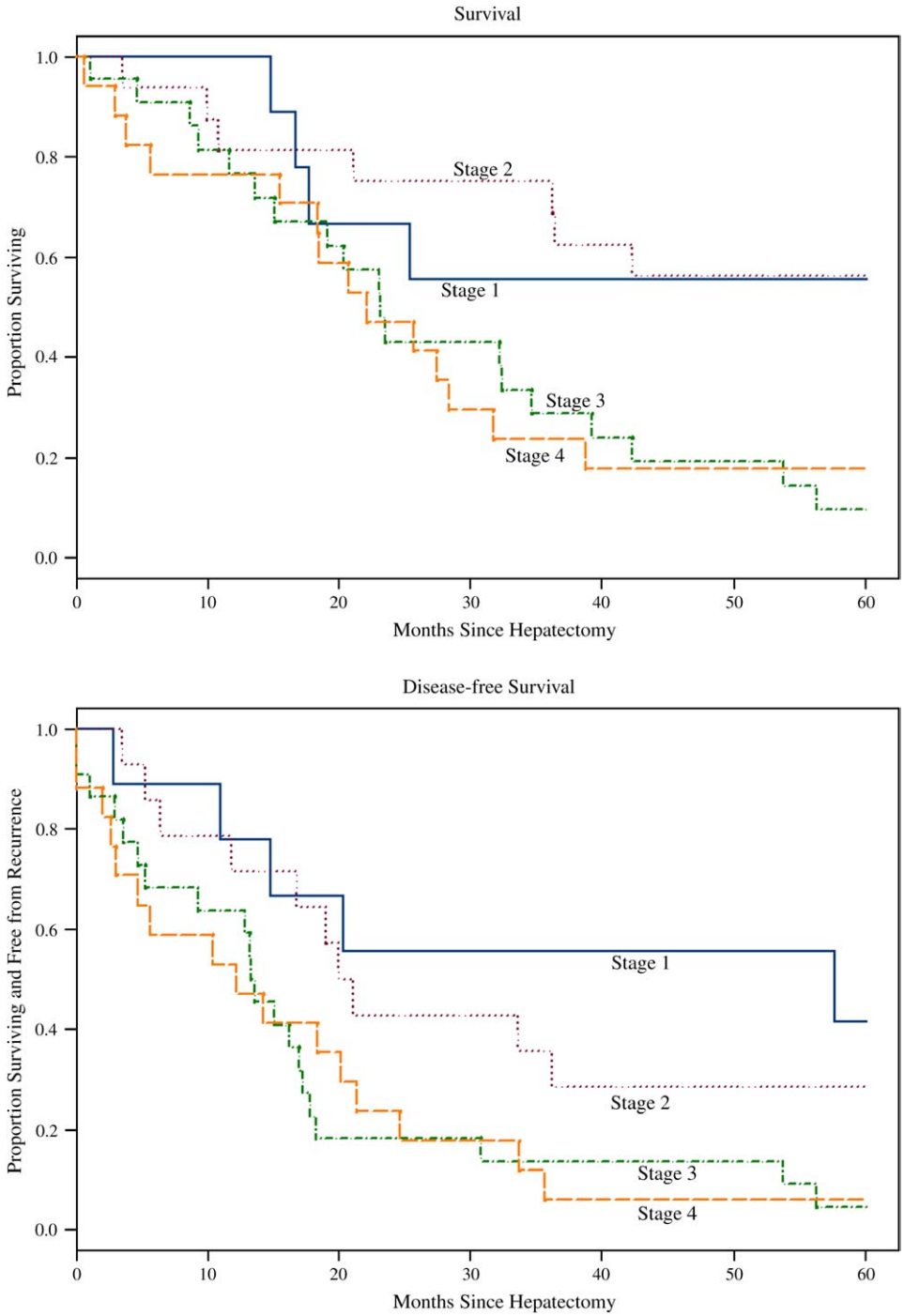

Fig. 3. Survival and disease-free survival by stage at presentation.

particular study, because of the time period when the resections were done, RFA was not used. Some authors suggest that RFA increases the resectability of certain tumors and can decrease the morbidity associated with the resection of metastatic tumors.[15] Certainly, RFA has shown to prolong survival in patients with metastatic colorectal carcinoma to the liver and can be a useful tool in the management of these patients.[16] However, we believe that RFA should not be advocated as a replacement for resection, which remains the gold standard for the treatment of liver tumors. A recent study from M. D. Anderson by Abdalla et al.[17] reviewed 428 patients with colorectal liver metastases. In that study, the 4-year survivals after resection alone, resection + RFA, and RFA alone were 65%, 36%, and 22%,

Table 6. Multivariate model for predictors of overall and disease-free survival

Variable (units)	Hazard ratio (95% confidence interval)	*P* value
Overall survival		
Pathologic stage of primary tumor (per 1 stage)*	1.6 (1.2–2.2)	0.001
Disease-free survival		
Pathologic stage of primary tumor (per 1 stage)†	1.6 (1.2–2.1)	0.001
Gastrointestinal primary (versus all others)	2.4 (1.2–4.8)	0.01

*Validated in >50% of models using the bootstrap resampling method.
†Validated in >60% of models using the bootstrap resampling method.

suggesting that the treatment of choice for metastatic colorectal cancer to the liver is resection. In that particular study, RFA alone provided survival only slightly superior to nonsurgical treatment. The extension of these results to patients with noncolorectal metastases would also favor resection in appropriate candidates. RFA is a useful tool for the management patients with metastatic disease to the liver, but surgical resection remains the treatment of choice in the appropriate candidates. Tumors deemed unresectable because of their number, location, and size relative to the liver volume should be considered for RFA.

Although over the last decade significant advances have been made with chemotherapy agents to treat a wide variety on cancers, surgery remains the cornerstone of effective management of most tumors. Progress in anesthetic and surgical techniques has significantly reduced the risk of many radical surgical procedures to levels that rival the current risk of systemic chemotherapy, and improved imaging techniques now allow selection of patients best suited for cytoreduction. In addition, the development of cryoablation and radiofrequency and laser ablation has provided additional surgical tools for safe cytoreduction. These factors have prompted a renewed consideration of surgical cytoreduction. Recently, repeated investigations on certain tumors such as ovarian, renal, and endometrial cancer have shown that surgical reduction of the tumor volume is highly correlated with prolongation of patient survival.[18–20]

If these factors are taken in consideration, it is possible that with the development of new and more effective chemotherapy agents, hepatic resection may become an important form of cytoreductive therapy for these and other types of cancer.

The only variables that consistently predicted worse prognosis after hepatic resection for these metastatic lesions were those that related to the time

Table 7. Surgical resection for non-colorectal, non-neuroendocrine hepatic metastasisi literature review

Study	Year	No. of patients	Median survival (mo)	5-year survival	Comments
Weitz et al.[10]	2005	141	17	30% (actuarial at 3 yr)	GU tumors and R0 resection favorable prognostic factors for survival. Includes patients from the series of Harrison et al. listed below.
Karavias et al.[21]	2002	18	NA	NA	14/18 (77%) alive at median follow up of 3.2 years. Stage IV presentation showed poor prognosis.
van Ruth et al.[22]	2001	28	21	35%	Stage at presentation, tumor type, and completeness of resection were not statistically significant for prognosis.
Hamy et al.[23]	2000	27	22	23%	Reproductive tumors gave longest median survival.
Lang et al.[24]	1999	127	20	16%	R0 resections associated with better survival.
Elias et al.[25]	1998	147	30	36%	Series included neuroendocrine tumor possibly favoring survival.
Harrison et al.[8]	1997	96	32	37%	GU tumors, disease-free interval, and R0 resection associated with better survival.

NA = not available; GU = genitourinary.

interval between diagnosis of the primary and resection of the liver metastases. The patients who presented with stage IV disease (liver metastases concurrent with the primary tumor) or with a short time interval between primary tumor resection and hepatic resection had worse overall and disease-free survival. This association probably reflects the biologic behavior of the tumor, with more "aggressive" tumors having earlier metastases and a worse prognosis. Other studies cited above have found similar associations, and have been ascribed to the ill-defined but important concept of "tumor biology."

CONCLUSIONS

Our results demonstrate a 30.2% 5-year survival after hepatic resection for NCNNE tumors metastatic to the liver in a selected group of patients. The operative mortality of this procedure was less than 2%. The major prognostic factors for overall and disease-free survival were uniformly related to the interval between the primary tumor resection and the resection of the hepatic metastases and the histologic stage of the tumor at presentation. Despite low overall resectability rates, in patients who are medically fit and have metastatic disease amenable to complete resection, these results justify an aggressive approach.

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Indications for Selective Intraoperative Cholangiography

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The indications for selective intraoperative cholangiography (IOC) include a clinical history of jaundice, pancreatitis, elevated bilirubin level, abnormal liver function test results, increased amylase levels, a high lipase level, or dilated common bile duct on preoperative ultrasonography. Although these clinical features are widely accepted as indications for IOC, they have not been tested for their ability to predict choledocholithiasis. Charts were reviewed for a 6-month time period in 2003 at Parkland Memorial Hospital for all patients undergoing cholecystectomy. Univariate analysis and logistic regression were used to determine which factors predicted choledocholithiasis. Of the 572 patients undergoing cholecystectomies during the study period, 189 underwent IOC and common bile duct stones were found in 57. Only preoperative hyperbilirubinemia or ultrasonograph identification of common bile duct dilation reliably predicted choledocholithiasis. There were 13 cases of choledocholithiasis that would not have been identified by preoperative hyperbilirubinemia or an enlarged common bile duct. However, common bile duct stones were clinically significant in only 2 of the 13 cases. One of these was treated with postoperative endoscopic retrograde cholangiopancreatography, and the other was treated with laparoscopic common bile duct exploration. Preoperative identification of a dilated common bile duct or elevated bilirubin levels can be the sole criteria for performing IOC on a selective basis in patients without malignancy. Reliance on a history of remote jaundice, pancreatitis, elevated liver function test values, or pancreatic enzymes results in unnecessary IOCs. (J GASTROINTEST SURG 2005;9:1371–1377)

KEY WORDS: Intraoperative cholangiography, choledocholithiasis, medical decision making

Cholecystectomy is one of the most frequently performed operations in the United States. Because many patients may have concomitant common bile duct stones, cholangiography may be performed as an additional procedure. Controversy exists regarding the need for cholangiography. Routine intraoperative cholangiography (IOC) has been advocated as a means to prevent common duct (CD) injury.[1,2] This notion has not been widely accepted, and recent hospital-based analysis has shown that a routine IOC strategy is used in only 11% of U.S. facilities. In contrast, in 70% of U.S. hospitals, IOC is performed with a selective approach.[3] Selective cholangiography is also performed when a patient presents with signs or symptoms suggestive of choledocholithiasis. These generally include a history of jaundice, biliary pancreatitis, a dilated common bile duct visualized with ultrasonography, or elevations in laboratory values of tests for liver or pancreatic function. With these or similar criteria, choledocholithiasis is identified in 46% to 62% of patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) or CD exploration.[4–10]

The criteria for performing selective cholangiography have been empirically derived from abnormalities associated with choledocholithiasis. A meta-analysis of 22 studies examining the relationship between clinical features associated with CD stones and the presence of choledocholithiasis showed that the presence of cholangitis, preoperative jaundice, and ultrasound evidence of common bile duct stones were predictive of choledocholithiasis. Less strongly correlated were hyperbilirubinemia and clinical jaundice. Elevated levels of alkaline phosphatase, pancreatitis,

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Table 1. Demographic features of the study patient population

Female (%)	85
Age (± SD, y)	38 ± 14
Race	
Hispanic (%)	66
African-American (%)	17
White (%)	16
Other (%)	1
Emergency admission (%)	67
Diagnosis	
Biliary colic (%)	39
Cholecystitis (%)	45
Pancreatitis (%)	16

SD = standard deviation.

cholecystitis, and hyperamylasemia were weakly correlated with the presence of choledocholithiasis.[11] The presence of any of these leads to IOC in clinical practice, resulting in a substantial number of unproductive IOCs.

However, the criteria for identifying choledocholithiasis during surgery were developed with a low diagnostic threshold because of concern for complications that could occur from undiagnosed, retained CD stones. Recently, it was demonstrated that the clinical manifestations of retained stones are less serious than previously thought. In a prospective analysis of routine IOC without subsequent duct exploration, approximately one half of positive IOCs were either false-positive results or the CD stones passed spontaneously. Of those with persistent choledocholithiasis, delay of stone removal for 6 weeks was not associated with complications.[12]

Given that the complications of retained CD stones are less than previously thought and that the indications of IOC result in substantial numbers of unproductive IOCs, we evaluated 10 of the most common indicators for selective cholangiography to refine the criteria for IOC.

METHODS

The medical records of 572 patients who underwent cholecystectomy at Parkland Memorial Hospital during a 6-month period in 2003 were reviewed. Clinical information was abstracted into an Excel spreadsheet (Microsoft, Redmond, WA) and then imported into an SAS (Statistical Analysis Software, Cary, NC) database. Clinical features represented by continuous variables were dichotomized into present or absent status using the following definitions: a history of jaundice (but not currently jaundiced), a diagnosis of biliary pancreatitis, the presence of a dilated CD on preoperative ultrasonography (defined as a CD diameter >0.5 cm), total bilirubin greater than 1.3 mg/dL, alkaline phosphatase greater than 126 mg/dL, aspartate aminotransferase (AST) greater than 40 mg/dL, alanine aminotransferase greater than 40 mg/dL, γ-glutamyltransferase (GGT) greater than 78 mg/dL, amylase greater than 108 mg/dL, or lipase greater than 59 mg/dL.

Although our primary intent was to assess which preoperative clinical features were associated with IOC diagnoses of choledocholithiasis, the consistency of our observations was assessed by comparison with preoperative ERCP findings. Patients

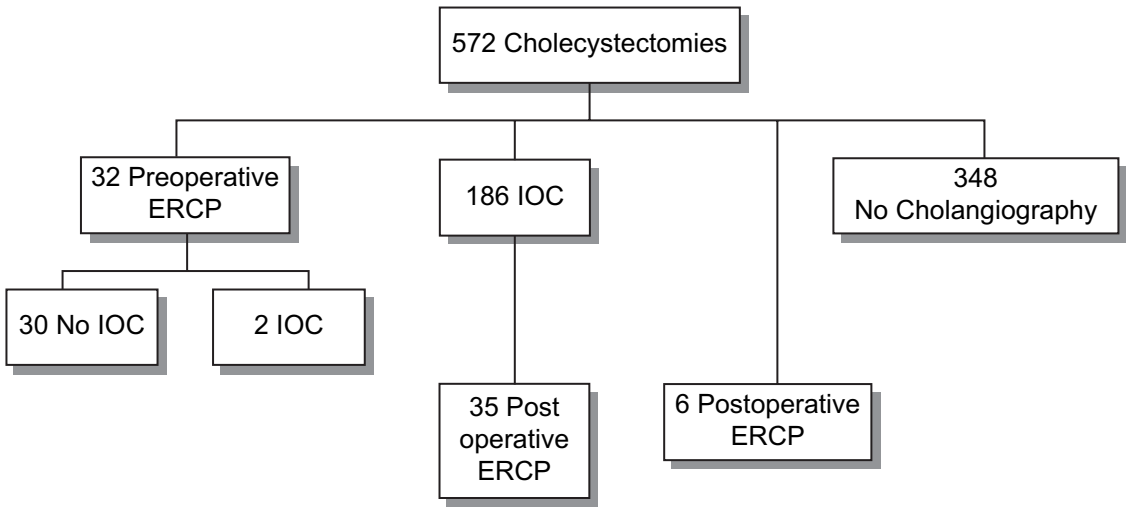

Fig. 1. Biliary imaging in the patient cohort studied. ERCP = endoscopic retrograde cholangiopancreatography; IOC = intraoperative cholangiography.

Table 2. Clinical features of the 32 patients who underwent preoperative ERCP

	Dilated CD	Elevated bilirubin	Choledocholithiasis	Biliary pancreatitis
1	(+)	(+)	(+)	(+)
2	(+)	(+)	(+)	
3	(+)	(+)	(+)	
4	(+)	(+)	(+)	
5	(+)	(+)	(+)	
6	(+)	(+)	(+)	
7	(+)	(+)	(+)	
8	(+)	(+)	(+)	
9	(+)	(+)	(+)	
10	(+)	(+)	(+)	
11	(+)	(+)	(+)	
12	(+)	(+)		(+)
13	(+)	(+)		(+)
14	(+)	(+)		(+)
15	(+)	(+)		(+)
16	(+)	(+)		
17	(+)		(+)	
18	(+)		(+)	
19	(+)		(+)	
20	(+)		(+)	
21		(+)	(+)	(+)
22		(+)	(+)	(+)
23		(+)	(+)	(+)
24		(+)		(+)
25		(+)		
26		(+)		
27		(+)		(+)
28		(+)		
29		(+)	(+)	
30		(+)	(+)	
31		(+)		(+)
32				(+)

CD = common duct; ERCP = endoscopic retrograde cholangio-pan-creatography.

undergoing preoperative ERCP were not included in the IOC analysis but were analyzed separately.

Patients undergoing IOC were classified into two groups on the basis of the presence or absence of choledocholithiasis. Univariate analysis was used to screen clinical indicator effectiveness for predicting choledocholithiasis. Clinical indicators (e.g., elevated liver function test values and pancreatitis) were assessed individually to determine whether their presence was associated with choledocholithiasis. Those found not to be significantly related to CD stones by chi-square analysis were not further considered.

Clinical features that were significantly correlated to choledocholithiasis in the univariate analysis were further assessed by multivariate analysis. The dichotomized variables were entered into a logistic regression equation (SAS LOGISTIC procedure) as independent variables with the regression's dependent variable being the presence of choledocholithiasis. Factors independently predictive for choledocholithiasis were identified following backward elimination logistic regression and were reported as odds ratios with 95% confidence intervals.

RESULTS

Table 1 summarizes the demographic features of the study population. Most were young, Hispanic females. There was a preponderance of cholecystitis diagnoses; the next most common diagnosis was biliary colic. Two-thirds of our admissions were emergent because we have a policy of emergently admitting patients with biliary colic.

Figure 1 summarizes biliary imaging use in our patient cohort. Of the 572 patients, 348 (61%) had no cholangiography of any kind. A total of 32 patients underwent an ERCP before cholecystectomy was performed (Table 2). Thirty-one of the 32 patients had a dilated CD or jaundice before the procedure. The remaining patient underwent preoperative ERCP for biliary pancreatitis without signs of CD obstruction and was not found to have significant CD pathology. The distribution of patients undergoing biliary imaging by disease is presented in Table 3.

A total of 186 patients underwent IOC during their cholecystectomy without preoperative ERCP. Of these patients, 56 (30%) were found to have common bile duct stones. Figure 2 demonstrates the univariate analysis for patients undergoing IOC. Each bar represents the presence of clinical features relied on for the decision to perform an IOC. Approximately equal numbers of patients with a history of jaundice, pancreatitis, elevated alkaline phosphatase, AST, alanine aminotransferase, GGT, amylase, and lipase levels were found to have choledocholithiasis as those without CD stones. Of those with abnormal cholangiogram results, 70% had dilated CDs and 46% had elevated bilirubin levels. In contrast, only 14% of those with normal IOC results had dilated CDs and 27% had elevated bilirubin levels. Of these factors, only CD dilation and elevated bilirubin, GGT, and AST levels were significantly associated with choledocholithiasis based on chi-square analysis. Logistic regression with backward elimination revealed that only a dilated common bile duct or elevated total bilirubin was independently predictive of common bile duct stones found with cholangiography (Table 4). GGT and AST levels failed to retain statistical significance during the backward

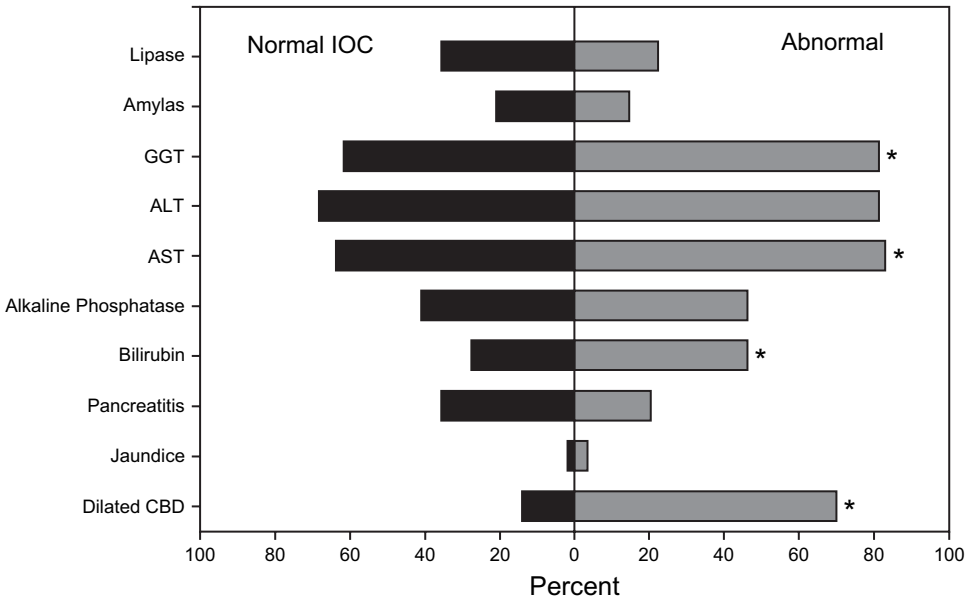

Fig. 2. Univariate analysis of clinical features used to determine the need for IOC. Each horizontal bar represents the proportion of patients having the clinical feature denoted on the vertical axis. Columns to the left of zero represent proportions for those having normal IOC results and those to the right represent abnormal IOC results. *$P < .05$, chi-square analysis. IOC = intraoperative cholangiography; GGT = γ-glutamyltransferase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CBD = common bile duct.

elimination procedure and were excluded from the final regression equation.

Because a dilated CD independently predicted choledocholithiasis, we further examined the patient data to evaluate what, if any, other preoperative findings should be considered as indications for IOC. Thirteen patients had abnormalities found with IOC and did not have IOC dilation preoperatively (Table 5). Of these patients, six had preoperative elevations of their total bilirubin. For the remaining seven patients, two were found to have an enlarged cystic duct at the time of surgery resulting in the surgeon's decision to perform a cholangiogram. Of the other five patients, two had small stones seen on cholangiography with good contrast flow into the duodenum, suggesting that these stones were not obstructive. One patient had no duodenal filling with IOC. A postoperative ERCP was performed with no CD abnormalities identified. Another patient had a nonobstructing, floating CD stone that was easily pushed into the duodenum with laparoscopic choledochoscopy. A single patient had an

Table 3. Distribution of biliary imaging studies by disease and modality

	Biliary colic	Cholecystitis	Pancreatitis
Preoperative ERCP	5	17	15
Choledocholithiasis found	4	11	5
IOC	55	94	59
Choledocholithiasis found	17	32	13
Postoperative ERCP	11	25	7
Choledocholithiasis found	6	16	4

ERCP = endoscopic retrograde cholangiopancreatography; IOC = intraoperative cholangiography.
Values are given as the numbers of patients undergoing the test. Biliary colic was the primary diagnosis only when pancreatitis or cholecystitis was absent. Sixty-one patients had both cholecystitis and pancreatitis. Thirty-seven patients underwent both IOC and ERCP.

Table 4. Logistic regression with backward elimination for the presence of common bile duct stones with intraoperative cholangiography on the various preoperative features

Feature	Odds ratio	*P*
Dilated CD	17.2 (7.7–38.7)	<.0001
Elevated bilirubin	2.8 (1.2–6.4)	.0143

Only those clinical features found to be significantly associated with the presence of choledocholithaisis found at IOC were entered into the regression equation. These included the preoperative identification of a dilated CD, elevated bilirubin, AST, or GGT. Elevated AST and GGT were eliminated by the regression procedure. CD = common duct.

Table 5. Clinical features for the 14 patients who had positive intraoperative cholangiography results and who did not have dilated common duct identified with preoperative ultrasonography

no.	Indication for IOC stated in out patient note	Preoperative bilirubin >1.3 mg/dL	IOC findings	Subsequent treatment
1	Biliary pancreatitis	Yes	No duodenal filling	ERCP papillotomy, no stones found
2	Question of nonobstruction CD stone on US	Yes	Small filling defect with slow contrast flow into the duodenum	ERCP stone extraction
3	Total bilirubin 2.3, elevated LFTs	Yes	CD stones	Failed attempt at lap CDE ERCP, stones extracted
4	Biliary pancreatitis	Yes	CD stones with good duodenal filling	Laparoscopic CDE, all stones removed
5	Pancreatitis, increased LFTs	Yes	Small filling defect with good duodenal filling	ERCP, biliary sludge only, no stones
6	Elevated LFTs	Yes	Small filling defect with good duodenal filling	ERCP, removed small stones
7	Intraoperative finding of dilated cystic duct	No	Distal CD stone with no duodenal filling	ERCP
8	Intraoperative finding of dilated cystic duct	No	Impacted stone at distal cystic duct	Open CDE, could not remove all of the stone fragments
9	Elevated LFTs	No	Floating CD stone	Laparoscopic CDE, stone pushed into duodenum
10	Minimally elevated LFTs	No	Small filling defect with good duodenal filling	ERCP stone extraction
11	Elevated LFTs	No	Distal CD stone with no duodenal filling	Failed attempt at lap CDE ERCP, stones extracted
12	Unclear anatomy	No	Good duodenal filling, small filling defect	ERCP, stone removal
13	Increased LFTs, alkaline phosphatase, GGT	No	No duodenal filling	ERCP, debris, no stones

IOC = intraoperative cholangiography; ERCP = endoscopic retrograde cholangiopancreatography; US = ultrasonography; CD = common duct; LFT = liver function test; CDE = common duct exploration; GGT = γ-glutamultransferase.

obstructing CD stone removed by postoperative ERCP.

DISCUSSION

We found that of the various clinical features used for deciding whether to perform an intraoperative cholangiogram, only the finding of a dilated common bile duct on preoperative ultrasound examination or elevated total serum bilirubin reliably predicted the presence of choledocholithiasis. A remote clinical history of jaundice or current pancreatitis was not predictive of a positive cholangiogram. Chemical abnormalities such as elevations in liver function test or pancreatitis test results were also not predictive of a positive cholangiogram. On our retrospective analysis, we found 13 patients with abnormalities on IOC who did not have a dilated CD. Of these patients, six had an elevated bilirubin level, which left seven who were found to have IOC abnormalities without preoperative hyperbilirubinemia or a dilated CD. Only two of these had potentially clinically significant choledocholithiasis. We found that 130 patients underwent an IOC that was negative and, therefore, unnecessary.

Our findings demonstrate that the traditional indications for IOC result in unnecessary procedures. Abnormal liver function and pancreatic function test results, or clinical histories of jaundice or pancreatitis do not predict CD pathology and should no longer serve as indications for IOC. Elevated serum bilirubin or a dilated CD noted on preoperative ultrasonography is strongly predictive of CD stones and should remain as the principal indicators for IOC during cholecystectomy. By using these criteria, there is little likelihood that clinically significant biliary pathology will be missed by not performing an IOC. The low risk of potentially retained choledocholithiasis was highlighted by a prospective analysis of 155 patients undergoing cholecystectomy who did

Table 6. Summary of recent prior publications examining the predictive ability of various clinical features in predicting choledocholithiasis

Year	Author	Reference	Clinical jaundice	Bilirubin	Cholangitis	Dilated CD	Pancreatitis	Amylase	SGOT
1996	Onken	20		X		X			X
1996	Robertson	21	X			X			
1996	Chan	22			X				
1997	Alponat	23	X		X	X			X
1998	Trondsen	24		X					
1999	Barr	25				X		N	
1999	Prat	26	X	X		X			X
	Prat <70 y/o					X			
	Prat >70 y/o					X			
2001	Wang	27		X					X
2003	Sarli	28	X						X
2004	Grande	29		X		X			
2004	Nathan	30		X		X		X	

X = denotes positive correlation with choledocholithiasis; XX = most highly correlated of the factors assessed; N = negative-negatively correlated with choledocholithiasis; E = equivoca—some but not a substantial effect on models predicting choledocholithiasis.

not undergo biliary imaging if the CD measured less than 10 mm with preoperative ultrasonography, they had normal liver function test results, and they had no history of pancreatitis or jaundice. With 3.5 years of follow-up, no patients had evidence of retained CD stones or complications related to potentially undiagnosed choledocholithiasis.[13]

Since Abboud and colleagues' meta-analysis assessing the efficacy of various clinical features in predicting choledocholithiasis,[11] a number of other series have been published. These are summarized in Table 6. The most consistent clinical feature associated with choledocholithiasis across studies is the preoperative finding of a dilated CD on ultrasonography. Although we found the presence of an elevated bilirubin level to be predictive of CD stones, this observation was more variable in prior studies. CD stones could be reliably predicted with a neural network model assessing 22 clinical variables,[14] but such a complex system is not practical for widespread clinical use. We sought to develop a simple algorithm that would facilitate the correct prediction of choledocholithiasis and minimize the risk of missing clinically significant CD stones. Our analysis and those summarized next suggest that only a minority of retained CD stones ever become clinically significant.

Retrospective and prospective analyses support the reduced use of IOC.[15] Aggregation of nine series including 4209 patients undergoing routine IOC found that only 4% had unsuspected choledocholithiasis. Of nine series reporting on 5179 patients undergoing selective IOC, only 0.6% presented with postoperative symptoms of residual stone disease, findings similar to other prior single institution and aggregated analyses.[16] A randomized controlled trial of routine versus selective cholangiography found that IOC changed management in only 7.5% of cases.[17] A prospective series of 999 patients undergoing cholecystectomy, all undergoing IOC, provides convincing data regarding the outcomes of abnormal IOC results. Forty-six patients (4.6%) undergoing cholecystectomy had abnormal IOC results. These patients had the IOC catheter left in place for 6 weeks postoperatively. Twelve of the 46 patients did not have CD abnormalities when restudied 48 hours after cholecystectomy. Another 12 patients had normal CDs 6 weeks postoperatively. Of the remaining 22 patients with persistent CD pathology at 6 weeks, 20 underwent ERCP with resolution of CD abnormalities.[12] This study convincingly demonstrated that post-cholecystectomy retained choledocholithiasis is well tolerated and unlikely to result in clinically significant complications.

Our findings support a more selective approach to biliary imaging than is currently practiced. Because relatively few CD stones result in significant pathology, it seems reasonable to proceed with preoperative ERCP or IOC concurrent to cholecystectomy only for patients who present with a dilated CD or elevated bilirubin level. Given the expense of preoperative ERCP and the fact that patients with symptomatic cholelithiasis will undergo cholecystectomy eventually, it may be more cost-effective to avoid preoperative ERCP in favor of cholecystectomy with IOC, reserving ERCP for those patients with biliary pathology that could not be addressed during cholecystectomy.[18] We studied patients who underwent cholecystectomy for cholelithiasis. Patients with jaundice and malignancy diagnoses were not included in our analysis. Jaundice can result from either choledocholithiasis or malignancy. ERCP is a good diagnostic test for pancreaticobiliary malignant disease

Table 6. (Continued)

SGPT	GGT	Alkaline phosphatase	Biliary colic	Cholecystitis	Dyspepsia	#of gallstones	US evidence of choledo-cholithiasis	Age	Fever	Male gender	Albumin
		X						E			
		X					X	X			X
							X				
							X				
X	X							X			
	X	E									
X	X	X		X				X	X		
	X			X				<70 y/o			
	X							>70 y/o	X		
	XX	XX									
			X	X	X	X	X				
		X				X					
		X					X	X		X	

so that preoperative ERCP can be recommended for patients with jaundice and suspected malignant disease.[19] Given this, we recommend preoperative ERCP for patients with jaundice and IOC for those patients with a dilated CD without jaundice.

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