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Long-term Outcome After Resection for Chronic Pancreatitis in 224 Patients

Hartwig Riediger · Ulrich Adam · Eva Fischer · Tobias Keck · Frank Pfeffer · Ulrich T. Hopt · Frank Makowiec

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Abstract
Introduction Organ complications like biliary or duodenal stenosis as well as intractable pain are current indications for surgery in patients with chronic pancreatitis (CP). We present here our experience with pancreatic resection for CP and focus on the long-term outcome after surgery regarding pain, exocrine/endocrine pancreatic function, and the control of organ complications in 224 patients with a median postoperative follow-up period of 56 months.
Methods During 11 years 272 pancreatic resections were performed in our institution for CP. Perioperative mortality was 1%. Follow-up data using at least standardized questionnaires were available in 224 patients. The types of resection in these 224 patients were Whipple (9%), pylorus-preserving pancreato-duodenectomy (PD) (PPPD; 40%), duodenum-preserving pancreatic head resection (DPPHR; 41%, 50 Frey, 42 Beger), distal (9%) and two central pancreatic resections. Eighty-six of the patients were part of a randomized study comparing PPPD and DPPHR. The perioperative and follow-up (f/up) data were prospectively documented. Exocrine insufficiency was regarded as the presence of steatorrhea and/or the need for oral enzyme supplementation. Multivariate analysis was performed using binary logistic regression.
Results Perioperative surgical morbidity was 28% and did not differ between the types of resection. At last f/up 87% of the patients were pain-free (60%) or had pain less frequently than once per week (27%). Thirteen percent had frequent pain, at least once per week (no difference between the operative procedures). A concomitant exocrine insufficiency and former postoperative surgical complications were the strongest independent risk factors for pain and frequent pain at follow-up. At the last f/up 65% had exocrine insufficiency, half of them developed it during the postoperative course. The presence of regional or generalized portal hypertension, a low preoperative body mass index, and a longer preoperative duration of CP were independent risk factors for exocrine insufficiency. Thirty-seven percent of the patients without preoperative diabetes developed de novo diabetes during f/up (no risk factor identified). Both, exocrine and endocrine insufficiencies were independent of the type of surgery. Median weight gain was 2 kg and higher in patients with preoperative malnutrition and in patients without abdominal pain. After PPPD, 8% of the patients had peptic jejunal ulcers, whereas 4% presented with biliary complications after DPPHR. Late mortality was analyzed in 233 patients. Survival rates after pancreatic resection for CP were 86% after 5 years and 65% after 10 years.
Conclusions Pancreatic resection leads to adequate pain control in the majority of patients with CP. Long-term outcome does not depend on the type of surgical procedure but is in part influenced by severe preoperative CP and by postoperative surgical complications (regarding pain). A few patients develop procedure-related late complications. Late mortality is high, probably because of the high comorbidity (alcohol, smoking) in many of these patients.

Keywords Chronic pancreatitis · Pancreatic resection · Long-term outcome · Endocrine function · Exocrine function

H. Riediger · U. Adam · E. Fischer · T. Keck · F. Pfeffer · U. T. Hopt · F. Makowiec (✉)
Department of Surgery, University of Freiburg, Hugstetter Str. 55, 79106 Freiburg, Germany
e-mail: Frank.Makowiec@uniklinik-freiburg.de

Introduction

The knowledge about the pathophysiology and origin of inflammation and pain in chronic pancreatitis (CP) has increased during the past decades. Progress in interventional procedures, such as endoscopic retrograde cholangiopancreatography, and improved cross-sectional imaging (computerized tomography [CT] and magnetic resonance imaging, [MRI]) has helped to delineate the inflammatory processes better. The

origin of pain was initially explained by pancreatic duct obstruction. Decompression was thought to control pain effectively. In a large analysis of more than 1,000 patients endoscopic drainage was successful in up to two-thirds of the patients.[1] A short preinterventional duration of CP was associated with a larger success rate of endoscopic drainage.[2] Nevertheless, a relevant number of interventionally treated patients had to undergo surgery because of failed symptom relief. Moreover, up to 50% of patients with chronic pancreatitis require surgical therapy during the long-term course of their disease.[3]

The hypothesis of pain origin by mechanisms other than increased intraductal pressure (e.g., fibrosis, alteration of pancreatic nerves, inflammatory enlargement) was strengthened during the last decade.[4] Detection and description of an inflammatory mass predominantly in the pancreatic head was a further step in the understanding of CP.[5,6] In an important follow-up analysis of patients with "failure of symptomatic relief after pancreatojejunal decompression", the cause for recurrent pancreatitis was localized in the pancreatic head, which was consecutively declared as a pacemaker of the disease.[6] With the publication of further disappointing results after surgical drainage, resectional procedures gained more importance in the treatment of CP, especially in patients with inflammatory enlarged areas of the pancreas. Duodenum preserving pancreatic head resections (DPPHR) as described by Beger[7] and Frey[8] and the pylorus preserving pancreatoduodenectomy (PPPD) as reintroduced by Traverso and Longmire[9] are current resectional procedures in chronic pancreatitis predominantly of the pancreatic head. Removal of the inflammatory pancreatic head mass resulted in substantial pain relief and control of other organ complications in up to 90% of patients.[10–14]

Pancreatic resection is now a procedure with acceptable morbidity and low mortality. In many centers, a perioperative mortality rate of clearly less than 5% has been reported. As a result of these advances in the perioperative course after pancreatic surgery, the debate on the indications and results of resectional surgery for CP now focuses on the long-term outcome (quality of life, pain control, endocrine and exocrine function, control of organ complications).

The aim of our study was to evaluate the long-term course after resection for CP in more than 200 patients with a median follow-up of almost 5 years. Risk-factor analyses were performed to search for potential parameters influencing the long-term outcome.

Patients and Methods

Patients and Indications for Surgery

From July 1994 to December 2005, 272 patients underwent pancreatic resection for CP. Postoperative histological examination confirmed CP in all cases. Of these 272 patients, prospective postoperative follow-up data using standardized questionnaires could be gained in 224 (82%). Three patients (1%) died of postoperative complications. Of the remaining 45 patients, nine (3%) died without postoperative follow-up, eight (3%) were not contacted because of a postoperative observation period of less than 6 months, and 28 (10%) were lost to follow-up.

The 224 patients (80% male) included in this study had a median age of 44 years (range 27–79 years) at the time of surgery. Median preoperative duration of CP was 36 (1–444) months. Further preoperative characteristics and (co-) indications for surgery are listed in Table 1.

The leading indications for surgery in the 224 patients were pain (chronic or recurrent; n=147, 66%), jaundice (n=36; 16%), duodenal obstruction (n=12; 5%), or one of various others (n=29; 13%). It is of note, however, that many patients had more than one indication for pancreatic resection. Two hundred eight (93%) patients had pain (chronic or during recurrent episodes of pancreatitis) as indication or coindication for surgery (Table 1).

During the evaluation of the preoperative status the intake of pain medication was documented (as yes or no). In contrast to the postoperative follow-up data, we have no further details on preoperative pain medication like frequency of intake or type of analgesic taken. Preoperatively, 87 patients (39%) of the entire study group (and 42% of the 208 patients with pain and/or recurrent attacks of pancreatitis) had documented pain medication.

Preoperative Assessment

All patients had at least one cross-sectional imaging modality before surgery (CT in 94%, MRI in 34%). During the last years of the study period MRI included MRCP and MR-angiography in the majority of patients. Until 2001, most patients preoperatively underwent conventional angi-

Table 1 Preoperative Characteristics of 224 Patients Undergoing Resective Surgery for Chronic Pancreatitis

	N (%)
Diabetes	68 (30%)
Alcohol abuse	165 (74%)
Body mass index (median, range)	22 (14.5–35.2)
Jaundice	57 (25%)
Bile duct stenosis (radiological/ERCP)	104 (46%)
Duodenal stenosis	35 (16%)
Pain	185 (83%)
Recurrent episodes of pancreatitis	180 (80%)
Pseudocysts	135 (60%)
Calcifications	152 (68%)
Pancreas divisum	15 (7%)
Regional or generalized portal hypertension	57 (25%)

ography because of the high percentage of vascular involvement in our patients. Since 2001, with better vessel imaging by multislice CT and/or MR-angiography conventional angiography is restricted to selected patients only (79% had angiography). One hundred fifty-seven patients (70%) had an ERCP preoperatively, and 61 (27%) had a preoperative biliary drainage. Of our patients only a few preoperatively underwent endoscopic stenting of the pancreatic duct. Endoscopic ultrasound was performed in 55% of our study group.

Anatomical Description of Chronic Pancreatitis

In the entire patient group, 74% had documented pancreatic duct stenosis, and 77% had pancreatic duct dilatation (large duct disease). Pancreatic duct stones were present in 44% of the patients. Sixty percent of the patients had pseudocysts (Table 1), reflecting the high percentage of patients with large duct disease in our study group. Sixty-eight percent had calcifications. Calcifications were more frequent in alcoholic CP (74%) than in nonalcoholic CP (53%; $p<0.01$). Seven percent of the patients had a pancreatic divisum (as potential etiology or coetiology of CP).

In the 201 patients undergoing pancreatic head resection (PD) or DPPHR, distal dilatation of the pancreatic duct was present in 78%. Sixty percent of those 201 patients had an inflammatory enlargement of the pancreatic head. Only 4% of the patients were documented as having neither an inflammatory mass of the pancreatic head nor a pancreatic duct dilatation nor a radiological stenosis of the common bile duct.

Surgery and Perioperative Management

The following types of pancreatic resection were performed in the 224 patients: PPPD ($n=89$; 40%), DPPHR ($n=92$; 41%; Beger 42 and Frey 50), classic Whipple operation ($n=20$; 9%), distal pancreatectomy ($n=21$; 9%), and two central pancreatic resections (1%).

The perioperative management of our patients has recently been described in detail.[15] After the resectional part, the pancreatic duct was always cannulated to exclude remaining pancreatic duct stones or stenosis. After PD pancreatic anastomosis was performed as a single-layer end-to-side pancreatojejunostomy (91%), a duct-to-mucosa-technique with a pancreatic duct catheter (6%) or as pancreatogastrostomy (3%). After DPPHR according to Beger, the pancreatic anastomosis was also performed in an end-to-side technique using interrupted full-thickness polydioxanone sutures. During the Beger operation, a bilioenteric anastomosis to the posterior wall of the jejunal loop was included in 24 (57%) of the 42 patients. After the Frey resection, reconstruction consisted in a side-to-side pancreatojejunostomy using running polydioxanone sutures.

The bilioenteric anastomosis after PD was performed in an interrupted technique with polydioxanone sutures in almost all patients. A few patients with large common hepatic duct caused by extensive cholestasis underwent end-to-side hepaticojejunostomy using running sutures.

After distal pancreatic resection, 16 of 21 (76%) patients had a single-layer pancreatojejunostomy and 5 (24%) had a suture closure of the pancreatic stump. Stapler closure of the pancreatic stump was not used in our study patients.

Perioperative octreotide was almost always applied for 5 to 7 days in the first years of this study period. Its routine use was abandoned in 2003. Before abdominal closure, flat silicon drains were placed at the pancreatic anastomosis (and at the bilioenteric anastomosis, when performed) and taken out through the abdominal wall. These drains were left in place for at least 3 postoperative days.

Definitions

Our standardized definition of pancreatic leakage was reported in detail before[15] and consisted in increased amylase in the drain output beyond the sixth postoperative day, the need of interventional drainage of abdominal fluid collections with a high amylase concentration or visible anastomotic insufficiency found during relaparotomy. All intraabdominal complications including gastrointestinal bleeding and wound infections were summarized as surgical complications.

The presence of diabetes was defined by the criteria of the WHO classification. Many patients underwent oral glucose tolerance tests or 24-h glucose profile determination.

Exocrine insufficiency was defined as the presence of steatorrhea and/or the need for oral pancreatic enzyme supplementation. In our complete study group, we did not routinely measure other parameters for exocrine function (e.g., stool elastase).

Follow-up

Postoperative follow-up examinations were performed in several chronological steps since 1996 in the form of mailed questionnaires (with or without additional telephone contact to the patient or home physician) or outpatient visits. They always included standardized questionnaires asking (among others) the presence of pain, pain intensity (including visual analog scales), pain frequency (none/daily/weekly/monthly/yearly), the presence of diabetes or steatorrhea, and the current specific medication (pancreatic enzymes, analgesics). In all follow-up questionnaires, the type of pain medications and the frequencies of their intake were evaluated. Furthermore, the need of reoperation was investigated.

In November and December 2005, mailed questionnaires were (again) sent to all eligible patients, and 130 surviving patients answered with completed questionnaires until end

Table 2 Postoperative Complications in 224 Patients after Resection for Chronic Pancreatitis

	N (%)
Total morbidity (all complications)	86 (38.4)
Surgical complications	62 (27.7)
Abdominal infection[a]	16 (7.1)
Wound infection	19 (8.5)
Bile leak	2 (1.3)
Pancreatic leakage	26 (11.6)
Postoperative bleeding	10 (4.5)
Gastrointestinal bleeding	6 (2.7)
Reoperation	16 (7.1)

[a] Intraabdominal abscess or peritonitis

of January 2006 (among those 21 patients without prior follow-up data).

Eighty-six patients included in this series were part of a randomized study comparing PPPD and DPPHR. These patients all had regular outpatient follow-up including the questionnaires mentioned above plus quality-of-life measurements and oral glucose tolerance tests. The specific results of this randomized study (especially regarding quality of life) are not included in this manuscript and will be reported separately.

For this study, the results of the last follow-up evaluation per patient were considered. Median postoperative follow-up in all 224 patients was 56.3 (4–141) months. Median follow-up was about 1 year longer after PD (57 months) than after DPPHR (44 months). This may reflect the fact that DPPHR was performed with increasing frequency during the later study period.

Statistics

All perioperative and outcome data were entered into a computerized database (SPSS 13.0, SPSS Inc., Illinois, USA). Data acquisition after pancreatic resection is performed prospectively in our department.[15] During subgroup analysis, comparisons were made by the chi-square and Mann–Whitney tests where appropriate. Potential risk factors for the long-term outcome parameters pain, endocrine and exocrine insufficiency were multivariately analyzed by a binary logistic regression model with a forward selection strategy using likelihood ratio statistics (inclusion and exclusion probability, $p=0.2$). For the subgroup analyses of the influence of the different types of surgery on the outcome ($n=222$ with PD, DPPHR, or distal resection), the two patients with segmental resection were excluded.

Results

Surgery and Perioperative Course

Median duration of surgery in all 224 patients was 400 min (range 160–870 min). Duration of surgery was 442.5 min (285–870) for PD, 377.5 min (195–740) for DPPHR, and 242.5 min (160–405) for distal resections.

The median number of intraoperatively transfused units of blood was 2 (range 0–36). The median of transfused units was 4 during PD and 2 during DPPHR and distal resection.

The total postoperative complication rate was 38%, with 28% of the patients having surgical complications (Table 2). The frequency of surgical complications did not differ significantly after PD, DPPHR, or distal resection. A reoperation for complications was necessary in 16 patients (7%). Median postoperative length of stay was 14 days (7–120).

Pain Assessment

At the last follow-up evaluation 134 (60%) patients reported no abdominal pain at all and 90 (40%) of the patients had abdominal pain. Of the 90 patients with abdominal pain, 12 (13%) had pain every day, 19 (21%) had pain at least once per week, 29 (32%) at least once per month, and 30 (33%) at least once per year. Subgroup analysis could not demonstrate a correlation between pain presence and type of surgery performed (Table 3). In addition, preoperative pain medication was not associated with pain or frequent pain during follow-up (Table 4). Univariate analysis of other potential risk factors for pain at the last follow-up evaluation showed that the absence of diabetes, a concomitant exocrine insufficiency, postoperative surgical complications, a shorter postoperative follow-up period (less than 5 years), and a shorter total duration of CP (less than 8 years) were significantly associated with a higher

Table 3 Pain and Pain Frequency at Last Follow-up Dependent on the Type of Surgery

Type of surgery	No pain	Pain				
		Total	Daily	Weekly	Monthly	Yearly
PD ($n=109$)	69 (63%)	40 (37%)	7	10	11	12
DPPHR ($n=92$)	52 (57%)	40 (43%)	3	8	17	12
Distal pancreatectomy ($n=21$)	12 (57%)	9 (43%)	2	1	0	6
All ($n=222$)	133 (60%)	89 (40%)	12 (5%)	19(9%)	28(13%)	30 (14%)

Table 4 Univariate Analysis of Potential Risk Factors for Pain in 224 Patients after Resection for Chronic Pancreatitis

Parameter			Pain		Frequent pain (weekly or daily)	
			N (%)	*p* value	*N* (%)	*p* value
Preoperative analgesics	Yes	87	38 (44%)	0.4	14 (16%)	0.44
	No	137	52 (38%)		17 (12%)	
Diabetes at last follow up	Yes	120	38 (32%)	0.005	13 (11%)	0,16
	No	104	52 (50%)		18 (17%)	
De novo Diabetes	Yes	57	17 (30%)	0.07	4 (7%)	0.08
	No	167	73 (44%)		27 (16%)	
Exocrine insufficiency at last follow up	Yes	146	66 (45%)	0.036	27 (19%)	0.006
	No	78	24 (31%)		4 (5%)	
De novo exocrine insufficiency	Yes	75	32 (43%)	0.59	17 (23%)	0.007
	No	149	58 (39%)		14 (9%)	
Gender	Male	179	69 (39%)	0.32	23 (13%)	0.39
	Female	45	21 (47%)		8 (18%)	
Portal hypertension	Yes	57	23 (40%)	0.97	8 (14%)	0.96
	No	167	67 (40%)		23 (14%)	
Surgical complications	Yes	62	33 (53%)	0.014	15 (24%)	0.005
	No	162	57 (35%)		16 (10%)	
Postop. Follow up	>60 months	104	32 (31%)	0.007	11 (11%)	0.18
	≤60 months	120	58 (48%)		20 (17%)	
Preoperative duration of chronic pancreatitis	>36 months	122	52 (43%)	0.41	18 (15%)	0.66
	≤36 months	102	38 (37%)		13 (13%)	
Duration of chronic pancreatitis	>8 years	117	39 (33%)	0.029	12 (10%)	0.10
	≤8 years	107	51 (48%)		19 (18%)	
Alcoholic pancreatitis	Yes	165	65 (39%)	0.68	20 (12%)	0.21
	No	59	25 (42%)		11 (19%)	
Preoperative BMI*	<20	51	20 (39%)	0.87	24 (14%)	0.97
	≤20	173	70 (41%)		7 (14%)	
Calcifications	Yes	145	63 (43%)	0.35	19 (13%)	0.38
	No	68	25 (37%)		12 (18%)	

*BMI=body mass index

rate of abdominal pain (Table 4). In multivariate analysis, the concomitant absence of diabetes, a concomitant exocrine insufficiency, postoperative surgical complications, and a short postoperative follow-up period were independent risk factors for abdominal pain at the last follow-up evaluation (Table 5).

To further assess pain after resection for CP, the subgroup of patients with frequent abdominal pain (pain daily or at

Table 5 Results of Multivariate Analysis of Risk Factors for Long-term Outcome Regarding Pain, Endocrine Insufficiency, and Exocrine Insufficiency in 224 Patients after Resection for Chronic Pancreatitis

	Risk factor	*p* value (multivariate)	Relative risk (Odd's ratio)	95% confidence interval
Pain, *n*=90 (40%)	Diabetes at last follow up	0.005	0.438	0.245–0.782
	Exocrine insufficiency at last follow up	0.016	2.410	1.177–4.931
	No surgical complications	0.02	0.477	0.255–0.890
	Postop. Follow up >60 months	0.015	0.488	0.274–0.869
Frequent pain(weekly or daily), *n*=31, (14%)	Exocrine insufficiency at last follow up	0.09	4.383	1.44–13.343
	No surgical complications	0.011	0.352	0.157–0.791
Exocrine insufficiency at final follow up, *n*=146 (65%)	Preoperative duration of CP	0.002	2.470	1.384–4.408
	Preoperative BMI >20	0.02	0.397	0.183–0.864
	No portal hypertension	0.032	0.449	0.217–0.933

No independent risk factor for diabetes could be identified.

Table 6 Endocrine and Exocrine Function at Last Follow-up in 222 Patients after Resection for Chronic Pancreatitis Dependent on the Type of Surgery

	Diabetes		Exocrine insufficiency	
	Total	De novo	Total	De novo
PD (n=109)	52 (48%)	24 (22%)	68 (62%)	37 (34%)
DPPHR (n=92)	54 (57%)	24 (26%)	69 (75%)	31 (34%)
Distal pancreatectomy (n=21)	12 (57%)	8 (38%)	8 (66%)	6 (29%)
All (n=222)	118 (53%)	56 (25%)	145 (65%)	74 (33%)

least once per week; n=31) underwent again uni- and multivariate evaluations. Here, uni- and multivariate analysis revealed that the presence of exocrine insufficiency and former postoperative surgical complications were independent risk factors for the occurrence of frequent pain (at least once per week; Tables 4 and 5).

Pain Medication at Follow-up

At the last follow-up evaluation, 65 of the 90 patients (72%) complaining of abdominal pain took pain medication (10 had analgesics every day, 17 at least once per week, 20 at least once per month, and 18 at least once per year). Of these 65 patients 38 (58%) had opioids and 27 (42%) had peripheral analgesics. Univariate analysis could not identify risk factors for the use of pain medication at follow-up (in those patients with pain).

Endocrine Function

Sixty-eight patients (30%) had documented endocrine insufficiency preoperatively. Of those 68 patients with preoperative diabetes, five (7%) had no evidence of diabetes at the last follow-up (two after PD, two after DPPHR, and one after distal resection).

At the last follow-up evaluation, a total of 120 patients (54%) had diabetes. Fifty-seven of the 156 patients (37%) without preoperative diabetes became diabetic (de novo diabetes). Of those 156 patients, seven (4%) developed diabetes directly after surgery (three after PD, three after

Table 7 Univariate Analysis of Risk Factors for Diabetes (all) or De Novo Diabetes after Resection for Chronic Pancreatitis

Parameter		Number of patients	Diabetes mellitus at final follow up		De novo diabetes mellitus	
			N (%)	p value	N (%)	p value
Exocrine insufficiency at final follow up	Yes	146	84 (58%)	0.104	36 (25%)	0.711
	No	78	36 (46%)		21 (27%)	
De novo exocrine insufficiency	Yes	75	39 (52%)	0.74	14 (19%)	0.1
	No	149	81 (54%)		43 (29%)	
Gender	Male	179	99 (55%)	0.3	46 (26%)	0.86
	Female	45	21 (47%)		11 (24%)	
Portal hypertension	Yes	57	36 (63%)	0.09	19 (33%)	0.11
	No	167	84 (50%)		38 (22%)	
Surgical complications	Yes	62	30 (48%)	0.34	15 (24%)	0.79
	No	162	90 (56%)		42 (26%)	
Postop. Follow-up (>60 months)	>60 months	104	61 (59%)	0.16	30 (29%)	0.28
	≤60 months	120	59 (49%)		27 (23%)	
Preoperative duration of CP (>36 months)	>36 months	122	68 (56%)	0.48	26 (21%)	0.12
	≤36 months	102	52 (51%)		31 (30%)	
Total duration of chronic pancreatitis	>8 years	117	68 (58%)	0.15	30 (26%)	0.94
	≤8 years	107	52 (49%)		27 (25%)	
Alcoholic CP	Yes	165	87 (53%)	0.67	40 (24%)	0.49
	No	59	33 (56%)		17 (29%)	
Preoperative BMI	<20	51	25 (49%)	0.46	16 (31%)	0.27
	≥20	173	95 (55%)		41 (24%)	
Calcifications	Yes	145	81 (56%)	0.23	39 (27%)	0.32
	No	68	32 (47%)		14 (21%)	

Table 8 Exocrine Insufficiency at Last Follow-up and De Novo Exocrine Insufficiency Depending on the Type of Pancreatic Resection

	Exocrine insufficiency at follow up		De novo exocrine insufficiency	
	N (%)	*p* value	N (%)	*p* value
PD, *n*=109	68 (63%)	0.775	17 (16%)	0.83
DPPHR, *n*=92	69 (75%)		11 (12%)	
Distal pancreatectomy, *n*=21	8 (38%)		3 (14%)	

DPPHR, and one after distal resection), whereas 50 (32%) became diabetic during the postoperative follow-up period.

Univariate (Tables 6 and 7) and multivariate analyses revealed no statistically significant risk factors for the presence of diabetes or the development of postoperative de novo diabetes, although patients with regional/generalized portal hypertension, after distal pancreatic resection or with concomitant exocrine insufficiency, had slightly higher risks to be diabetic at the end of follow-up.

Exocrine Function

Preoperatively, 71 patients (32%) were assessed to have exocrine pancreatic insufficiency. During the postoperative follow-up period, 75 other patients (33.5%) developed exocrine insufficiency leading to a total number of 146 patients (65.2%) with exocrine pancreatic insufficiency at the last follow-up assessment (Table 8).

Univariate analysis revealed that the presence of portal-venous hypertension at the time of surgery, a longer preoperative duration of CP, and malnutrition (preoperative body mass index <20) were univariately associated with a higher probability of exocrine insufficiency at the last follow-up (Table 9). These three parameters were also independent risk factors for exocrine insufficiency after multivariate analysis (Table 5).

Malnutrition (low BMI) was the only risk factor for postoperative de novo exocrine insufficiency (Table 8). A multivariate analysis was, therefore, not performed for this entity.

Table 9 Univariate Analysis of Risk Factors for Exocrine Insufficiency and De Novo Exocrine Insufficiency at Last Follow-up after Pancreatic Resection in 224 Patients

Parameter		Number of patients	Exocrine insufficiency at final follow up		De novo exocrine insufficiency	
			N (%)	*p* value	*N* (%)	*p* value
Diabetes at last follow up	Yes	120	84 (70%)	0.1	39 (33%)	0.74
	No	104	62 (60%)		36 (35%)	
Gender	Male	179	114 (64%)	0.35	58 (33%)	0.5
	Female	45	32 (71%)		17 (38%)	
Portal hypertension	Yes	57	45 (79%)	0.012	19 (33%)	0.98
	No	167	101 (61%)		56 (34%)	
Surgical complications	Yes	62	43 (69%)	0.42	23 (37%)	0.48
	No	162	103 (64%)		52 (32%)	
Postop. Follow up	≥60 months	104	64 (62%)	0.29	32 (31%)	0.42
	<60 months	120	82 (68%)		43 (36%)	
Preoperative duration of chronic pancreatitis (>36 months)	<36 months	122	91 (75%)	0.001	42 (34%)	0.74
	≤36 months	102	55 (54%)		33 (32%)	
Duration of chronic pancreatitis (>8 years)	≥8 years	117	77 (66%)	0.84	36 (31%)	0.37
	<8 years	107	69 (65%)		39 (36%)	
Alcoholic pancreatitis	Yes	165	109 (66%)	0.64	51 (31%)	0.17
	No	59	37 (63%)		24 (41%)	
Preoperative BMI	<20	51	41 (80%)	0.009	23 (45%)	0.045
	≥20	173	105 (61%)		52 (30%)	
Calcifications	Yes	145	99 (68%)	0.18	45 (31%)	0.3
	No	68	40 (59%)		26 (38%)	

Postoperative Weight Change

In all 224 patients, the median weight gain from the time of surgery to the last follow-up evaluation was 2 (range −31 to +37) kg. In subgroup analysis, patients without frequent pain (median weight difference +2 kg vs. −2 kg in patients with daily or weekly pain; $p<0.001$) and patients with a preoperative BMI below 20 (median weight gain +4 kg vs. +1 kg in patients with a BMI>20) had significantly higher weight gain. In other subgroup analyses (endocrine and exocrine function, type of surgery, surgical complication), no statistical differences regarding the postoperative body weight changes were found between the groups.

Anatomical Classification

The preoperative anatomical classification of CP (pancreatic duct dilatation, presence of an inflammatory mass of the pancreatic head, calcifications) did not show any influence on the main outcome parameters. The presence or absence of these mentioned characteristics had no effect on long-term pain, pain classification, exocrine or endocrine function (data not shown).

Late Morbidity/Organ Complications

Eleven patients underwent surgery for incisional hernia and two for small bowel obstruction caused by postoperative adhesions. The reasons for surgery for specific complications after pancreatic resection are listed in Table 10.

Symptomatic peptic ulcer disease was documented in nine patients (4%) during follow-up. After PPPD, seven (8%) of 109 patients developed peptic anastomotic or jejunal ulceration leading to reoperation in one of those. After classical Whipple procedure ($n=20$), one patient had a peptic ulcer requiring surgery and one patient required completion pancreatectomy for recurrent CP.

Relevant biliary complications occurred in seven (3%) of the 224 patients (two stenoses [one after Beger procedure, one after Frey procedure]) and two impacted common bile duct stones (Frey) after DPPHR treated by surgery or ERCP; two stenoses of the bilioenteric anastomosis after PPPD; one new stenosis of the distal bile duct after former distal pancreatic resection as a result of recurrent CP in the pancreatic head.

Table 10 List of Specific Complications Requiring Surgery after Pancreatic Resection for CP (Seven of 224 Patients)

Initial type of surgery	Late complication	Treatment
Whipple	Peptic ulcer	Y-Roux-reconstruction
Whipple	Recurrent CP	Pancreatectomy
PPPD	Peptic ulcer	Resection of anastomosis
PPPD	Biliary stenosis	Redo bilioenteric anastomosis
DPPHR ($n=2$)	Biliary stenosis	Hepaticojejunostomy
Distal resection	Biliary stenosis	Hepaticojejunostomy

Late Mortality

Long-term survival could be evaluated in the 224 patients of this study and nine more patients who died during the postoperative period without undergoing clinical follow-up examination. Thirty of those 233 patients died, a median of 3.2 years after pancreatic resection. Reasons for death were cardiac ($n=6$), pancreatic cancer ($n=3$), liver cirrhosis ($n=3$), suicide ($n=3$), ENT cancer ($n=2$), one lung cancer, one bile duct cancer, and complications of diabetes in another patient. Reason for death is unknown in 10 of 30 patients.

Five- and 10-year survival rates were 86 and 65%, respectively, in these 233 patients with a median age of 44 years at the time of pancreatic resection.

Discussion

In this study, we report the long-term outcome after pancreatic resection for CP in 224 patients with a postoperative follow-up period of up to 11.7 years. Prospective documentation of many perioperative parameters and standardized follow-up questionnaires enabled us to perform risk-factor analyses for the late postoperative outcome.

Most patients were referred by gastroenterologists, frequently after failed nonsurgical therapy of pain or complications of adjacent organs. The high frequencies of large duct disease, inflammatory mass, pseudocysts, and bile duct stenoses reflect the fact that many patients had a rather severe and/or advanced form of CP. Patients with small duct disease without complications other than pain were exceptional in our study group.

Although there is an ongoing debate on the type of surgery in subgroups of patients with large duct disease (resection versus drainage procedures alone), we believe that the majority of our patients had a clear indication for resection as defined by the aforementioned criteria (together with suspicion of malignancy in some of them). Our results, therefore, cannot be compared with studies also reporting good outcomes after surgical drainage alone.[16,17]

Follow-up evaluation was performed in several steps since 1996. By analysis of the questionnaires and/or documentation of postoperative outpatient visits, we judge the data regarding the presence of pain and diabetes as very reliable. About half of the patients pre- and postoperatively had oral glucose tolerance tests further determining endocrine function. Regarding pain, however, one cannot exclude that postoperative pain has other reasons than

recurrent or ongoing pancreatitis (like, e.g., postoperative bowel adhesions) as indicated by the fact that postoperative surgical complications were a risk factor for abdominal pain. By analyzing the questionnaires or by telephone contact with the home physicians, however, we had the strong impression that many severe pain attacks in the postoperative period were caused by episodes of recurrent CP and often related to alcohol abuse. The evaluation of postoperative alcohol intake by patient interview or questionnaire has certainly a strong bias and was not performed in our study.

Because some patients were treated for postoperative (non-surgical) problems by their home-gastroenterologists or in the initially referring hospital, we have certainly not recorded all recurrent attacks of pancreatitis. We, therefore, did not include a detailed analysis of recurrent CP episodes in this paper. Regarding necessary reoperations, however, we probably have sufficient data of all patients.

Our results confirm many other reports that pancreatic resections can be performed with a very low mortality (1% in our overall patient group) and an acceptable morbidity in patients with chronic pancreatitis.[10,13,18–20] Therefore, the long-term control of pain is the most important outcome parameter after surgical treatment of CP. In our patients, we could achieve a complete or satisfactory control of pain in more than 80%. Pain origin in patients with CP is not fully understood. Beyond increased intraductal pressure other factors like nerve alterations, fibrosis, or an inflammatory mass per se ("pacemaker") may be responsible[4,6]. A concomitant exocrine insufficiency was one of two strong risk factors for abdominal pain in our study. Possible explanations here are advanced CP leading to exocrine insufficiency by parenchyma destruction and to recurrent pain by one of the aforementioned mechanisms. As already mentioned, former postoperative surgical complications were also an independent risk factor for late pain. The explanation for this phenomenon is difficult, and, we cannot exclude that intraabdominal adhesions may be (in part) responsible for pain in these patients. By uni- and/or multivariate analysis, a longer duration (total and preoperative, respectively) of CP was associated with fewer pain at last follow-up compared to patients with a shorter CP history. It is possible that in these patients with long-standing disease a "burn-out" of CP may be responsible as suggested by Ammann et al.[21] or Layer et al.[22]

At the end of the follow-up period, 54% of the patients were diabetic. About half of those developed diabetes during the postoperative period (de novo diabetes). Only a small minority of de novo diabetes occurred directly as a consequence of surgery, but most de novo endocrine insufficiency developed later, probably as a consequence of further ongoing parenchyma destruction by CP.[12,13,23] Surprisingly, we could not identify any risk factor for diabetes or de novo diabetes in our patients. It has been well described that distal resection bears a higher risk of postoperative diabetes,[23] but we could at best see a tendency for a higher rate of de novo diabetes after distal resection. The presence of calcifications as risk factor for diabetes as described by Malka et al.[23] was not confirmed in our patients.

As already reported by Beger et al.,[20] we also had five patients with improvement of endocrine function after surgery, four after head resection, and even one after distal resection. This may be because of removal of an inflammatory process and/or pancreatic duct stenosis with subsequent improvement of function in the remaining pancreas.

Similar to endocrine insufficiency, about one-third of the patients presented with exocrine insufficiency before surgery and half of the remaining patients developed exocrine insufficiency during the postoperative follow-up period of almost 5 years. In contrast to diabetes, we could identify risk factors for late exocrine insufficiency. Patients with preoperative malnutrition (as defined by a BMI <20) developed postoperative de novo exocrine insufficiency significantly more frequently than patients with a BMI above 20 (although the rate of exocrine insufficiency was comparable before surgery). Summarized with further risk factors like a longer preoperative duration of CP or the presence of portalvenous hypertension, we can suggest that more severe or advanced forms of CP bear a higher risk to develop exocrine insufficiency.

It is somewhat surprising that the anatomical classification of CP (presence or absence of large duct disease, inflammatory mass, or calcifications) and the different types of surgery did not influence the main outcome parameters. Since 1996, the type of surgery was always adapted to the underlying pathological condition (also in the 86 patients randomized for PPPD or DPPHR) with the intention to leave as much pancreatic tissue as possible after removal of the part potentially responsible for the complication leading to surgery. It is, therefore, possible that this adapted surgical strategy may be responsible for those comparable long-term results.

Reoperation or reintervention for pain or recurrence of organ complications was rarely necessary in our patients. As published before,[24] peptic ulcer disease, often in the form of jejunal ulcers, is a specific complication after PD (especially after PPPD) and required reoperation in two cases. Biliary complications occurred in a few patients because of stenoses of a bilioenteric anastomosis, recurrent CP, or insufficient biliary decompression during primary Frey operation.

Late mortality in patients with CP is clearly higher than in the normal population.[3,12,22,24] The 10-year survival in our patient group was even poorer than the data reported from the Mayo group,[25] although the median age of our patients was almost 4 years lower than in their study. Strate

et al.[12] from Hamburg reported a mortality rate of 24% of the patients during a follow-up of almost 9 years. Although patients with alcoholic CP were found to have a higher mortality rate this was not the case in our patients. The reasons for death were heterogeneous, and we had only a few patients dying from pancreatic cancer in our series. The high late mortality rate is probably mainly caused by a high comorbidity from alcohol abuse and smoking.[3,12,24]

During the last decade, several reports of the long-term outcome after surgery for chronic pancreatitis with follow-up periods between 34 and 104 months and relevant number of patients (57–504 patients) have been published.[12,13,16–18,20,24,26,27] One study randomized patients to undergo either the Frey or the Beger procedure; all other studies reported results of one or more types of surgery (including also drainage operations without resection;[16,17]) in a nonrandomized fashion.[12] Complete or substantial pain relief was reported in between 65 and 91%. The frequency of exocrine and endocrine insufficiency (total or postoperative de novo) was not reported in all of those papers. However, when data were available, between 30%[19] and almost 60%[12] of the patients had diabetes at the end of the observation period, in part dependent on the length of follow-up. In our patients, one-third of preoperatively nondiabetic patients developed de novo diabetes (25% of all patients), which is in the range of other reports. In analysis of the literature, it seems evident that the majority of postoperative de novo diabetes is not related to surgery itself, but develops during the later disease course even in patients without pain. Long-term exocrine insufficiency is reported at even higher rates than diabetes with frequencies up to 83%[12] in patients with long follow-up periods. As for diabetes, there seems to be no or less impact of surgery on the development of exocrine insufficiency, but rather an effect of longstanding disease as outlined by Ammann et al.[21]

There is still a controversial discussion about the appropriate type of surgery in CP, especially in those patients with large duct disease and absence of other organ complications, large inflammatory masses, or suspicion of malignancy. In North America, many centers prefer drainage procedures, whereas other centers in Europe promote resectional procedures. Arguments to favor resections may be a frequent failure of pain relief by drainage procedures reported in some studies.[6,21,28]

Because of rather comparable results especially regarding pain relief, the current literature, however, suggests that the choice of the type of surgery depending on the underlying complications other than pain (e.g., duct stenosis, portalvenous compression) and anatomy (size of the pancreatic duct, inflammatory masses) may be the most important measure to obtain these rather good outcome reported in most studies. In the only randomized study with a substantial follow-up time, the group from Hamburg found comparable long-term outcomes after the Frey or the Beger procedure after almost 9 years.[12] In our study, outcomes were comparable between the different procedures. During the initial learning curve of our group, more PDs were performed for CP predominantly of the pancreatic head. Outside of our randomized study performed between 1997 and 2001, we since then have chosen the type of head resection dependent on the presence and type of local complications with less extended operations (Frey) preferred when possible.

Conclusion

Pancreatic resection leads to adequate pain relief and control of organ complications in the majority of patients with CP. Long-term outcome does not depend on the type of surgical procedure, but is in part influenced by severe and/or advanced preoperative CP and by postoperative surgical complications (regarding pain). As reported before, however, the majority of patients develop exocrine and endocrine insufficiency unrelated to surgery. A few patients develop procedure-related late complications requiring reintervention. Late mortality is high, probably because of the high comorbidity (alcohol, smoking) in many of these patients.

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DISCUSSION

Dr. W. Nealon (Galveston, TX): Dr. Kaufman, Dr. Joseph, members and guests. The authors from Freiburg have contributed yet another thorough review of their outcomes in the management of chronic pancreatitis. They report today their experience with 272 resections, including pancreaticoduodenectomy, classic Whipple, and duodenum-preserving pancreatic head resection either by the Frey or Beger technique, and they provide detailed follow-up data, including measures of pain relief, functional derangements, and nutritional outcomes.

Their operative mortality of 1% is striking, particularly considering their inclusion of patients with portal vein or splenic vein thrombosis, which they have seen in 25% of their resected patients, and often this entity raises considerably the risk for hemorrhage in these resections. Complete abolition of pain was achieved in 60% and some reduction in pain was achieved in 86%. These are superb outcomes. Two-thirds of the patients had steatorrhea in follow-up, half acquired after surgery; 37% developed new-onset diabetes. Again, all these are acceptable rates. Notably, 86% of patients survived 5 years and 65% 10 years, reflecting the known chronic nature of this disease and the likely ongoing ravages of alcoholism. Nutritional improvements were noted in a high percentage of patients independent of their pancreatic function after operation, an observation we have made in the past, and a striking one, I believe.

I have three questions. Can you review with us your formula for choosing among the operations that are available for resection, particularly how you decide between a Frey's procedure and a Begar procedure?

Number two, you don't mention narcotics use, and I must say in my huge experience with these patients this overshadows every bit of patient management. I am wondering if you monitor this and whether you have any thoughts on the impact of narcotic dependence on the management of these patients after surgery?

And number three, regarding exocrine insufficiency, there are some mysteries you have exposed. It was shown patients with postop exocrine insufficiency correlated with persistent pain, and your patients with low pre-op BMI had a higher chance of developing exocrine insufficiency and a higher risk of persistent pain. Do you have any thoughts on the connection between this exocrine function and the pain?

I congratulate you and your colleagues on a thorough and superbly analyzed review of these very complex patients.

Dr. Makowiec: Thank you, Dr. Nealon. Your first question was about the choice of the type of operation.

After all the experience we have with our patients and our studies, we try to perform the less invasive or the smallest operation possible. That means, in ascending order of complexity, the Frey operation, the Beger operation and PD depending on the anatomy of chronic pancreatitis and the organ complications. For example, patients with pancreatic duct dilatation and a small or medium sized pancreatic head mass will receive a Frey operation. If a patient has a larger mass, a lot of fibrosis in the pancreatic head with concomitant bile duct stenosis, he will undergo Beger's procedure. In more than 50% of the Beger operations in our series a bilioenteric anastomosis was included, with good results in the vast majority regarding clearance of the bile ducts. If patients have a suspicion of malignancy or an enormous inflammatory mass of the pancreatic head with duodenal destruction, destruction of the antrum of the stomach, we perform a pylorus-preserving pancreaticoduodenectomy.

Regarding the second question, we have no reliable data on the use of narcotics before and after surgery. Preoperatively about one-fourth of the patients took opioids, and three-fourths of the patients had some form of peripheral analgesics. In our follow-up examinations we always asked for the use of narcotics and other analgetics, but I think that the results were not very reliable, again related to the use of alcohol. So we don't think that the data given by the patients regarding pain medications are scientifically reliable.

Your third question was about the correlation between pain, exocrine insufficiency, and body mass index. We saw that patients with exocrine insufficiency had, more frequently, pain. I think that this is a sign of advanced disease. Another reason is probably, especially patients with severe pain and frequently recurrent pain, the continuous use of alcohol. As for informations about pain medication, this information is also not very reliable. However, during many phone contacts with home physicians, we heard that in most cases with severe or frequent pain these patients continue to drink.

Low body mass index is probably a sign of severe and advanced chronic pancreatitis. These patients are malnourished just because they can't eat more due to abdominal pain.

Dr. L. Traverso (Seattle, WA): Frank, thank you for showing us all these details in just 10 minutes. I have three questions. When we reviewed our patients after a five-year follow-up following the Whipple operation for chronic pancreatitis those with diabetes preoperatively had better pain relief. Did you see that association?

Number two, we found it valuable to compare the patient's pre-op pain to their post-op pain and whether they had received some benefit from the operation. With that method we observed every patient indicated they were improved and 76% had complete pain relief.

Third, we all have to be accountable when deciding to resect the head of the pancreas in these patients with documented chronic pancreatitis and chronic abdominal pain. The best way is to make sure they really have severe chronic pancreatitis before resection. The first slide you showed on preoperative ductal anatomy indicated that about 87% or so had large duct disease. From that slide, a little over 10% of your patients could have had a normal pancreatic duct. Was that the case? Did they all have abnormal pancreatic ducts? The method that we used was the Cambridge Classification of Image Severity described by Axon (Axon ATR, Classen M, Cotton PB, et al. Pancreatography in chronic pancreatitis: international definitions. Gut 1984; 25:1107-1112). To qualify for resection a patient had to have the worst case of image severity, i.e., stage IV. The minimal disease required to have that category was a major pancreatic duct stricture in the head, with or without stones, with or without duct dilatation. When pancreatic surgeons are accountable that way then almost every patient after head resection for true chronic pancreatitis will get pain relief, provided they don't start drinking alcohol again.

Dr. Makowiec: Regarding our data, I can confirm some inverse correlation between the presence of diabetes and pain. We found that the absence of diabetes was a risk factor for pain. I have no explanation for this phenomenon.

Many of the patients who noted to have pain on their questionnaires also noted that they are satisfied with the operation because they had clearly less pain. Regarding pain assessment we had one problem: In 60% of our patients we had no complete preoperative pain documentation regarding frequency or a visual analog scale. So we can hardly compare the data.

About 20 % of our patients had no relevant dilatation of the pancreatic duct. However, most of those had inflammatory masses and/or bile duct stenosis. I, therefore, agree completely with you that pancreatic head resection may be appropriate in these cases, even without large duct disease.

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Efficacy of Preoperative Combined 18-Fluorodeoxyglucose Positron Emission Tomography and Computed Tomography for Assessing Primary Rectal Cancer Response to Neoadjuvant Therapy

Genevieve B. Melton · William C. Lavely · Heather A. Jacene · Richard D. Schulick · Michael A. Choti · Richard L. Wahl · Susan L. Gearhart

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Abstract
Purpose Efficacy of F-18 fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG-PET/CT) for determining neoadjuvant therapy response in rectal cancer is not well established. We sought to evaluate serial FDG-PET/CT for assessing tumor down-staging, percentage residual tumor, and complete response or microscopic disease with rectal cancer neoadjuvant therapy.
Methods Patients with rectal cancer undergoing neoadjuvant therapy, definitive surgical resection, and FDG-PET/CT before and 4–6 weeks after neoadjuvant treatment were included. Tumors were evaluated pretreatment and on final pathology for size and stage. FDG-PET/CT parameters assessed were visual response score (VRS), standardized uptake value (SUV), PET-derived tumor volume (PETvol), CT-derived tumor volume (CTvol), and total lesion glycolysis (δTLG).
Results Twenty-one rectal cancer patients over 3 years underwent neoadjuvant treatment, serial FDG-PET/CT, and resection. Complete response or microscopic disease (n=7, 33%) was associated with higher ΔCTvol (AUC=0.82, p=0.004) and ΔSUV (AUC=0.79, p=0.01). Tumor down-staging (n=14, 67%) was associated with greater ΔPETvol (AUC=0.82, p<0.001) and ΔSUV (AUC=0.82, p<0.001). Pathologic lymph node disease (n=7, 33%) correlated with ΔCTvol (AUC=0.75, p=0.03) and ΔPETvol (AUC=0.70, p=0.08).
Conclusion FDG-PET/CT parameters were best for assessing tumor down-staging and percentage of residual tumor after neoadjuvant treatment of rectal cancer and can potentially assist in treatment planning.

Keywords Rectal cancer · Neoadjuvant therapy · FDG-PET/CT

This work was presented in the plenary session of the 47th Annual Meeting of the Society for Surgery of the Alimentary Tract at the Digestive Disease Week in Los Angeles, CA on 24 May 2006.

G. B. Melton · R. D. Schulick · M. A. Choti · S. L. Gearhart (✉)
Department of Surgery, The Johns Hopkins Hospital,
600 North Wolfe Street,
Baltimore, MD 21287, USA
e-mail: sdemees1@jhmi.edu

W. C. Lavely · H. A. Jacene · R. L. Wahl
Department of Nuclear Medicine,
Johns Hopkins Medical Institutions,
Baltimore, MD, USA

Improved long-term outcomes in locally advanced rectal cancer have resulted from the use of multimodality therapies.[1–3] In particular, advanced locoregional primary rectal cancer management frequently involves neoadjuvant chemotherapy and/or radiation therapy followed by definitive surgical tumor resection.[2] Despite these advances, up to 38% of patients with rectal cancer will eventually die having recurrent disease.[4,5] This outcome may be in part caused by inaccurate disease staging, which in turn effects treatment decisions. With respect to management of primary disease, accurate staging of the response to neoadjuvant therapy can potentially help to direct surgical therapy, prognosticate long-term outcomes, or modify chemotherapy or radiation treatment in those patients with less than optimal response.

Presently, assessment of rectal cancer extent can incorporate several types of diagnostic testing. Conventional staging modalities include digital rectal examination, spiral computed tomography (CT), magnetic resonance imaging (MRI), and endorectal ultrasound (TRUS). Local staging of rectal cancer with TRUS or MRI with endorectal coil for the initial tumor provides an accuracy of 82% for bowel wall invasion (T-staging) and 70% for lymph node involvement (N-staging).[6] Although CT can be somewhat limiting with respect to pelvic disease evaluation, spiral CT can accurately detect up to 85% of metastatic hepatic lesions >1 cm.[7]

The use of F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) has been accepted for the detection of recurrent or metastatic colorectal cancer.[8] FDG-PET has been demonstrated to accurately detect pelvic recurrence ranging from 74 to 96%.[9,10] In addition, FDG-PET combined with CT (FDG-PET/CT) appears to have better accuracy for detecting locally recurrent and metastatic colorectal cancer when simultaneous CT was performed.[11,12]

Recently, FDG/PET was demonstrated to have a complementary role to conventional imaging modalities for the initial staging of rectal cancer. We have previously shown that the use of pretreatment FDG-PET/CT in the initial staging of rectal cancer resulted in a deviation in the proposed treatment plan in 27% of patients.[13] Alterations in treatment planning included radiation fields and the extent of surgical resection.

The goal of this study was to assess the ability of serial FDG-PET/CT to be used as a diagnostic tool for tumor response to neoadjuvant therapy, which may further alter the management of this disease. Specifically, the ability of FDG-PET/CT to discriminate tumor down-staging, microscopic disease or complete responders, presence of lymph nodes, and the percentage of pathological response in resected specimens of advanced primary rectal cancer was evaluated.

Patients and Methods

From May 2003 to March 2006, all patients with previously untreated, biopsy-proven adenocarcinoma of the rectum were identified from the Johns Hopkins Colorectal Cancer Database, which is a Johns Hopkins Medical Institutions Institutional Review Board-approved database to study outcomes in colorectal cancer. Patients were included if they had known locoregional disease, neoadjuvant chemotherapy, and/or radiation therapy before definitive surgical resection, as well as two serial FDG-PET/CT scans performed before and after neoadjuvant therapy at our institution. Those patients with early tumors (T1 or T2) without nodal disease and patients who had previous chemotherapy or radiation therapy were excluded. Radiation therapy consisted of 4,500 cGy to the true pelvis with a boost of 540 cGy to the tumor. Chemotherapy consisted of concurrent continuous infusion 5-FU or capecitabine with radiation, or 5-FU, leucovorin +/− oxaliplatin.

Patients underwent initial-staging TRUS or with MRI of the pelvis, as well as initial FDG-PET/CT before the induction of neoadjuvant chemotherapy or radiation therapy. Patients then underwent a second FDG-PET/CT scan 4–6 weeks after completion of neoadjuvant therapy and rectal cancer resection approximately 4 weeks after this. Initial locoregional staging along with final pathological analysis were used as the gold standard of locoregional tumor response.

Initial Tumor Staging

On initial evaluation, all tumors were staged locally with TRUS or with MRI of the pelvis with an endorectal coil. In all cases, TRUS was performed by a single investigator. Patients were placed in the left lateral position and a 10-Mhz crystal on an ultrasound probe (model 1846; Bruel and Kjaer, Marlboro, MA) was inserted into the anorectum. The rectal tumor was evaluated for distance from the anal verge, degree of bowel wall thickening (T stage), and lymph node disease within the mesorectum (N stage). Tumors were classified as low (≤6 cm), mid (7–10 cm), and high (≥10 cm) according to their distance from the anal verge. Although there is inconsistency among clinical studies regarding the classification of low, mid, and high lesions, the classification used in this study was based on the location of the three valves of Houston at approximately 7, 10, and 12 cm from the anal verge. As such, the valves of Houston served as additional confirmatory markings for determining the distance of the tumor from the anal verge.

In addition to local staging, patients underwent a triple-phase standard spiral CT scan of the chest, abdomen, and pelvis for evaluation of distal metastasis. Stages were reported using the tumor-node-metastasis classification according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual.[14]

PET/CT Imaging

Patients underwent a FDG-PET combined with nonintravenous contrast CT using standard protocols. Specifically, all patients underwent a 4-h fast before the examination, although water intake was allowed. Blood glucose level was checked in patients, and the study was not performed if the blood glucose exceeded 150 mg/dL. Patients were then initially injected with 15 to 20 mCi of FDG intravenously and then drank oral CT contrast lacking glucose with a 13% barium concentration. In the supine position, patients were imaged from the skull base to the mid thigh with the GE Discovery LS PET/CT system (GE Medical Systems, Milwaukee, WI). Simultaneous noncontrast CT images were

obtained generally at 140 kV, 80 mA, and 0.8 s per CT rotation, with a pitch of 6 and a table speed of 22.5 mm/s.

Pathologic Assessment

Pathologic tumour, node, metastasis (TNM) staging was performed on a retrospective basis on the surgical specimen to assess for tumor down-staging and lymph node status. Tumor response was assessed based on tumor viability and the extent of fibrosis and inflammation. A grading scale, pathological response score (PRS), was used to characterize the extent or percentage of tumor with response: grade 0: no response; grade 1, <33%; grade 2, 34–66%; grade 3, 67–95%; and grade 4, 96–100%. This grading scale is derived from a validated measure of pathologic response in patients with nonsmall-cell lung cancer receiving chemotherapy.[15]

Imaging Interpretation

FDG-PET/CT scans were interpreted at a workstation where images could be displayed with or without attenuation correction for PET with the registered noncontrast CT scan. Findings were determined and qualified by an experienced nuclear medicine physician who was blinded to the results of the pathologically assessed response to therapy and to other clinical details.

The standard uptake value (SUV) is defined as the uptake of FDG-18 normalized by the administered injected FDG dose and by the weight of the patient, using the following equation:

$$\mathrm{SUV} = \frac{\text{Decay corrected dose (mCi)/tumor (mL)}}{\text{Injected dose (mCi)/body weight (g)}}.$$

SUV_{max} was determined using the maximal pixel value in a region. ΔSUV_{max} has been recommended by the 1999 European Organization for Research and Treatment of Cancer position paper as a method to assess metabolic response of tumors with FDG-PET.[16] ΔSUV_{max} was calculated using the SUV_{max} results of the pretreatment and posttreatment scans to calculate the percent decreased between SUV_{max} on the posttreatment scan versus the pretreatment scan.

In addition to ΔSUV_{max}, the PET-derived tumor volume (PETvol) and CT-derived tumor volume (CTvol) were calculated on pre- and posttreatment studies, and the percent change in volumes were calculated: ΔPETvol and ΔCTvol, respectively. To determine the ΔPETvol, in each patient the background FDG activity within the liver served as the lower limit of FDG activity. Then using a software program developed by GE medical systems, the volume of PET activity in a particular lesion of interest was calculated and compared between scans. Primary tumor response was the only variable able to be addressed. Similarly, to determine ΔCTvol, the volume of abnormal tissue on noncontrast CT before and after neoadjuvant therapy was compared.

Change in total lesion glycolysis (δTLG) is analogous to the change in metabolically active tumor and can be considered a global measure of the response of the entire tumor to treatment.[17] To compute δTLG, the ratio of the posttreatment tumor metabolic rate over the pretreatment tumor metabolic rate was calculated. If one assumes that there is no change in glucose arterial blood concentration and body composition, the PETvol can be multiplied by SUV to calculate the percent δTLG during the interval of neoadjuvant treatment:

$$\delta\mathrm{TLG} = \frac{\mathrm{SUV_{post}} \times \mathrm{PETvol_{post}}}{\mathrm{SUV_{pre}} \times \mathrm{PETvol_{pre}}} X100$$

Visual response score (VRS) is a visually graded response measure, which represents the global response of the entire tumor. Specifically, VRS was assessed as a percentage of response of tumor to neoadjuvant therapy by comparing pretreatment and posttreatment images using the following scale: 0, no response or disease progression; 1, <33%; 2, 34–66%; 3, 67–95%; and 4, 96–100% response.

Statistical Analysis

Statistical analysis was performed using SAS® (Cary, NC) software. Statistics were considered significant for p value ≤0.05. Differences in continuous diagnostic variables for each categorical outcome measure were assessed using receiver operating characteristic (ROC) analysis. ROC can be conceptualized as the ability of a diagnostic test or clinician to discriminate a particular dichotomous outcome.[18] For example, in this study ROC analysis was performed using ΔSUV (the diagnostic test) to assess its ability to discriminate tumor down-staging (the outcome). A higher ROC area under the curve (AUC) indicates a better discriminatory power. An AUC of 0.50 indicates that the test is as good as random chance for discriminating an outcome, whereas an AUC of 1.0 indicates perfect discrimination of the test (sensitivity and specificity of 100%). Whereas, AUC indicates the overall ability of the parameter to predict a particular outcome, different points in the ROC graphical curve elucidate sensitivity, specificity, and likelihood ratios for particular threshold values of the test. According to which characteristics of the test are most important (i.e., low false positives or high sensitivity), different cutoff values in the curve may be optimal. In our analysis, the variables with the highest AUC with respect to tumor down-staging, minimal microscopic disease only or no residual disease, and presence of lymph nodes on final

pathology were analyzed to delineate sensitivity, specificity, positive likelihood ratio (LR), obtaining optimal threshold values to maximize the positive likelihood ratio (LR). Correlations between variables and PRS were made using Spearman's rho coefficient of rank correlation.[19]

Results

Patient Characteristics

From May 2003 to March 2006, 21 patients with primary rectal cancer (13 male, median age 61 years) underwent neoadjuvant treatment (2 chemotherapy, 2 radiotherapy, 17 chemoradiotherapy) and serial FDG-PET/CT (Table 1). Two patients received only radiotherapy because of intolerance to chemotherapy, and two received only chemotherapy because of distant metastatic disease. Using TRUS (n=20) and MRI (n=1), preoperative locoregional staging demonstrated uT2N1 (3, 14%), uT3N0 (3, 14%), uT3N1 (11, 52%), cT3N2 (1, 5%), uT4N1 (2, 10%), and uT4N2 (1, 5%). Tumors were localized as low (n=14), mid (n=5), and high (n=2). Patients underwent definitive surgical treatment with the following procedures: abdominoperineal resection (n=5) and low anterior resection (n=16). Table 2 depicts the tumor and nodal down-staging observed in the study group.

Table 1 Patient Characteristics

	n (%)
Total	21 (100)
Male	13 (62)
Age (Median in years±Std. dev)	61±12
Neoadjuvant therapy	
Chemotherapy alone	2 (10)
Radiotherapy alone	2 (10)
Both chemotherapy and radiotherapy	17 (80)
Location of Tumor (cm from anal verge)	
Low (0–6 cm)	14 (66)
Mid (7–10 cm)	5 (24)
High (>10 cm)	2 (10)
CEA level before neoadjuvant therapy (median±Std. dev)	4±11
Pre-operative stage of tumor	
uT2N1	3 (14)
uT3N0	3 (14)
uT3N1	11 (52)
T3N2	1 (5)
uT4N1	2 (10)
uT4N2	1 (5)
Type of surgery	
Low anterior resection	16 (76)
Abdominoperineal resection	5 (24)

Table 2 Tumor and Nodal Locoregional Down-staging with Neoadjuvant Therapy

		Initial staging with transrectal ultrasound*				
		uT2	uT3	uT4	uN0	uN1
Final Pathological Staging	pT0	1	4	–		
	pT1	2	2	–		
	pT2	–	6	1		
	pT3	–	2	2		
	pT4	–	1	–		
	pN0				3	11
	pN1				-	7

*Single patient was staged using MRI.

Minimal Microscopic Disease Only or Complete Pathological Response

On final pathological evaluation, 33% of patients (n=7) demonstrated either a complete pathological response or had only minimal microscopic disease (5 complete response, 2 microscopic disease) (Table 3). Overall, the percentage of residual tumor in resected specimens (PRS 0–4) correlated best with ΔCTvol (r=0.62, p=0.003), VRS (r=0.58, p=0.006), and ΔPETvol (r=0.57, p=0.007) (Table 4). All tumors had a high SUV_{max} before neoadjuvant therapy, the average value being 14.4 (±6.2), which fell significantly to 5.2 (±3.3) after treatment (P<0.001). The presence of microscopic disease or no residual disease was associated with higher average ΔSUV (75% vs. 57%; AUC=0.79, p=0.01) and ΔCTvol (79% vs. 37%; AUC=0.82, p=0.004). Taking the two most highly associated variables, ROC analysis demonstrated optimal cutoff values for ΔCTvol of greater than 80% (LR 10.0, sensitivity 71.4%, specificity 100%) and ΔSUV of greater than 75% (LR 5.57, sensitivity 85.7%, specificity 84.6%; Table 5). This is depicted graphically using an ROC curve (Fig. 1).

The likelihood ratio was used to help estimate positive predictive value (PPV), also known as the posttest probability, for ΔCTvol and ΔSUV as diagnostic tests for complete pathological response or only minimal microscopic disease. Best estimates of the percentage of patients who will be complete responders is between 20 and 30% with current regimens,[1,20–22] although higher rates have been reported with oxaliplatin-based chemoradiation. Using 30% as an estimate of pretest probability, the PPV from Bayes theorem can be directly calculated using the likelihood ratio. For ΔCTvol >80% with LR 10, the PPV was 81%, Similarly for a ΔSUV >75% with LR of 5.57, the PPV was 70% for correctly being able to discriminate patients with microscopic disease or complete response. Figure 2 depicts a nomogram for these relationships.

Table 3 Mean Values of FDG-PET/CT Variables for Tumor Down-staging, Microscopic Disease or Complete Responders, Lymph Nodes

	Tumor down-staging			Microscopic disease only/ complete responders			Metastatic lymph nodes on final pathology		
	Yes 14 (67%)	No 7 (33%)	AUC (p value*)	Yes 7 (33%)	No 14 (67%)	AUC (p value*)	Yes 7 (33%)	No 14 (67%)	AUC (p value*)
ΔCTvol	69%	14%	0.79 (0.005)	79%	37%	0.82 (0.004)	16%	68%	0.75 (0.03)
ΔPETvol	69%	12%	0.82 (<0.001)	66%	42%	0.66 (0.22)	17%	67%	0.70 (0.08)
ΔSUV	72%	44%	0.82 (<0.001)	75%	57%	0.79 (0.01)	49%	69%	0.70 (0.10)
δTLG	83%	17%	0.70 (0.10)	89%	55%	0.65 (0.27)	21%	81%	0.55 (0.74)
VRS	2.9	1.7	0.75 (0.02)	3.1	2.2	0.73 (0.06)	1.9	2.9	0.67 (0.15)

AUC = Area under the Receiver Operating Characteristic curve
*p value, probability that AUC equals 0.5

Tumor Down-staging

Tumor down-staging from the initial clinical stage to the pathological final stage occurred in 67% (n=14) of patients. Tumor down-staging was most strongly associated with a greater average ΔSUV (72% vs. 44%; AUC=0.82, p< 0.001) and ΔPETvol (69% vs. 12%; AUC=0.82, p< 0.001). Furthermore, VRS correlated significantly with tumor response by down-staging (AUC=0.75, p=0.02) and PRS (r=0.58, p=0.006) only. ROC analysis demonstrated that a decrease in the SUV_{max} of >70% was associated with an optimal likelihood ratio (LR) of 3.86 (sensitivity 64.3%, specificity 100%). If PETvol decreased by >75%, down-staging of the primary tumor on final pathology was associated with an optimal LR of 5.5 (sensitivity 78.6%, specificity 85.7%).

Lymph Node Involvement

None of the FDG-PET/CT parameters on initial scanning (SUV_{max}, PET_{vol}, or CT_{vol}) correlated with the presence of positive lymph nodes on staging TRUS before induction therapy. There were seven patients (33%) who had lymph node metastasis present in the final pathology specimen. The finding of metastatic lymph nodes on final pathology was associated only with less average ΔCTvol (16% vs. 68%; AUC=0.75, p=0.03) and trended toward significance with less ΔPETvol (17% vs. 67%; AUC=0.70, p=0.08).

Table 4 Correlation of FDG-PET/CT Variables with Degree of Pathologic Response using Pathologic Response Score

	Pathologic response score (PRS) Correlation coefficient (p value)
ΔCTvol	0.62 (0.003)
ΔPETvol	0.57 (0.007)
ΔSUV	0.52 (0.02)
δTLG	0.49 (0.03)
VRS	0.58 (0.006)

Optimization of the ROC curve for likelihood ratios demonstrated that a decrease in CTvol and PETvol of less than or equal to 50 and 70%, respectively, were associated with LR 8.0 (sensitivity 57.1%, specificity 92.9%) and 2.5 (sensitivity 71.4%, specificity 71.4%), although the sensitivity was poor with ΔCTvol (57.1%) and the likelihood ratio of ΔPETvol was small (LR=2.5).

Discussion

In the current study, serial FDG-PET/CT parameters show potential for discriminating tumor response to neoadjuvant therapy for primary rectal cancer. Specifically, FDG-PET/CT parameters were most effective at discerning the presence of minimal microscopic disease only or complete pathological response and tumor down-staging, but were less effective with lymph node status. When the most significant parameters were examined for each outcome, we were able to use ROC analysis to derive optimal likelihood ratios for each of the main outcomes with the three variables of ΔSUV, ΔCTvol, and ΔPETvol. Interestingly, analyzing tumor response to neoadjuvant therapy using a subjective visual response score by one blinded radiologist was only effective in determining the presence of tumor down-staging and degree of tumor pathological response. As such, the use of a combination of three parameters (ΔSUV, ΔCTvol, and ΔPETvol) has the potential for use as a predictive model for neoadjuvant therapy response in primary rectal cancer.

While TRUS and MRI have both been demonstrated to have great accuracy for assessing initial tumor staging, these modalities are much less effective in staging rectal tumors after radiation therapy and were not used in our study design to assess response to neoadjuvant treatment.[23,24] As such, the use of FDG-PET has emerged as a promising modality to assess neoadjuvant therapy response. Several investigators have demonstrated that serial FDG-PET may be effective at assessing response to neoadjuvant

Table 5 Receiver Operating Characteristic Analysis for Most Highly Associated FDG-PET/CT Parameters for Each Outcome

Outcome measure	FDG-PET/CT parameter	Cutoff value[a] (%)	Sensitivity (%)	Specificity (%)	LR+
Tumor down-staging	ΔPETvol	>75	78.6	85.7	5.50
	ΔSUV	>70	64.3	100.0	3.86
Microscopic disease only/complete responders	ΔCTvol	>80	71.4	100.0	10.0
	ΔSUV	>75	85.7	84.6	5.57
Metastatic lymph nodes on final pathology	ΔCTvol	≤50	57.1	92.9	8.00
	ΔPETvol	≤70	71.4	71.4	2.50

LR+, positive likelihood ratio.
[a] Optimal cutoff values for each parameter maximizing the positive likelihood ratio of the outcome

therapy for rectal cancer and predicting long-term outcomes.[25–27] In an analogous fashion, FDG-PET has emerged as an initial staging tool and as a modality to assess neoadjuvant response with other malignancies.[28–30]

FDG-PET with CT appears to be superior to FDG-PET alone for detecting locally recurrent and metastatic colorectal cancer.[11,12] Several reports looking at other malignancies have also demonstrated greater utility of using FDG-PET with CT scanning as compared to FDG-PET alone.[31–34] Data in the use of combined FDG-PET with CT in initial staging and restaging of primary rectal cancer is limited. We have previously demonstrated that pretreatment FDG-PET/CT in the initial staging of rectal cancer resulted in a deviation in the proposed treatment plan in 27% of patients.[13] Few have reported on the efficacy of FDG-PET/CT in rectal cancer for assessing neoadjuvant therapy response.

We focused on three outcomes in this study: tumor down-staging, lymph node status, and complete response or microscopic disease only. These variables have each been demonstrated to have clinical significance. Although the current study does not have a large enough sample size to generalize FDG-PET/CT parameter correlation with tumor downstaging, effective preoperative neoadjuvant therapy for distal rectal carcinomas with tumor down-staging has been associated with very high rates of sphincter-preserving surgery without local failure.[35,36] Although more definitive studies are needed,[37] sphincter-preserving surgery has been associated with enhanced patient quality of life.[38–40] Furthermore, newer data have suggested that survival after a more radical resection is comparable to less radical procedures (transanal excision) or nonoperative management in patients with complete pathological response after neoadjuvant therapy.[41] Depending on the neoadjuvant therapy given, up to 30% of patients will have a complete pathological response, which in many series is associated with better survival rates.[1,20–22] These data are supported by the finding that patients with a complete pathological response or microscopic disease only after neoadjuvant therapy have improved overall and disease-free survival such that the 5-year overall survival for these patients approaches 95%.[4,42] Whereas ideally we would have been

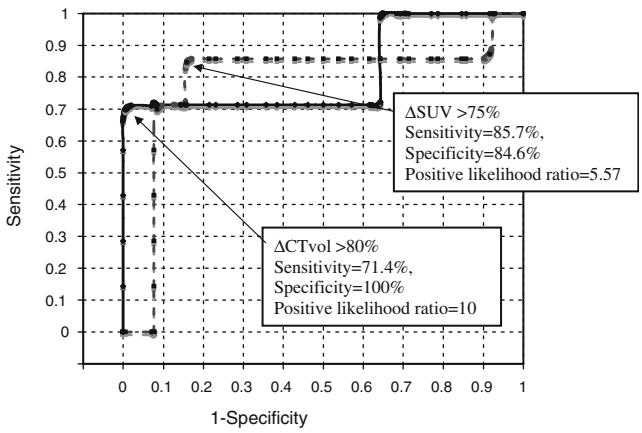

Figure 1 FDG-PET/CT scan with axial and sagittal views before (a) and after (b) neodjuvant therapy demonstrating of patient response to neoadjuvant therapy.

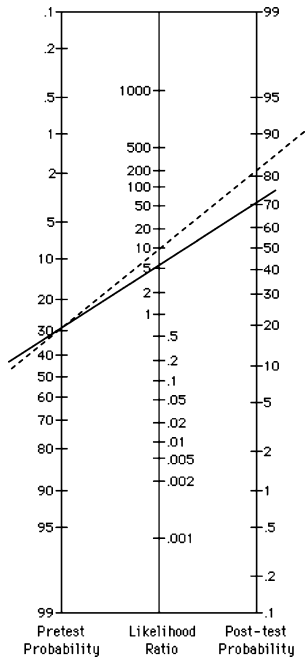

Figure 2 Receiver-operating characteristic curve of ΔCTvol (solid) and ΔSUV (dashed) for the detection of complete responders/microscopic disease only. With a threshold of >80% for ΔCTvol, positive likelihood ratio was optimized, with a sensitivity of 71.4%, specificity of 100%, and positive likelihood ratio of 10. Using a threshold of >75% for ΔSUV, the positive likelihood ratio was 5.57, with a sensitivity of 85.7% and specificity of 84.6%.

able to stratify patients according to microscopic disease only, complete responders and the combination of the two, the two were combined only for our analysis because of small patient numbers in this study. Similarly, the presence of lymph nodes after neoadjuvant therapy has been associated with worse long-term outcomes, with respect to disease-free and overall survival.[4] We have previously noted that FDG-PET/CT found evidence of lymph node metastases far more often that conventional CT.[13] Whereas serial FDG-PET/CT parameters would ideally predict lymph node status, it is not surprising that the presence of lymph nodes was not well predicted by serial FDG-PET/CT, as these are often microscopic. If it is proven in future studies that clinicians can better predict response with accurate serial staging with neoadjuvant regimens using FDG-PET/CT or other staging modalities, then serial FDG-PET/CT and perhaps some combination of other studies could potentially be used to improve local control rates, sphincter preservation rates, and possibly survival by allowing for patient-tailored oncologic treatment, including altering surgical management and the creation of neoadjuvant protocols customized according to the type of response detected on serial imaging.

Importantly, this study demonstrates that a decrease in the VRS was only associated with tumor down-staging and not with lymph node status or the achievement of a complete pathological response or microscopic disease only. FDG-PET/CT parameters of ΔSUV, ΔPETvol, and/or ΔCTvol were, however, effective for discriminating tumor down-staging, complete pathological response/microscopic disease, and the presence of metastatic lymph nodes on final pathology.

The parameter ΔCTvol was used in our study as another means to assess changes to the tumor after neoadjuvant therapy. Indeed, this parameter was significantly altered for all outcomes studied. This finding would suggest that it is not necessarily the FDG-PET that provides prognostic information, but that the associated CT is most important in defining tumor response. In fact, this finding would suggest that CT alone is effective in determining response. However, our study has not effectively removed the bias of the knowledge of the registered FDG-PET/CT findings by the reviewing radiologist when determining the ΔCTvol. Another work at our institution[7] has demonstrated that CT findings alone are limited with respect to assessment of pelvic disease. Our data would suggest that FDG-PET alone would not be as effective at determining tumor response as combined FDG-PET/CT.

The results of this study are limited by the small number of patients in the study. Because of the small numbers in this study, the results of this study at this time do not warrant a change in clinical management based on serial FDG-PET/CT results for patients undergoing neoadjuvant therapy for rectal cancer. We were also unable to distinguish response on serial FDG-PET/CT for various neoadjuvant regimens. Furthermore, this study does not assess whether response by FDG-PET/CT predicts long-term outcome. We chose to evaluate serial FDG-PET/CT results by looking at this modality before and after neoadjuvant treatment. A potential greater benefit may be achieved with regards to treatment alterations if a second FDG-PET/CT were performed at an earlier time point such that chemoradiation therapy might be modified. We plan to take the analysis from this study to prospectively validate each of the most strongly associated parameters for tumor down-staging, microscopic disease or no residual disease, and lymph node status with a large patient cohort. If these measures can be demonstrated in a prospective fashion to be effective predictors of response, surgeons might be able to modify surgical treatment to less-invasive, sphincter preserving treatments.

Conclusion

Serial FDG-PET/CT parameters are effective for assessing tumor down-staging, complete responders or those with only microscopic disease, percentage of residual tumor after preoperative treatment of primary rectal cancer, and, to a lesser extent, lymph node status. Additional studies are needed to confirm the efficacy of FDG-PET/CT for determining tumor response to neoadjuvant therapy for primary rectal cancer. Accurate assessment of tumor response to neoadjuvant therapy can potentially assist in treatment planning for rectal cancer.

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DISCUSSION

Dr. M. Dayton (Buffalo, NY): That was a very nice presentation, Dr. Melton. I would like to thank the authors for the opportunity to review the manuscript in advance of the meeting.

Dr. Melton and her colleagues have conducted this study to ascertain the value of PET–CT in assessing the response of rectal cancer to neoadjuvant therapy. Most of us in the recent past have used PET–CT or PET scans to assess patients for recurrence or metastatic disease. That has been its major application for the majority of surgery. This work represents a possible new use of PET–CT, particularly if it is found to accurately predict downstaging, complete response, and nodal status. The results of your study seem to suggest that it does that at least in two of the three categories, downstaging and complete response. It probably isn't quite as good in predicting nodal status. The study also seems to corroborate a previous study from your group that found that 27% of the time using this modality changes the strategic plan for therapy. An example might be downstaging an APR to a sphincter–sparing operation. This technique does have the potential to impact our management of rectal cancer, and I applaud the authors for conducting the study.

Just a couple of questions and maybe a minor criticism of the design. First, the PET–CT scan obtained post–neoadjuvant therapy was done four to six weeks post–operation, but surgery wasn't done for four weeks beyond that. Why not get the PET–CT scan 8 to 10 weeks post–treatment instead of 4 to 6? It seems like it might more accurately predict the impact of the neoadjuvant therapy.

Second, I noticed in the manuscript that there were multiple, different chemotherapy regimens. Some patients just received 5–FU or capecitabine, others got 5–FU, leucovarin, and oxaliplatin. In a small study like this in which there are multiple regimens, it seems that that would muddy the waters quite a bit. The authors ought to either have a larger N or limit the study to one regimen. Similarly, it seems that patients who had radiation only or chemo only ought to be eliminated from your study group.

Third, was there any difference in response to neoadjuvant therapy based on the level of the rectal cancer? It might be a little bit early to predict that.

And finally, 67% of the patients responded to neoadjuvant therapy. Is it your experience that those that don't respond at all have a poorer prognosis?

Again, I very much enjoyed the paper. I think it outlines a possible new use for PET CT scanning in rectal cancer.

Dr. Melton: Dr. Dayton, thank you for reviewing our manuscript and also for your insightful comments and questions.

The first question was specifically about the timing of the FDG–PET–CT scans. There are studies in the radiation oncology literature that you get maximal tumor shrinkage all the way out to 12 weeks. Therefore, one could argue that you could do it later, and that may be helpful for surgery planning. We believe that not only is four to six weeks a reasonable time to repeat the FDG–PET/CT but also repeating it earlier may be helpful for other parts of treatment planning. For example, if a FDG–PET–CT scan is completed a few weeks into a regimen, at that point, depending on the results of the scan you might be able to actually alter the neoadjuvant regimen. There is some evidence in the nuclear medicine literature that doing the scan earlier actually may be better. Both rationales make sense. The protocol that we have been using is the four to six weeks, and that I think is partially historical where tumor downstaging and tumor response was found to be maximized in some studies early on at about six weeks.

The next question was about different neoadjuvant regimens, specifically chemotherapeutic regimens, that patients were on. The authors acknowledge that this is a limitation of the study. I think this study can be strengthed if we conduct a follow-up study on a prospective basis and actually validate these measures. Another way to make the cleanest study would be, as you are saying, to make it a single regimen to take out those confounders.

The third question was about level of tumor and differences in response, and we did not see any difference in response according to the level of tumor.

The final question was about prognosis with the non-responders, and we don't actually have that data. We do know that those that do not respond generally don't do as well and that the two strongest markers for having better long–term disease–free and overall survival are going to be lymph node status and having a complete pathological response.

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Echogenic Appearance of Colorectal Liver Metastases on Intraoperative Ultrasonography is Associated with Survival After Hepatic Resection

Michelle L. DeOliveira · Timothy M. Pawlik · Ana L. Gleisner · Lia Assumpcaom · Gaspar J. Lopes-Filho · Michael A. Choti

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Abstract Survival after resection of colorectal liver metastases has traditionally been associated with clinicopathologic factors. We sought to investigate whether echogenicity of colorectal liver metastasis as assessed by intraoperative ultrasound (IOUS) was a prognostic factor after hepatic resection. Prospective data on tumor IOUS appearance were collected in 84 patients who underwent hepatic resection for colorectal liver metastasis. Images were digitally recorded, blindly reviewed, and scored for echogenicity (hypo-, iso-, or hyperechoic). The median tumor number was 1 and the median tumor size was 5.0 cm. At the time of surgery, the IOUS appearance of the colorectal liver metastases were hypoechoic in 35 (41.7%) patients, isoechoic in 37 (44.0%) patients, and hyperechoic in 12 (14.3%) patients. Traditional clinicopathologic prognostic factors were similarly distributed among the three echogenicity groups (all $p>0.05$). Patients with a hypoechoic lesion had a significantly shorter median survival (30.2 months) compared with patients who had either an isoechoic (53.2 months) or hyperechoic (42.3 months) lesion ($p=0.005$). The 5-year survival after hepatic resection of colorectal liver metastasis was also associated with the echogenic appearance of the lesion (hypoechoic 14.4 vs isoechoic 37.4 vs hyperechoic 46.2%) ($p<0.05$). Intraoperative ultrasound echogenicity should be considered a prognostic factor after hepatic resection of metastatic colorectal cancer.

Keywords Colorectal metastases · Intraoperative ultrasound · Echogenicity · Prognosis

Introduction

The liver is the most common site of colorectal metastases, with approximately half of all colorectal patients eventually developing liver lesions.[1] Hepatic resection remains the only potentially curative therapeutic option with 5-year survival rates ranging between 26 and 58%.[2–5] Currently, there are few criteria for predicting which patients will derive greater benefit from liver resection and which will have early recurrence. Clinicopathologic factors were used to predict patient survival after hepatic resection.[6–8] Traditionally, primary tumor stage, preoperative carcinoembryonic antigen (CEA) level, hepatic tumor size, number of liver metastases, time from primary tumor treatment to diagnosis of hepatic metastases (disease-free interval), and presence of extrahepatic disease were reported to be predictors of survival after resection.[6,7] However, data regarding the prognostic importance of these clinicopathologic factors are inconsistent and conflicting.[9] As such, there has been no complete agreement concerning which clinicopathologic factors are the most relevant to prognosis after resection.

Recently, echogenicity of colorectal liver metastases on intraoperative ultrasonography (IOUS) was reported to be an independent prognostic factor of survival.[10–12] Specifically, Gruenberger et al.[10] reported that echogenicity of liver metastases from colorectal cancer was associated with

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M. L. DeOliveira · T. M. Pawlik · A. L. Gleisner · L. Assumpcaom · G. J. Lopes-Filho · M. A. Choti (✉)
Department of Surgery, The Johns Hopkins University School of Medicine,
600 North Wolfe Street, Halsted 614,
Baltimore, MD 22187-6681, USA
e-mail: mchoti@jhmi.edu

long-term outcome in patients treated surgically. However, in this study only a minority of patients (n=52) were treated with resection alone, whereas the majority of patients (n=91) were treated with resection combined with cryotherapy to lesions not amenable to further resection. Although echogenicity was confirmed to be prognostically important in patients with colorectal liver metastases treated with regional chemotherapy,[11] to our knowledge, the prognostic role of echogenicity after surgical resection has not been independently corroborated. Given this, the objective of the current study was to validate echogenicity of colorectal liver metastases as a prognostic factor. More specifically, we sought to evaluate the relative prognostic power of echogenicity compared to other standard clinicopathologic factors in a large cohort of patients who had undergone hepatic resection alone (e.g., not combined with ablative therapy) for colorectal metastases.

Patients and Methods

Data on the IOUS appearance of colorectal liver metastases were collected prospectively on 147 patients at Johns Hopkins Hospital between 1998 and 2004. All patients included in the study underwent resection and IOUS by a single surgeon (M.C.). Only patients with colorectal liver metastasis confirmed on histology were included in the study. Patients who underwent resection combined with ablative therapy, who underwent resection combined with regional chemotherapy pump placement, or who had concurrent extrahepatic disease were excluded from consideration, thereby leaving 84 patients for further analyses. The study was approved by the Johns Hopkins Institutional Review Board.

The following data were collected for each patient: demographics; primary tumor site; preoperative CEA; history of preoperative chemotherapy; tumor size, number, and location; operative details; and echogenicity of liver lesions. Specifically, IOUS of the liver was performed in each case using a 4.0- to 8.0-MHz curvilinear transducer probe (Phillips ATL HDI-5000). The IOUS was performed according to a well-established protocol as previously described by our group.[13,14] Metastases were imaged in both the longitudinal and transverse planes to define the relation of the lesion to surrounding structures such as the portal or hepatic veins and biliary structures. Ultrasound settings were kept consistent for image capture using standard presets, including (1) the depth of field, (2) the location of focal zones, (3) the gain setting, (4) the scan orientation, and (5) the image zoom settings.[14] In each case, all evaluable IOUS images were digitally recorded and coded randomly. Images were retrieved and two reviewers who were blinded to the clinical and operative details graded the echogenic appearance of the metastases: hypoechoic, isoechoic, or hyperechoic. In case of disagreement between the reviewers, the sonographic images were reevaluated for a final consensus. For patients with more than one metastasis, the tumor echogenicity was based on the average scored echogenicity of all measured lesions.

Summary statistics were obtained using established methods. Student's t test was used for comparison of continuous variables. The χ^2 test was used to compare categorical variables. The odds ratio (OR) and the 95% confidence intervals (CI) were estimated and a P value <0.05 was considered to be statistically significant. Survival was analyzed using the Kaplan–Meier method (univariate log rank) and the Cox proportional hazards model (multivariate). All statistical analyses were performed using SPSS version 11.5 (Chicago, IL, USA).

Results

Table 1 shows the clinicopathologic features of the 84 patients in the study. There were 44 (52.4%) men and 40 (47.6%) women. The median patient age was 64 years old (range 22–87 years). The primary tumor site was colon in 59 (70.2%) patients and rectal in 25 (29.8%) patients. The median number of hepatic metastases per patient was 1 (range 1–6) and the median size of the largest hepatic lesion was 5.0 cm (range 1.5–9.0 cm). The majority [n=54 (64.3%)] of patients were treated with neoadjuvant chemotherapy before surgical resection. At the time of surgery, 14 (16.7%) patients underwent a nonanatomical wedge resection, 29 (34.5%) patients underwent an anatomical segmentectomy, whereas 41 (48.8%) underwent either a hemihepatectomy or an extended resection. On final pathology, the surgical margin was microscopically negative (R0) in 78 (92.9%) patients and microscopically positive (R1) in 6 (7.1%) patients. No patient underwent a resection with gross residual disease (R2). At 30 days, there were two deaths for a perioperative mortality rate of 2.4%.

On IOUS, there were 35 (41.7%) patients with hypoechoic lesions, 37 (44.0%) patients with isoechoic lesions, and 12 (14.3%) patients with hyperechoic lesions. A significantly higher percentage of hepatic steatosis was observed in the group with hypoechoic lesions (60.1%) compared with nonhypoechoic lesions (27.6%) (p=0.03). Patients with mucin-producing lesions also tended to have more hypoechoic lesions (14.3%) than isoechoic or hyperechoic lesions (5.4%) (p=0.11), although this did not reach statistical significance. The distribution of other prognostic factors, such as nodal status of the primary tumor, disease-free interval, tumor number, size of largest hepatic lesion, preoperative CEA level, and margin status, were similar among the three echogenic groups (Table 2).

Table 1 Clinical and Morphologic Features of Patients (n=84)

Variable	No. of Patients (%)[a]
Age	
Median (year)	64
Sex	
Female	40 (47.6)
Male	44 (52.4)
Race	
White	71 (84.5)
Black	8 (9.5)
Others	5 (6.0)
Primary tumor site	
Colon	59 (70.2)
Rectum	25 (29.8)
Preoperative chemotherapy	
No	30 (35.7)
Yes	54 (64.3)
Preoperative CEA (ng/ml)	
<100	8 (9.5)
≥100	76 (90.5)
Tumor size	
Median (cm)	5.0
Tumor number	
Median	1
Tumor location	
Unilobar	74 (88.1)
Bilobar	10 (11.9)
Type of liver resection	
Wedge	14 (16.7)
Segmentectomy	29 (34.5)
Hemihepatectomy/extended resection	41 (48.8)
Microscopic resection margin	
Negative	78 (92.9)
Positive	6 (7.1)
Echogenicity of lesion	
Hypoechoic	35 (41.7)
Isoechoic	37 (44.0)
Hyperechoic	12 (14.3)

[a] Unless otherwise specified
CEA = Carcinoembryonic antigen

With a median follow-up of 26.2 months, the overall median actuarial survival for the entire cohort (n=84) was 53.3 months. At the time of analysis, 56 patients (66.7%) were alive, whereas 28 (33.3%) patients had died. This corresponded to a 1-, 3-, and 5-year actuarial survival rate of 95.2, 63.4, and 34.1%, respectively (Fig. 1). On univariate analysis, no traditional clinicopathologic factor was associated with survival (Table 3). Specifically, primary tumor site, primary tumor node status, disease-free interval, CEA level, and size and number of hepatic metastasis were not associated with long-term outcome (all p>0.05). However, an association between IOUS tumor echogenicity and survival after hepatic resection was observed. Patients with hypoechoic metastases on IOUS had a significantly shorter median survival (30.2 months) compared with patients who had either an isoechoic (53.2 months) or hyperechoic (42.3 months) lesion (p= 0.005). Differences in 5-year survival after hepatic resection of colorectal liver metastasis were also associated with the echogenic appearance of the lesion (hypoechoic 14.4 vs isoechoic 37.4 vs hyperechoic 46.2%) (p<0.05; Fig. 2)

On mutivariate analysis, echogenicity remained independently associated with long-term prognosis. Specifically, after adjusting for traditional prognostic factors (e.g., the clinical score of 6), such as nodal status of primary tumor, disease-free interval, tumor number, size of largest hepatic metastasis, and CEA level, patients with hypoechoic colorectal metastases were at significantly higher risk of disease-specific death (OR=5.11, p=0.006, 95% CI 1.59 to 16.44) (Table 3). As seen in the adjusted Cox proportional survival curves (Fig. 3), after controlling for other clinical factors, lesion echogenicity remained a powerful factor on which to stratify patient prognosis.

Discussion

Intraoperative ultrasound was demonstrated to be an important tool for accurately staging liver tumors and frequently impacts intraoperative decision making.[15–19] The superiority of IOUS over preoperative radiologic images and intraoperative inspection and palpation by the liver surgeon was demonstrated in numerous studies.[13,17,20,21] Specifically, compared with preoperative radiologic findings, IOUS was reported to identify at least one additional malignant lesion in 10–12% of cases.[21] As such, IOUS may have an oncologic benefit by more accurately identifying all the malignant hepatic disease, thereby allowing for complete resection of all existing macroscopic disease.[20] In fact, findings discovered by IOUS were reported to change or guide surgical treatment in up to 67% of procedures.[17,18,20,21] Little data exist, however, regarding whether IOUS tumor characteristics themselves—such as echogenicity—affect overall prognosis. Such data would have important implications, suggesting that IOUS during resection of colorectal liver metastases is essential not only for technical reasons but also as a means to ascertain relevant prognostic information.

Seifert and Morris[12] reported that pretreatment echogenicity of colorectal liver metastases predicted survival after hepatic cryotherapy. In that study, patients with hyperechoic metastases had a median survival twice as long as that of patients who were found to have hypoechoic lesions (50 vs 24 months, respectively). This same group also reported that echogenicity was an important prognostic survival parameter in patients treated with liver resection plus cryotherapy[10] or regional chemotherapy.[11] To our knowledge, however, the relative prognostic importance of IOUS tumor

Table 2 Distribution of Prognostic Variables Among Echogenicity Groups

Variable	Echogenicity (%)			*p* value
	Hypoechoic, *n*=35	Isoechoic, *n*=37	Hyperechoic, *n*=12	
Primary tumor site				
Colon	24 (68.6)	21 (56.8)	7 (58.3)	0.50
Rectum	11 (31.4)	16 (43.2)	5 (41.7)	
Nodal status of primary				
Negative	10 (28.6)	12 (32.4)	4 (33.3)	0.94
Positive	25 (71.4)	25 (67.6)	8 (66.7)	
Disease-free interval (months)				
<12	9 (25.7)	16 (43.2)	6 (50.0)	0.15
≥12	26 (74.3)	21 (56.8)	6 (50.0)	
Tumor number				
Solitary	7 (20.0)	15 (40.5)	5 (41.7)	0.13
Multiple	28 (80.0)	22 (59.5)	7 (58.3)	
Size of largest hepatic lesion (cm)				
≤5	29 (82.9)	22 (59.5)	10 (83.3)	0.15
>5	6 (17.1)	15 (40.5)	2 (16.7)	
Preoperative CEA (ng/ml)				
<100	33 (94.3)	32 (86.5)	11 (91.7)	0.50
≥100	2 (5.7)	5 (13.5)	1 (8.3)	
Preoperative chemotherapy				
No	9 (25.7)	12 (32.4)	4 (33.3)	0.79
Yes	26 (74.3)	25 (67.6)	8 (67.7)	
Microscopic resection margin				
Negative	33 (94.3)	34 (91.9)	11 (91.7)	0.92
Positive	2 (5.7)	3 (8.1)	1 (8.3)	

CEA = Carcinoembryonic antigen

echogenicity has never been independently corroborated in a large cohort of patients undergoing resection alone for colorectal liver metastases. In the current study, we report that echogenic appearance of colorectal metastases is an independent prognostic factor of survival after curative hepatic resection. In particular, patients with a hypoechoic tumor on IOUS had a significantly shorter median survival (30.2 months) compared with patients who had either isoechoic (53.2 months) or hyperechoic (42.3 months) metastases (p=0.005). In fact, patients with hypoechoic lesions on IOUS had a 5-year survival rate of only 14.4%.

Traditionally, clinicopathologic factors were combined to formulate prognostic scoring systems in an attempt to derive more prognostic information.[6,7] Nordlinger et al.[7] proposed a prognostic scoring system that incorporated seven clinicopathologic factors: age greater than 60 years, stage of primary tumor, synchronous disease, size of largest lesion greater than 5 cm, more than four liver metastases, CEA level greater than 30, and positive margins. Fong et al.[6] developed a clinical risk score based on (1) positive nodal status of the primary tumor, (2) multiple tumors, (3) disease-free interval of less than 12 months between primary tumor and diagnosis of liver metastasis, (4) CEA level greater than 200, and (5) tumor size greater than 5 cm. Although these clinicopathologic factors were validated by some groups,[22,23] other investigators have shown that patients with similar clinicopathologic characteristics may have different long-term outcomes.[24,25] In the current study, the distribution of these traditional clinicopathologic factors

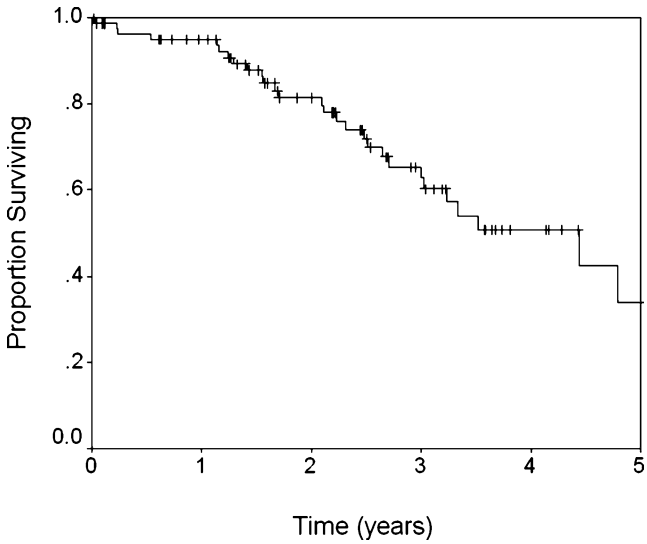

Figure 1 With a median follow-up of 26.2 months, the overall median actuarial survival for the entire cohort (n=84) was 53.3 months. At the time of analysis, 56 patients (66.7%) were alive, whereas 28 (33.3%) patients had died. This corresponded to a 1-, 3-, and 5-year actuarial survival of 95.2, 63.4, and 34.1%, respectively.

Table 3 Clinicopathologic Factors Influencing Prognosis After Hepatic Resection of Colorectal Metastases

Prognostic Factor	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Primary tumor site (rectal)	1.56	0.73–3.34	.25	1.62	0.65–4.06	0.30
Nodal status primary (positive)	1.47	0.65–3.35	.36	1.35	0.56–3.23	0.50
Disease-free interval (>12 month)	1.45	0.67–3.17	.35	1.21	0.49–2.98	0.67
Tumor number (multiple)	1.15	0.54–2.46	.72	1.97	0.74–5.24	0.18
Size of largest hepatic lesion (>5 cm)	1.05	0.46–2.38	.91	2.09	0.67–6.56	0.20
Preoperative CEA level (≥100 ng/ml)	2.51	0.85–7.44	.10	1.00	0.76–3.93	0.96
Preoperative chemotherapy (yes)	0.95	0.43–2.10	.90	1.12	0.36–2.23	0.75
Microscopic resection margin (positive)	1.46	0.34–6.23	.61	1.57	1.08–3.99	0.57
Echogenicity						
Isoechoic	–	–	–	–	–	–
Hyperechoic	1.66	0.48–5.74	.42	2.00	0.44–9.07	0.37
Hypoechoic	3.59	1.48–8.70	.005	5.11	1.59–16.44	0.006

CEA = Carcinoembryonic antigen, CI = confidence interval

was the same in each of the three echogenicity groups (Table 2). In addition, none of these clinicopathologic factors were found to influence overall survival. Intraoperative ultrasound tumor echogenicity, however, was a powerful predictor of survival. One explanation for these findings may be related to sample size. The relative ability of IOUS tumor echogenicity compared with traditional clinicopathologic factors to stratify patients in our relatively small sample set (n=84) suggests that echogenicity has more discriminatory prognostic power. This independent prognostic power of tumor echogenicity was borne out on the multivariate analyses. In Cox proportional hazards analysis, which controlled for traditional clinicopathologic prognostic factors, IOUS tumor echogenicity became an even more potent predictor of outcome (Table 3).

The confirmation of IOUS echogenicity as an independent predictor of prognosis for patients with colorectal metastases after hepatic resection raises the following question: What is it about tumor echogenicity that dictates outcome? Other investigators have suggested that hypoechoic lesions were significantly more likely to be mucin-producing tumors.[10] Mucin secretion is an established negative prognostic factor for primary colorectal cancer.[26,27] In the current study, we similarly noted that hypoechoic lesions were more likely to be mucin-producing, although this did not reach statistical significance.

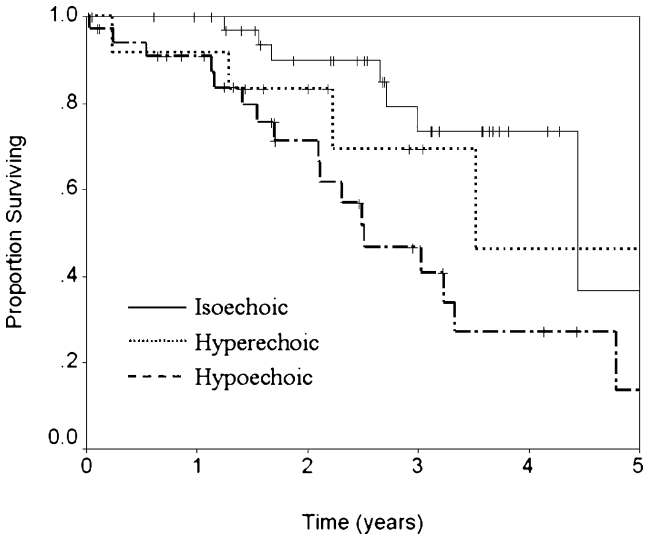

Figure 2 Univariate log-rank Kaplan–Meier analysis of overall survival stratified by IOUS appearance of metastatic lesion. The 3- and 5-year survival following hepatic resection of colorectal liver metastasis was associated with the echogenic appearance of the lesion (hypoechoic 47.4 and 14.4%, isoechoic 79.3 and 37.4%, hyperechoic 70.1 and 46.2%, respectively) (all p<0.05).

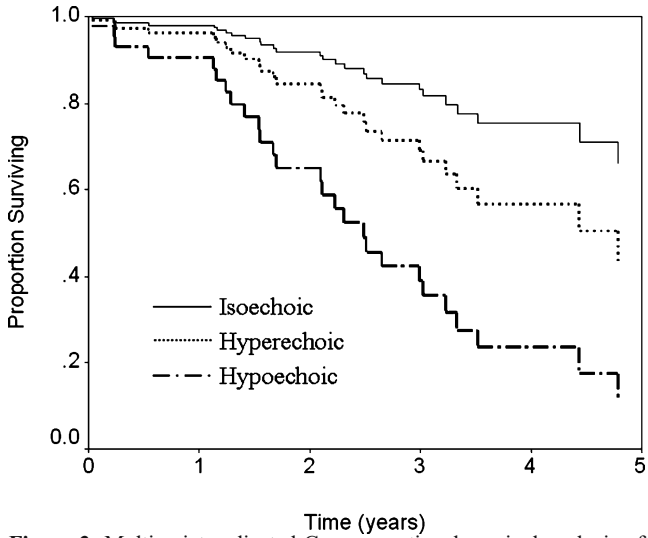

Figure 3 Multivariate adjusted Cox proportional survival analysis of overall survival stratified by IOUS appearance of metastatic lesion. After adjusting for traditional prognostic factors (e.g. the clinical score6) lesion echogenicity remained a powerful factor on which to stratify patient prognosis (p<0.05).

Other potential prognostically important parameters such as treatment with preoperative chemotherapy or response to chemotherapy were not associated with tumor echogenicity. Collectively, these data suggest that an intratumoral biologic characteristic—such as mucin, or an as-yet-undefined factor—contributes to a lesion's hypoechogenicity and, in turn, its associated worse prognosis.

A potential weakness of the current study was our use of the average echogenicity to categorize patients with multiple lesions. Unlike previous studies,[10,11] we did not use a "mixed echogenicity" category for such patients. However, it is unlikely that this practice introduced any misclassification bias into the study. First, most patients had a solitary hepatic colorectal metastasis. Second, previous work done by our group had demonstrated that there is a high concordance in the IOUS echogenic appearance of multiple lesions within the same patient[14]. Therefore, as expected, a "mixed echogenicity" category did not have any additional discriminatory prognostic power. Relative strengths of our study include the fact that, rather than scoring echogenicity at the time of the operation, we recorded all IOUS images prospectively and assessed echogenicity in a masked fashion.

Conclusion

Intraoperative ultrasound tumor echogenicity was associated with prognosis and long-term survival after hepatic resection with curative intent for colorectal metastases. Traditional clinicopathologic factors such as nodal status of the primary tumor, disease-free interval, tumor number, size of largest hepatic metastasis, and CEA level were not associated with outcome. Rather, prognosis was dependent on IOUS echogenicity. Specifically, patients with hypoechoic hepatic metastases on IOUS were at significantly higher risk of disease-specific death and had a significantly shorter median survival. Hypoechoic lesions tended more often to be mucin-producing, which is a known adverse prognostic factor of primary colorectal cancer. Future investigations will need to better elucidate the pathogenesis underlying the basis for the differential echogenic appearance of hepatic lesions, thereby providing insight into the reason for the worse prognosis of hypoechoic lesions.

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DISCUSSION

Bruce D. Schirmer, M.D. (Charlottesville, Vir): Did you actually look at the histology of the metastatic lesions themselves? In other differentiated and perhaps had tumor necrosis as a reason for their hypoechoicness?

Michelle DeOliveira, M.D. (Baltimore, Md): Dr. Shirmer, thank you very much for your question regarding a possible correlation between histology and echogenicity of the liver metastases. Indeed, we evaluated the histology, but failed to identify significant correlation as moderated or poorly differentiated tumors were similarly distributed in the three types of echogenicity.

David M. Nagorney, M.D. (Rochester, Minn): Isn't echogenicity relative? It is relative to the surrounding liver that you are looking at. Did you look at the texture and echogenicity of the regular liver and compare this also to outcomes?

Dr. DeOliveira: Thank you very much, Dr. Nagorney, for your important question about the interpretation of the echogenicity of the liver lesions. Yes, the echogenicity of lesions depend not only on the tumor characteristic but also on the surrounding liver. We tried to characterize the surrounding liver regarding features such as steatosis, etc. We could not find any influence of variable echogenicity of the surrounding tissue on our results.

J. Nicolas Vauthey, M.D. (Houston, Tex): You looked at preoperative chemotherapy as a possible factor. I was wondering about the type of chemotherapy agents you included in the preoperative chemotherapy group? As you know, there is quite a difference between preoperative chemotherapy, with just 5-FU, which does not induce a significant response in liver metastases, and the other end of the spectrum, FOLFOX or FOLFIRI with bevacizumab, where we really see a loss of vascular enchancement of the lesions.

Dr. DeOliveira: Thank you very much, Dr. Vauthey, for your remark. This is an important point. We report on the use of chemotherapy but did not stratify for the specific agents. We plan to analyze the effects of FOLFOX or FOLFIRI relationship to echogenicity.

Howard S. Kaufman, M.D. Michelle, one final question. You have told us about a phenotype, which is echogenicity as a prognostic factor. Your group has done some work in the past with molecular markers. Have you looked at the genetics of these tumors to see if there is a genetic expression or a genetic correlation with echogenicity?

Dr. DeOliveira: Thank you, Dr. Kaufman, for this question. We have not yet studied molecular markers in this set of patients and the relationship of echogenicity.

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Gallstones Containing Bacteria are Biofilms: Bacterial Slime Production and Ability to Form Pigment Solids Determines Infection Severity and Bacteremia

Lygia Stewart · J. McLeod Griffiss · Gary A. Jarvis · Lawrence W. Way

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Abstract
Objective Gallstone bacteria provide a reservoir for biliary infections. Slime production facilitates adherence, whereas β-glucuronidase and phospholipase generate colonization surface. These factors facilitate gallstone formation, but their influence on infection severity is unknown.
Methods Two hundred ninety-two patients were studied. Gallstones, bile, and blood (as applicable) were cultured. Bacteria were tested for β-glucuronidase/phospholipase production and quantitative slime production. Infection severity was correlated with bacterial factors.
Results Bacteria were present in 43% of cases, 13% with bacteremia. Severe infections correlated directly with β-glucuronidase/phospholipase (55% with vs 13% without, $P<0.0001$), but inversely with slime production (55 vs 8%, slime <75 or >75, $P=0.008$). Low slime production and β-glucuronidase/phospholipase production were additive: Severe infections were present in 76% with both, but 10% with either or none ($P<0.0001$). β-Glucuronidase/phospholipase production facilitated bactibilia (86% with vs 62% without, $P=0.03$). Slime production was 19 (±8) vs 50 (±10) for bacteria that did or did not cause bacteremia ($P=0.004$). No bacteria with slime >75 demonstrated bacteremia.
Conclusions Bacteria-laden gallstones are biofilms whose characteristics influence illness severity. Factors creating colonization surface (β-glucuronidase/phospholipase) facilitated bacteremia and severe infections; but abundant slime production, while facilitating colonization, inhibited detachment and cholangiovenous reflux. This shows how properties of the gallstone biofilm determine the severity of the associated illness.

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L. Stewart (✉)
Department of Surgery (112), University of California
San Francisco, 4150 Clement Street,
San Francisco, CA 94121, USA
e-mail: lygia.stewart@med.va.gov

L. Stewart
Surgical Service, San Francisco VA Medical Center,
San Francisco, CA, USA

J. M. Griffiss · G. A. Jarvis
Center for Immunochemistry, San Francisco VA Medical Center,
San Francisco, CA, USA

J. M. Griffiss · G. A. Jarvis
Department of Laboratory Medicine, University of California,
San Francisco, San Francisco, CA, USA

L. W. Way
4 Koret Way, L-101, Box 0475, University of California
San Francisco, San Francisco, CA 94143, USA

Keywords Gallstone · Bacteremia · β-Glucuronidase · Phospholipase · Biofilm · Slime · Glycocalyx

Bacterial microcolonies in gallstones provide a reservoir for biliary infections. Bacterial slime production facilitates adherence, whereas β-glucuronidase (bG) and phospholipase (PhL) production generate colonization surface. The importance of bacterial bG was first noted by Maki[1] who reported that bacterial bG caused precipitation of Ca-bilirubinate. After this, numerous studies documented the importance of bG and bacterial PhL (leading to Ca-palmitate precipitation) to pigment stone formation and biliary infections.[2–6] Our group was the first to identify bacterial microcolonies in pigment stones, the pigment portions of mixed stones, and the pigmented centers of certain predominantly cholesterol stones.[7] Others have reported similar findings.[8–10] We studied these biliary bacteria and found that they possessed P1-fimbriae, and usually produced slime, which facilitated pigment stone

formation.[11–13] These bacterial microcolonies were associated with infectious manifestations, and certain bacterial factors determined bacterial virulence.[14–16] To cause severe infections these bacteria need to detach from the biofilm, and to cause cholangitis they need to reflux into the systemic circulation (cholangiovenous reflux). We previously studied these cholangiovenous reflux pathways, and noted that abundant slime inhibited cholangiovenous reflux in rats.[17–19] But the influence of these bacterial factors (slime, bGPhL production) on the development of severe infections and bacteremia in a clinical setting has not been studied. In this study, we examined the influence these factors on infection severity and bacteremia.

Methods

Two hundred ninety-two cases with gallstone disease were prospectively studied. There were 48 women and 244 men whose average age was 61 years (range 17–104). 84% were Caucasian, 9% were Hispanic, 4% were Asian, and 3% were African American. Forty percent had biliary colic, 31% had acute cholecystitis, 16% had choledocholithiasis or pancreatitis, and 13% had cholangitis.

Gallstones, bile, and blood (when clinically indicated) were cultured. Gallstones were obtained under sterile conditions at surgery [or endoscopic retrograde cholangiopancreatography (ERCP)], rinsed with normal saline, crushed, and cultured in Tryptic Soy broth for 24–48 h. One hundred twenty-four gallstones were also examined under scanning electron microscopy (SEM) for the presence of bacterial microcolonies using our previously described technique.[7] Bile was obtained for culture at surgery, ERCP, or during percutaneous transhepatic cholangiography. Blood cultures were obtained when clinically indicated. Patients whose gallstones or bile cultured bacteria, or whose gallstones contained bacteria on SEM examination, were considered to have biliary bacteria. Patients whose stones and bile were sterile and devoid of bacterial microcolonies on SEM were considered to have a sterile biliary tract.

Illness severity was staged as: none (no clinical infection or inflammatory manifestations, included in this group were patients with jaundice but no clinical manifestations of infection); moderate [fever (T>100°F), leukocytosis (white blood cells>11 K/cm^2), or focal necrosis of the gallbladder wall]; and severe (cholangitis, bacteremia, hypotension, organ failure, abdominal abscess, hepatic abscess, or empyema/gangrenous/emphysematous gallbladder).

Bacterial Slime Production

Quantitative bacterial slime production was determined using a previously described assay.[20] Glass test tubes (American Scientific Products, McGaw Park, IL, USA) containing 1 ml of Tryptic Soy broth supplemented with 10% (vol/vol) glucose were inoculated with a single colony of bacteria and incubated stationary at 37°C for 48 h. Each tube was decanted and washed two times with 1 ml of H_2O and reacted with Carnoy's solution (absolute ethanol, chloroform, glacial acetic acid 6:3:1). After this, 1 ml of safranin was added to the tubes, and the tubes were gently rotated to uniformly coat the adherent material (slime). Excess stain was removed by washing with 3 ml of H_2O two times. One milliliter of 0.2 M NaOH was added and the sample was heated for 1 h at 85°C. Samples were then vortexed, cooled to room temperature, and the optical density (OD) determined at 530 nm. Optical density directly correlates with the amount of slime present. Test tubes containing only Tryptic Soy broth supplemented with 10% (vol/vol) glucose were used as negative controls. Analysis was done in triplicate for each bacterial species.

Bacteria were analyzed individually and in two groups: those with low slime production (LowSL <75 OD) and those with high slime production (HighSL >75 OD). In cases where there was more than one bacterial isolate present in the biliary tree, the minimum level of slime production present in the biliary tree determined the classification.

Bacterial β-Glucuronidase and Phospholipase Production

Bacterial bG production was assessed using the method of Jackson et al.[21] Bacteria were pour-plated on MacConkey agar containing 100 mg of 4-methylumbelliferyl-β-D-glucuronide per liter. They were incubated at 37°C for 24 h and colonies were then examined under ultraviolet light at 360 nm. Bacterial colonies producing bG appeared fluorescent when examined under ultraviolet light.

Gallstone composition of 187 representative gallstones was determined using Fourier-transformed infrared spectroscopy (FTIR), as previously described.[13] Stones were air-dried, crushed in an agate mortal and pestle, mixed with potassium bromide in a shaking device, and pressed into pellets at 3,000 psi. They were then examined under FTIR. Standards of varying concentrations were made using commercially available cholesterol, conjugated bilirubin (bilirubin isomers, Sigma), Ca-palmitate, Ca-carbonate, and Ca-bilirubinate. Multiple standards were made to fully bracket the range of compositions of all the gallstones. Presence of Ca-palmitate documented bacterial PhL production in the gallstone biofilm.

Cases were separated into two groups based on bacterial bG or PhL (bGPhL) production. Cases with one or more bG producing bacteria or Ca-palmitate present in the gallstone were considered to have bGPhL production. Cases without either were considered to be devoid of bGPhL-producing bacteria.

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) or the Student's *t* test for interval data, and the chi-squared or Fischer Exact test for variables on a nominal scale (rates and proportions).

Results

Biliary bacteria were present in 126 (43%) of 292 cases. Bacteria were present in gallstones and bile in 122 (42%) and 93 (32%) of the cases, respectively. Among cases with biliary bacteria, one bacterial isolate was present in 42%, two were present in 24%, and three or more were present in 34%. Blood cultures were positive in 13% of the cases with biliary bacteria. Among cases with bacteremia, one bacterial isolate was present in 59%, two were present in 35%, and three were present in 6%.

Influence of Slime Production and on Bactibilia and Bacteremia

Bacteria with higher slime production were less likely to be recovered from bile or blood cultures. Average slime production of bacterial species present vs not present in bile was 33 (±7) vs 70 (±13), $P=0.009$, ANOVA). Among bacteria present in bile, only 17% had slime production >75. Average slime production was 19 (±8) vs 50 (±10) for bacteria that were or were not present in the blood ($P=0.002$, Student's *t* test), and no bacteria with slime production > 75 were recovered from blood cultures.

This data was also analyzed by individual patients to determine whether the properties of the bacterial biofilm (gallstone) as a whole influenced the development of bactibilia and bacteremia. The incidence of bactibilia was 79 vs 42% for cases with LowSL- vs HighSL-producing bacteria ($P=0.028$, Fischer exact test). Bacteremia was present in 22 vs 0% of cases with LowSL- vs HighSL-producing bacteria ($P=0.061$, Fischer exact test).

Influence of β-Glucuronidase and Phospholipase on Bactibilia and Bacteremia

Bactibilia and bacteremia were more common in cases with one or more bGPhL-producing bacteria present in the biliary tree. Bactibilia was present in 86 vs 61% of cases with or without bGPhL-producing bacteria, respectively ($P=0.004$, chi-square test). There was a trend toward a higher incidence of bacteremia in cases with bGPhL bacteria (19 vs 5%, with or without bGPhL bacteria, $P=0.091$, chi-square test).

Only the bGPhL properties of the biofilm as a whole reflected the incidence of bactibilia or bacteremia; bG production of individual bacterial species did not seem to influence whether they were present in bile or blood. Seventy-nine vs seventy-seven percent of bacteria that did or did not produce bG were cultured from the bile, whereas 11 vs 9% of bacteria that did or did not produce bG were cultured from blood.

Combined Interactions: Slime and β-Glucuronidase/ Phospholipase Production

The combined influence of slime and bGPhL production on bactibilia and bacteremia is shown in Fig. 1. As shown in Fig. 1a, cases with LowSL- and bGPhL-producing bacteria had the highest incidence of bactibilia (90%, $P<0.0001$ vs all others, chi-square test). In contrast, cases with HighSL without bGPhL production had the lowest level of bactibilia (20%), whereas in the other groups bactibilia was present in about 50% of the cases. Similarly (Fig. 1b), cases with LowSL- and bGPhL-producing bacteria had the highest

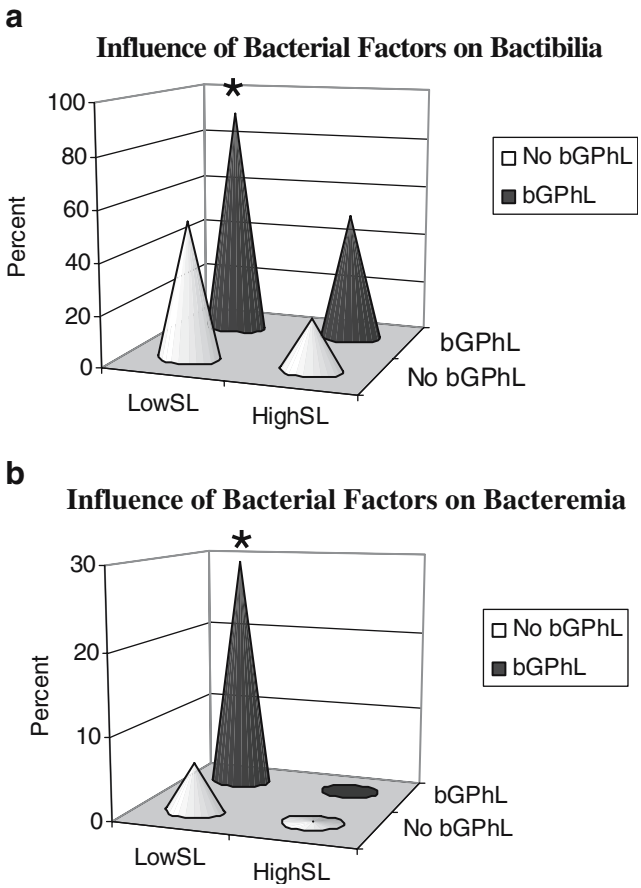

Figure 1 **a** Influence of bacterial factors on bactibilia. The asterisk indicates $P<0.0001$ vs all others (chi-square test). **b** Influence of bacterial factors on bacteremia. The asterisk indicates $P=0.002$ vs all others (chi-square test). **a**, **b** bGPhL = Presence of one or more bacteria with either bG or PhL production, LowSL = slime production <75 OD, HighSL = slime production >75 OD.

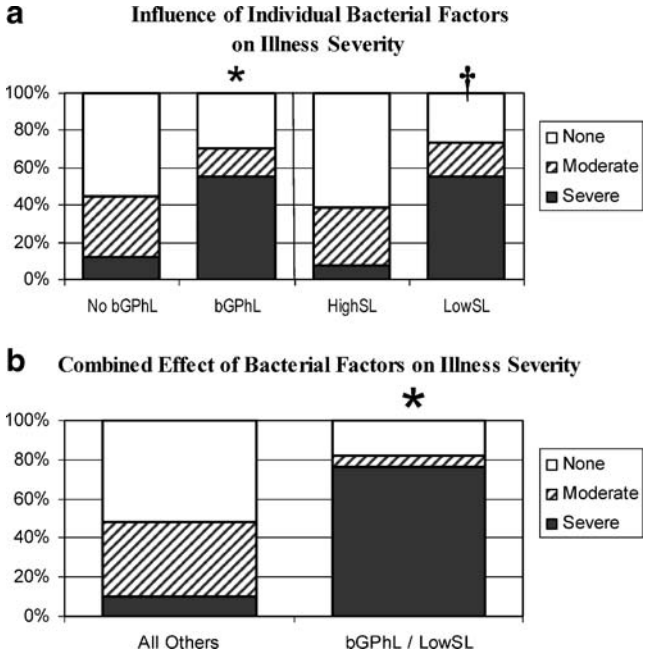

Figure 2 **a** Influence of individual bacterial factors on severity of illness. Only cases with biliary bacteria are included in the graph. Illness severity levels are defined in the text. Influence of each bGPhL or slime production on entire group is shown. The asterisk indicates $P<0.0001$, no bGPhL vs bGPhL (chi-square test). The dagger indicates $P=0.008$, HighSL vs LowSL (chi-square test). **b** Combined effect of bacterial factors on severity of illness. The asterisk indicates $P<0.0001$, bGPhL and LowSL vs all others (chi-square test). **a**, **b** bGPhL = Presence of one or more bacteria with either bG or PhL production, LowSL = slime production <75 OD, HighSL = slime production >75 OD.

incidence of bacteremia (29%, $P=0.002$ vs all others, chi-square test). Bacteremia was not seen in any cases with HighSL, and it was uncommon in cases with LowSL without bGPhL-producing bacteria.

Infectious and Inflammatory Manifestations

Cases with a sterile biliary tract had a less severe illness compared to those with biliary bacteria: sterile, none 80% and moderate 20%; biliary bacteria, none 37%, moderate 21%, and severe 42% ($P<0.0001$, chi-square test).

Among cases with biliary bacteria, severity of illness varied directly with presence of bGPhL-producing bacteria in the biofilm (Fig. 2a). Illness severity was none 29%, moderate 15%, and severe 55% vs none 55%, moderate 33%, and severe 12% for cases with and without bGPhL bacteria, respectively ($P<0.0001$, chi-square test). These relationships held true for cases with bacteria-laden common bile duct (CBD) stones as well. Illness severity was none 22%, moderate 9%, and severe 69% vs none 58%, moderate 17%, and severe 25% for cases with and without bGPhL bacteria in CBD stones, respectively ($P=0.021$, chi-square test).

Illness severity inversely correlated with the level of slime production present (Fig. 2a). Illness severity was none 27%, moderate 18%, and severe 55% vs none 62%, moderate 31%, and severe 8% for cases with LowSL vs HighSL bacteria ($P=0.008$, chi-square test). These relationships held true for cases with bacteria-laden CBD stones as well. Illness severity was none 16%, moderate 0%, and severe 84% vs none 80%, moderate 20%, and severe 0% for cases with LowSL vs HighSL bacteria in CBD stones, respectively ($P<0.0001$, chi-square test).

Level of bacterial slime production and presence of bGPhL production had a combined influence on illness severity (Fig. 2b). Cases with LowSL- and bGPhL-producing bacteria had the most severe illnesses; whereas those with LowSL without bGPhL-producing bacteria, or HighSL (with or without bGPhL bacteria), had less severe illnesses (Fig. 2b). As shown, cases with LowSL- and

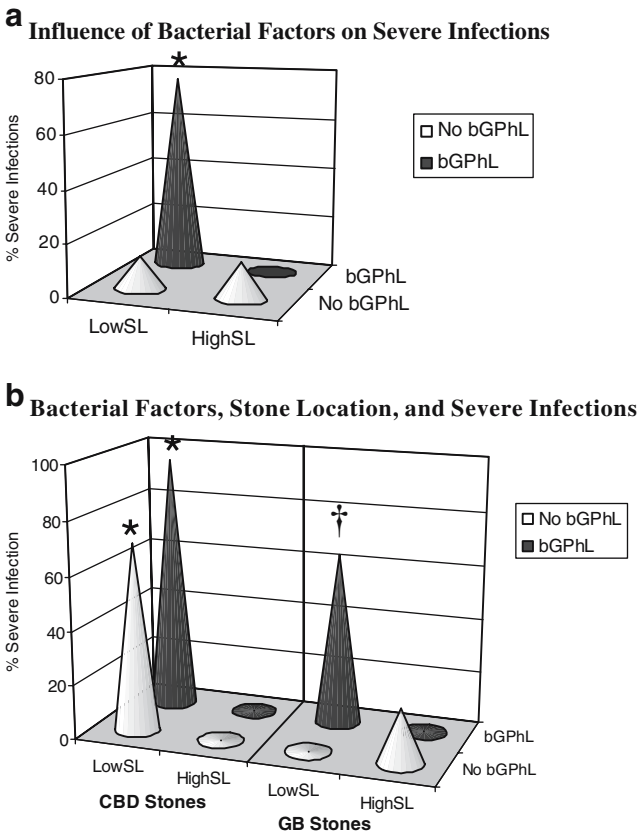

Figure 3 **a** Influence of bacterial factors on the development of severe infections. The asterisk indicates $P<0.0001$, bGPhL and LowSL vs all others (chi-square test). **b** Influence of bacterial factors on the development of severe infections for cases with bacteria-laden gallstones in the CBD or confined to the gallbladder (GB). The asterisk indicates $P=0.001$, LowSL vs HighSL (Fischer exact test). The dagger indicates $P=0.001$, LowSL and bGPhL vs all others (Fischer exact test). **a**, **b** bGPhL = Presence of one or more bacteria with either bG or PhL production, LowSL = slime production <75 OD, HighSL = slime production >75 OD.

bGPhL-producing bacteria usually (76%) had a severe illness, whereas all other groups infrequently (10%) had severe illnesses, and usually (52%) had no infectious manifestations ($P<0.0001$, chi-square test).

The influence of the various bacterial factors on severe illness is shown in Fig. 3a for the entire group, and in Fig. 3b for cases with bacteria-laden CBD stones or gallstones confined to the gallbladder. As shown, cases with LowSL- and bGPhL-producing bacteria had the most severe illnesses whether the bacteria-laden stones were in the CBD or gallbladder. In cases with bacteria-laden CBD stones, all cases with LowSL had more severe illnesses compared to cases with HighSL ($P=0.011$, LowSL vs HighSL, Fisher exact test).

Discussion

A number of factors influence the ability of bacteria to cause infections. They need to be able to access and persist in the host environment. For successful colonization of the biliary tree, bacterial pathogens must be capable of surface adhesion and avoidance of natural host defenses.[11,12,22–26] Organisms that cannot attach to host cells are swept away in normal biliary secretions, which possess antibacterial properties (bile salts, IgA).[11,12,22–26] Even bacteria that are able to attach to epithelial cells are removed when cells slough. Stasis (which prevents washout of bacterial species) and bacterial attachment to a foreign body (stent, gallstone) eliminate this natural bacterial removal and facilitate bacterial colonization.

Two well-described adhesion factors are fimbriae and slime production. Fimbriae bind to epithelial cells and foreign bodies. Biliary bacteria possess P1-fimbriae[11] and they are also associated with bacteria causing pyelonephritis.[25] Bacterial slime, or glycocalyx, also allows bacteria to adhere to host surfaces, and facilitates their survival.[1,12,13,22–30] This anionic glycoprotein facilitates bacterial adhesion and the adherent bacteria form microcolonies held together and coated by the slime. Bacterial slime protects bacteria against antibodies, phagocytosis, antibiotics, and surfactants and acts as an ion-exchange resin for nutrient transport. Slime production has been shown to be an important virulence factor, especially in foreign body infections.[1,12,22–30] We demonstrated that most biliary bacteria produced slime, whereas only about 8% of bacteria from stool produce slime.[12]

The presence of surface for colonization has been shown to be one of the most important factors in the facilitation of bacterial growth. In aquatic environments, bacterial concentrations on surfaces can be 10^3 to 10^6 higher than the surrounding fluid.[23,24] So bacteria that elaborate bG or PhL would have a survival advantage by causing the precipitation of Ca-bilirubinate or Ca-palmitate, thus creating more surface area for bacterial colonization. This, of course, also leads to pigment stone formation. In addition, foreign bodies are more easily colonized than epithelial cells,[22–30] and positively charged cations (like Ca) enhance biofilm formation because of attachment of negatively charged bacteria to the positively charged surface.[23,24] This last mechanism is likely to be very important in pigment stone formation and bacterial incorporation into pigment stones because most gallstone pigments contain Ca (Ca-bilirubinate, Ca-palmitate, Ca-carbonate, or Ca-phosphate). We, in fact, demonstrated that bacteria preferentially adhere to the surfaces of pigment stones, rather than cholesterol stones.[7]

The above suggests that bacteria that produce slime and elaborate bG or PhL should have a survival advantage in the biliary tree. Moreover, bacteria producing bG or PhL should have an increased ability to propagate because of their ability to create surface for colonization. This increased bacterial load could increase the possibility of bactibilia. In this study, we found that bGPhL-producing bacteria were associated with a higher incidence of bactibilia. Similarly, severe infections and bacteremia (as a trend) were also more common in cases with bGPhL-producing bacteria present in the biliary tree. Several studies have reported an association between bacterial bG or PhL production and pigment stone formation, but none of these studies were able to determine whether it alone was a determinant of illness severity.[2–6,31–33] Because pigment stones are associated with severe biliary infections, bacterial bG and PhL production are considered to be important factors in the development of severe biliary infections. But because our patient comparison groups *all* had bacteria present in their biliary tree, we were able to determine that bGPhL production is associated with increased illness severity, apart from facilitating infectious stone formation.

But for bacteria to cause infectious manifestations, they must first detach from the biofilm (in this case the gallstone). And bacteria that are also able to reflux into the systemic circulation (cholangiovenous reflux) would be the most likely to cause a severe illness. There is extensive research documenting the association between bacterial biofilm formation and chronic infections, foreign body infections, and medical device infections (central lines, urinary catheters, endotracheal tubes, etc.).[22–30] And there is evidence that bacteria that produce large amounts of slime have increased adherence properties.[33] But the factors that allow detachment of these bacteria from the biofilm have not really been studied in clinical disease.

Bacteria exist in two modes, the mobile planktonic mode and the slime-enclosed microcolony (biofilm).[22,24,26,34] In nature, the microcolony mode is the most common. Factors that favor the microcolony growth mode include hypoxia, decreased nutrient levels, and intact host immune defenses.[22,24,26,34] Intact host defenses force the bacteria to adopt the defensive, microcolony mode of growth; whereas

immunocompromised hosts allow bacteria to remain in the planktonic mode. Bacterial biofilm formation may be associated with decreased systemic spread of bacteria. One of the few well-studied examples is *Pseudomonas aeruginosa*, an organism that is capable of lush slime production. This species can be associated with severe infections (including bacteremia) or chronic (even asymptomatic) persistent colonization, depending on the host defenses. Nearly all patients with cystic fibrosis develop chronic pulmonary infections with *P. aeruginosa*.[34–37] Because these patients have normal immune defenses, the *Pseudomonas* exists in well-established biofilms, and *Pseudomonas* bacteremia is extremely rare.[34–37] In contrast, when this organism colonizes the compromised patient (e.g., burn, neutropenic, cancer, or transplant patients), a severe, even overwhelming, infection can occur.[34,37]

In this study, we found an association between quantitative bacterial slime production and the subsequent development of bactibilia, bacteremia, and the severity of the associated illness. High slime production, while facilitating gallstone formation and bacterial colonization, was associated with decreased bacterial detachment from the gallstone biofilm. High slime-producing bacteria were less often recovered from bile cultures, and none were cultured from the blood. In contrast, cases with low slime-producing bacteria were associated with a higher incidence of bactibilia and bacteremia. This finding correlates with our previous studies in a rat model of cholangitis, where we noted that bacteria with HighSL exhibited decreased cholangiovenous reflux even when the bacteria were injected retrograde into the rat CBD at pressures high enough to cause cholangiovenous reflux.[17] These findings, taken together, suggest that HighSL limits bacterial detachment. And even if bacteria with HighSL escape into the bile, their ability to reflux into the systemic circulation is limited. The liver's filtering effect is likely to play a role in this as well.[17–19]

The bacterial factors had a synergistic effect. The combination of bacteria with LowSL with the presence of bacteria-producing bG or PhL was the most virulent combination. Cases with this combination of bacterial factors had a higher incidence of bactibilia, bacteremia, and the most severe illnesses, whether the bacteria-laden gallstones were located in the CBD or confined to the gallbladder. The combination of bacterial factors creating surface for colonization, coupled with low enough slime production to facilitate bacterial detachment, set the stage for a more serious infection. In cases with multiple bacterial isolates, one isolate with the ability to produce bG or PhL and one with LowSL was sufficient for this effect. There were several cases where these bacterial properties came from different bacterial species. And, in multiple-species biofilms with both high slime- and low slime-producing bacteria, the isolates with LowSL were the ones most commonly recovered from bile cultures, and only low slime-producing bacteria were recovered from the blood.

Conclusion

Bacteria-laden gallstones are biofilms whose characteristics influence bacterial colonization, detachment, and cholangiovenous reflux. Factors creating colonization surface (bGPhL) facilitated bacteremia and severe infections; but abundant slime production, while facilitating colonization, inhibited bacterial detachment and cholangiovenous reflux. High slime-producing isolates were recovered from bile, but not blood. This underscores the liver's filtering effect, which inhibited cholangiovenous reflux of high slime-producing bacteria. These data show how the properties of the gallstone biofilm determine the severity of the associated clinical illness.

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DISCUSSION

Dr. D. Soybel (Boston, MA): I appreciate you sending me the manuscript to review, and I actually would like to commend the discussion in this paper. For anybody who thinks that we can beat bacteria at their own game simply with antibiotics, I think the discussion here actually was very enlightening.

There are two comments that I wanted to make and with questions. The first one is, you and of course Henry Pitt's group have been interested in whether bacteria might find cholesterol versus pigment stones more hospitable, and both groups have shown that pigment stones are much more hospitable to bacteria than are cholesterol stones. In this study you lumped all stones together. But just for the sake of understanding the patient population, what were the percentages, among patients who actually had bacteria, for cholesterol versus pigment stones? Also do you have any thoughts about why cholesterol stones are less hospitable than pigment stones?

The second question is whether certain species of bacteria tend to lose the ability to make slime more than others? Are we looking at some sort of general environmental thing where essentially any bacteria could make slime or not make slime? What do you think is in the environment that is actually inducing them to stop making slime so that they can become more pathogenic?

Dr. Stewart: Thank you. Very good questions. With regards to the cholesterol-stone question, you can find bacteria in cholesterol stones that have a pigmented center about 35% of the time. We have an up-coming paper on this that shows that when the bacteria are in the center of a predominantly cholesterol stone, they are associated with less infectious manifestations.

But what is interesting, is that the bacteria in pigment and cholesterol stones make different amounts of pigment-forming factors, and bacteria preferentially stick to the surface of pigment stones. We studied this question some time ago by placing pigment and cholesterol stones into Tryptic-soy broth with 10^7 bacteria/cc. We found that the bacteria didn't stick to the cholesterol stones even though there were 10^7 bacteria/cc,

but they did stick to the pigment stones. Why is that? Well, go back to the biofilm research, and what you find is that positively charged ions are an attractor for negatively charged things (like bacteria). And if you look at a pigment stone, it is made of calcium salts: calcium bilirubinate, calcium palmitate, calcium phosphate, calcium carbonate. These calcium salts attract the bacteria, and this, by the way, is known across biofilm research in general. It doesn't matter whether the biofilm is in a pipe or in a vein. Not only do the calcium salts in the pigment stone allow the bacteria to stick, but the bacteria facilitate pigment formation because they make beta-glucuronidase or pholpholipase. So it is a combination of things.

Now, you raise a very important issue of why do some bacteria make more slime than others. Is it the environment? Is it the bacteria? Can they actually lose their ability to make slime? What is the issue here? We know that intact host immune responses promote biofilm formation, as do factors like low nutrients, low oxygen, etc. And there is some evidence that bacterial species that are able to make abundant slime (like Pseudomonas) cause chronic infections in the intact host, but are more likely to cause severe illnesses in immunocompromised patients (burn, transplant, cancer patients, etc.). Why? Because the immune mechanism isn't making that Pseudomonas go into the biofilm, change its genes and make the slime.

But in our study, all the bacteria were from the biliary tree. The environment of the biliary tree drives bacteria into biofilm formation because it has low nutrients, low oxygen, bile salts that are antibacterial, and IgA. So, there are several factors pushing those bacteria into the biofilm mode. We found that there was diversity among the bacteria, some made abundant slime while others didn't. We have noted, in prior publications, that biliary bacteria make more slime than stool bacteria. So it seems, when you get right down to it, that both are important, the tendency of the bacteria to make slime and the environment both play a role.

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Inferior Vena Cava Stenting: A Safe and Effective Treatment for Intractable Ascites in Patients with Polycystic Liver Disease

Jayleen Grams · Swee H. Teh · Vicente E. Torres · James C. Andrews · David M. Nagorney

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Abstract We performed a retrospective study of seven patients with polycystic liver disease who underwent stenting of the inferior vena cava for intractable ascites. All patients had symptomatic ascites and inferior vena cava stenosis demonstrable by venography. The mean pressure gradient across the inferior vena cava stenosis before stenting was 14.5 mm Hg (range 6–25 mm Hg) and significantly decreased to a mean pressure gradient of 2.8 mm Hg (range 0–6 mm Hg, $p=0.008$) after stenting. Two patients also had stenting of hepatic venous stenoses after unsuccessful inferior vena cava stenting. After a mean follow-up of 12.2 months (range 0.5–39.1 months), five of the seven patients have had maintained clinical improvement, defined as decreased symptoms, diuretic requirements, and frequency of paracentesis. Four patients have required no further intervention. The other patient was lost in follow-up. Patients with clinical improvement had an overall larger mean pressure gradient before stenting (19.2 vs. 9.8 mm Hg) and a larger Δ pressure gradient (15.8 vs. 7.8 mm Hg) compared to those in whom stenting was unsuccessful. These results suggest inferior vena cava stenting is safe and effective and should be considered as a first-line intervention in the treatment of medically intractable ascites in select patients with polycystic liver disease.

Keywords IVC stent · Polycystic liver disease · Intractable ascites

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J. Grams · D. M. Nagorney (✉)
Department of Surgery, Mayo Clinic College of Medicine,
200 1st Street SW,
Rochester, MN 55905, USA
e-mail: nagorney.david@mayo.edu

S. H. Teh
Department of Surgery, Oregon Health Sciences University,
Portland, OR, USA

V. E. Torres
Division of Nephrology and Hypertension,
Department of Medicine, Mayo Clinic College of Medicine,
Rochester, MN, USA

J. C. Andrews
Department of Vascular and Interventional Radiology,
Mayo Clinic College of Medicine,
Rochester, MN, USA

Introduction

Polycystic liver disease (PLD) is a relatively rare disorder with a prevalence of 0.13–0.6%[1,2] and is the most common extrarenal manifestation of autosomal dominant polycystic kidney disease (ADPKD). Although PLD is usually asymptomatic, progressive hepatomegaly and intractable ascites can become debilitating. Numerous approaches have been employed for the treatment of symptomatic PLD. The efficacy of these approaches has varied depending upon the extent and distribution of hepatic involvement and the presence of concurrent chronic hepatic dysfunction. Most commonly, we have selectively employed hepatic resection and cyst fenestration to palliate symptomatic hepatomegaly in patients with PLD. Although perioperative morbidity may be significant,[3] most patients have noted a marked and durable improvement in quality of life. Orthotopic liver transplantation also effectively relieves symptomatic hepatomegaly;[4–6] however, it necessitates chronic immunosuppression and impacts organ allotment for patients who rarely develop liver failure. Intractable ascites can develop de novo or after hepatic resection.[7–9] Management of patients with PLD and symptomatic

ascites can be particularly challenging, especially in the presence of renal failure from ADPKD, and may be refractory to maximal medical therapy of fluid and sodium restriction, diuretics, and repeated paracentesis.

We have observed that some patients with PLD have compression of the inferior vena cava (IVC), the hepatic veins (HVs), or both and develop large intrahepatic collateral veins evident on dimensional imaging of the liver. Consequently, we hypothesized that venous obstruction of the IVC, HV, or both could be a major cause of intractable ascites in some patients with PLD and postulated that these patients might be treated safely and effectively with percutaneous venous stenting to relieve venous obstruction. This study reviews our experience with venous stenting for patients with intractable ascites associated with PLD.

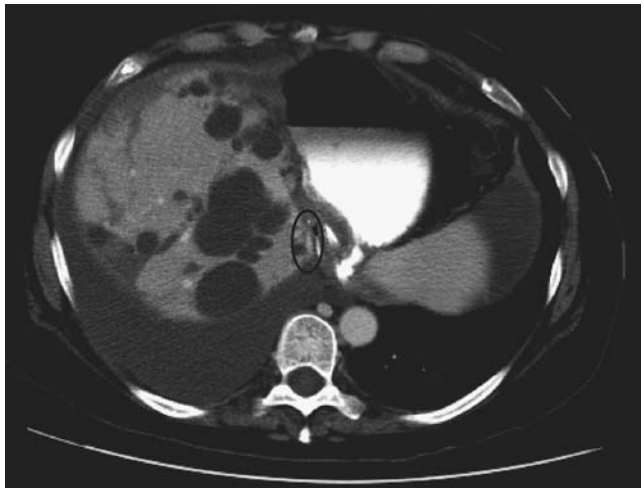

Figure 1 Computed tomography scan demonstrating compression of the intrahepatic IVC.

Material and Methods

Patients

We performed a retrospective chart review of patients with PLD who underwent stenting of the IVC or HV for intractable ascites from January 1996 through January 2005 at the Mayo Clinic in Rochester, MN. Patients were considered to have intractable ascites if they remained symptomatic from their ascites with decreased quality of life despite maximal medical therapy (diuretics, low salt diet, repeat paracenteses). The study was approved by the Institutional Review Board. All patients were assessed clinically and radiologically by abdominal ultrasonography, computed tomography, or magnetic resonance imaging (Fig. 1). All seven patients ultimately underwent venography to assess the IVC and HV for patency, compression, or stenosis. Venous pressure, proximal and distal to angiographically recognized sites of inferior vena caval or hepatic venous compression or stenosis, was measured directly and venous pressure gradients were calculated before and after stenting. Demographic data, symptoms, and number and type of hepatic operations were abstracted from clinical records. The site of the vein stented, the number of stents, treatment-related morbidity, follow-up interval, additional interventions, and efficacy were also obtained. Follow-up was obtained via clinic visit, physician correspondence, and/or telephone contact. Follow-up interval was calculated from the time of stent deployment to the time of latest follow-up or to the time of the secondary intervention (Leveen shunt in patients 1 and 2, liver transplantation in patient 5). Follow-up was calculated from the deployment of the second stent in the two patients requiring sequential deployment of stents.

Procedure

All interventional vascular procedures were performed under fluoroscopic guidance with conscious sedation. Vascular access was obtained via the right common femoral vein in all patients. One patient also underwent ultrasound-guided access via the right internal jugular vein during a second interventional procedure. Briefly, venous access was obtained after sterile preparation of the groin or neck using a modified Seldinger technique. The access tract was dilated and catheterized for venography of the IVC or HV. An expandable metallic Gianturco Z stent (Cook Medical, Bloomington, IN, USA) was deployed across a confirmed stenosis after size determination by balloon dilatation. A Palmaz stent (Cordis Endovascular, Warren, NJ, USA) was used to stent the right HV in one patient. Venograms were obtained before and after stenting and velocity gradients across the stenosis and stent were also measured. The sign rank test was used to determine whether there was a significant difference between venous pressure gradients before and after stenting.

Results

General

There were seven patients with PLD who underwent IVC stenting with or without HV stenting for intractable ascites from January 1996 through January 2005 (Table 1). There were six women and one man, with a mean age of 55 years (range 36–75 years). All had refractory ascites. Clinical features varied and included the following: early satiety, abdominal pain, malnourishment, nausea/vomiting, bilateral lower extremity edema, hypotension on dialysis, gastroin-

Table 1 Demographic, Prestent, and Poststent Data

Patient	Sex	Age	Prestent Resection	Vein Stented	Prestent Gradient (mm Hg)	Poststent Gradient (mm Hg)	Δ mm Hg	Efficacy	Safety	Secondary Procedure	Follow-up (Months)
1	M	64	+	IVC	6	3	3	−	+	Leveen shunt	0.5
				Right HV	17	3	14	−	+		
2	F	61	+	IVC	8	0	8	−		Leveen shunt	2.7
3	F	36	+	IVC	8	2	6	−	+	–	10.5
				Right HV	25	6	19	+	+		
4	F	75	−	IVC	14	0	14	+	+	–	0.6
5	F	43	+	IVC	NR	5	–	+	+	–	4.6
6	F	56	−	IVC	24	6	18	+	+	–	39.1
7	F	47	+	IVC	14	2	12	+	+	–	27.6

testinal reflux, and spontaneous bacterial peritonitis. Two patients developed intractable ascites de novo without prior hepatic resection. Both of these patients had resolution of their ascites after IVC stenting. One of these patients had undergone bilateral nephrectomy prior to anticipated renal transplantation. Five patients developed intractable ascites after partial hepatectomy and cyst fenestration: two patients underwent left hepatectomy and fenestration of right-lobe cysts and three patients underwent right hepatectomy and fenestration of left-lobe cysts. The mean interval from hepatic resection to stent placement was 55.4 days (median of 41 days, range 32–96 days). These five patients represented 8.6% of all patients with PLD undergoing hepatic resection and cyst fenestration for symptomatic hepatomegaly at our institution between January 1996 and January 2005. IVC stenting was successful in three of these five patients, but it was unsuccessful in the remaining two patients.

Venography in all seven patients demonstrated compression or stenosis of the IVC and all seven patients subsequently underwent stenting of the IVC (Fig. 2). The mean pressure gradient across the venous compression or stenosis before stenting was 14.5 mm Hg (range 6–25 mm Hg). Stents were deployed successfully within the IVC in all patients. Six patients were stented with 25-mm×5-cm (diameter × length) stents, and a single patient was stented

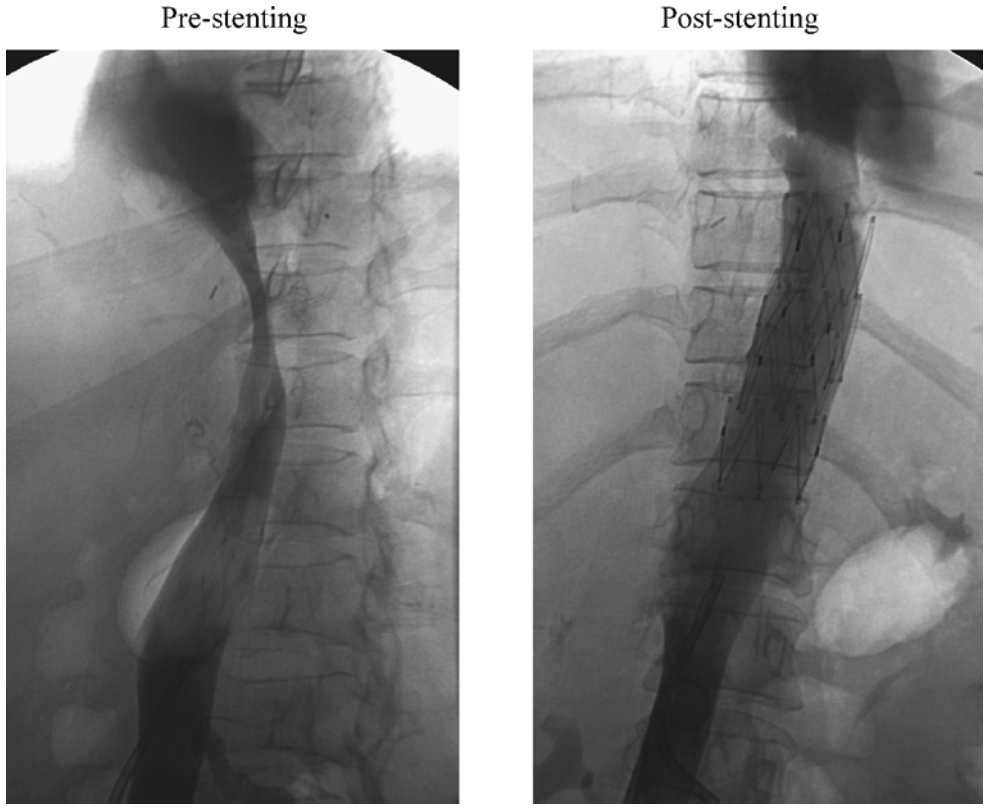

Figure 2 A venogram demonstrating a stenotic segment of IVC before stenting and subsequent resolution of the stenosis after deployment of multiple intravascular stents.

with a 20-mm×5-cm stent. The number of stents varied from one stent in three patients, two stents in three patients, and four stents in one patient. Four patients had clinical improvement after IVC stenting alone. Four patients also had hepatic venous stenoses on initial venography. IVC stenting alone was unsuccessful in two patients with hepatic venous stenoses. These two patients underwent subsequent stenting of the right HV. One of these patients showed improvement after HV stenting and has not required further treatment. The other patient failed to improve and a Leveen peritoneovenous shunt was inserted, followed by clinical improvement. Overall, there was a significant decrease in the venous pressure gradient after stenting to a mean of 2.8 mm Hg (range 0–6 mm Hg, $p=0.008$) and the mean Δ pressure gradient was 11.8 mm Hg (range 3–19 mm Hg).

Laboratory values demonstrated that renal and hepatic function was maintained. Serum albumin level trended upward presumably from improved appetite and decreased protein wasting from repeated paracenteses. There were no deaths. Treatment-related morbidity occurred in one patient (patient 6) who was on dialysis. She had undergone bilateral nephrectomy complicated by hemorrhagic shock and cardiac arrest approximately 2 months prior to her stenting. She developed pulmonary edema several hours following stenting of the IVC and responded to dialysis and supplemental oxygen. Interestingly, her pressure gradients before and after stenting were 24 and 6 mm Hg, respectively (Δ pressure gradient of 18 mm Hg). The pulmonary edema presumably was secondary to increased venous return.[10] She had permanent resolution of her ascites. She underwent renal transplantation approximately 6 months after stenting of the IVC and has remained well after 3 years. The other two patients with end-stage renal disease on dialysis (patients 1 and 4) had no morbidity associated with stenting.

The mean follow-up was 12.2 months (range 0.5–39.1 months). Patency of all stents has been confirmed at follow-up by Doppler ultrasonography. Five patients (71.4%) achieved clinical improvement, defined as a decrease of symptoms, diuretic use, and frequency of paracentesis.

Comparison of nonresponders vs. responders

Overall, five patients improved and two patients failed to improve clinically. The nonresponders were older (64 and 61 years old) than the responders (mean age of 51 years old; range 36–75 years). Both nonresponders had undergone prior hepatic resection and cyst fenestration vs. three of five of the responders. The mean follow-up for the responders and nonresponders was 16.2 and 1.2 months, respectively. Unresolved ascites after stenting and initiation of other therapy was defined as stent failure, which ended further stent-specific follow-up, accounting for the brief duration of follow-up for the nonresponders. Of the nonresponders, one patient subsequently improved after insertion of a Leveen peritoneovenous shunt. The other patient failed to improve after both Leveen peritoneovenous and saphenoperitoneal shunts and died approximately 1 year after IVC stent placement.

Of the responders, one patient who failed to improve after IVC stenting subsequently responded to stenting of a right HV and has required no further intervention. Four of the five patients with clinical improvement of ascites after stenting of the IVC required no additional intervention. One of these patients underwent orthotopic liver transplantation approximately 4.6 months after her IVC stent for refractory symptomatic hepatomegaly but without ascites. The remaining patient was lost in follow-up after 0.6 months.

Clinical response to stenting may be related to the Δ pressure gradient across the IVC. The mean pressures in the IVC below the stenosis before stenting for responders and nonresponders were 19.2 and 9.8 mm Hg, respectively. The mean Δ pressure gradients across the stenosis for responders and nonresponders were 15.8 and 7.8 mm Hg, respectively.

Discussion

Massive hepatomegaly is an infrequent complication of PLD that can cause severe abdominal discomfort and decrease the quality of life. There is no current effective medical treatment for hepatomegaly. Hepatomegaly can be reduced only by cyst aspiration, cyst fenestration, hepatic artery embolization, hepatic resection, or hepatic transplantation. Cyst aspiration with or without chemical sclerosis may be effective in patients with a small number of large dominant cysts but is seldom useful in patients with massive hepatomegaly and lacks durability.[11–15] Selective hepatic artery embolization for the treatment of massive PLD has only been used in Japan.[16] In select patients with favorable hepatic anatomy, laparoscopic or open fenestration or hepatic resection combined with cyst fenestration can be effective.[17–25] Combined resection and fenestration has been reported to be effective in over 80% of patients at long-term follow-up;[23] however, perioperative mortality has ranged from 3 to 11% and morbidity has been significant (37–83%). Although orthotopic liver transplantation has been performed for hepatomegaly in PLD patients with promising durability,[26–30] perioperative mortality remains significant (20–33%), immunosuppression is required, and organ allotment issues for patients without liver failure remain controversial.[31] Only hepatic transplantation for the treatment of hepatomegaly in patients with PLD can reliably eliminate ascites.

Intractable ascites is a rare complication of PLD but can develop de novo or postoperatively.[32–34] Management of ascites is confounded by its multifactorial cause and the associated degree of renal dysfunction in patients with PLD. Hepatic resection or cyst fenestration may alleviate de novo ascites through the reduction of inferior venal caval or hepatic venous pressure gradients by removal of the sites of venous compression in the polycystic liver. Postoperative ascites, although more common, usually respond to medical management. Only 5 of 58 patients (8.6%) who have undergone combined hepatic resection and cyst fenestration for PLD within our study period have developed refractory ascites. Although a rare patient may harbor residual cysts that cause significant venous obstruction and can be approached by repeat resection, fenestration or aspiration, and sclerosis, the limits of resection and fenestration usually have been reached in most patients and usually preclude repeat operative intervention. This clinical setting should prompt the consideration of intravascular stenting.

Intractable ascites and other signs of hepatic venous outflow obstruction have been observed in several clinical conditions, including patients with Budd–Chiari syndrome (BCS) and mechanical narrowing following liver transplantation. With the recent advances of percutaneous transluminal angioplasty and stent technology, small case series have demonstrated the safety and effectiveness of IVC stenting for the treatment of BCS[35–38] and IVC compression or torsion following liver transplantation.[39,40] Ascites associated with PLD are likely multifactorial: hepatic venous outflow obstruction (torsion, compression, operative narrowing), cyst secretion, lymphatic leak, chronic liver disease, and chronic renal disease. All of our patients had IVC stenoses. We hypothesized that relieving obstruction in the IVC may improve hepatic venous drainage into the IVC by dilatation of clinically occult but clinically significant obstruction of the orifices of some of the HVs at their junction with the IVC. Moreover, stenting of IVC reduces renal outflow pressure, which may also enhance renal clearance of ascites. To our knowledge, our study is the first to address this clinically challenging subgroup of PLD patients with this novel approach. Our results in this initial series have been encouraging. We have shown that IVC stenting is both relatively effective (70%) in palliating intractable ascites and safe with a morbidity rate of 14%. More than half of the patients so treated required no further intervention. Although all patients had an IVC with or without HV stenosis with associated elevated pressure gradients, IVC stenting was unsuccessful in two patients. These failures suggest that other factors contribute to intractable ascites, such as (1) lymphatic leakage after resection, (2) secretion from residual cysts, or (3) concomitant chronic liver disease or hepatic fibrosis. Although we attempt to ligate the prominent perihepatic lymphatics and ablate the residual exposed cyst epithelium during hepatectomy, we cannot assess the impact of these operative maneuvers on outcome in these patients. Our study is too small to define fully who will not respond to IVC stenting. However, our data suggest that an initial venous pressure gradient of >10 mm Hg across the stenosis before stenting and Δ pressure gradient of >10 mm Hg may be predictive of outcome.

Conclusions

Intractable ascites in patients with PLD are a challenging clinical problem. Our results suggest that percutaneous IVC stenting is safe and effective and should be considered as a first-line treatment for intractable ascites in select PLD patients. Hemodynamic evidence of hepatic venous outflow obstruction clinically identifies potential candidates for stenting and a venous pressure gradient >10 mm Hg may be predictive of outcome. Further studies will be required to confirm long-term efficacy and address various technical issues related to IVC stenting in this challenging patient group.

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Distal Pancreatectomy for Chronic Pancreatitis: Risk Factors for Postoperative Pancreatic Fistula

Thomas Schnelldorfer · Patrick D. Mauldin · David N. Lewin · David B. Adams

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Abstract One of the most common complications after distal pancreatectomy is a fistula from the pancreatic remnant. Factors influencing the development of a pancreatic fistula after distal pancreatectomy have not been clearly elucidated. The records of 91 patients who underwent distal pancreatectomy for chronic pancreatitis between 1995 and 2003 were retrospectively reviewed and analyzed. Average daily volume and amylase concentration between postoperative days 2 and 20 from drains located at the pancreatic resection site were compared to clinical variables. Out of 137 pre- and intraoperative clinical variables, multivariate analysis showed serum creatinine (t=3.05, p=0.004), history of intraabdominal operation (t=−2.68, p=0.01), right-sided pancreatic duct dilation (t=2.65, p=0.01), synchronous cholecystectomy (t=2.53, p=0.02), and serum albumin (t=−2.19, p=0.04) to be independently associated with drain volume. Drain amylase concentration was linked to serum creatinine (t=8.55, p<0.001), blood urea nitrogen (t=−3.43, p=.001), preoperative parenteral nutrition (t=2.56, p=.01), and serum alkaline phosphatase (t=2.51, p=0.01). There was no correlation between the degree of fibrosis and drain output. Technique of pancreatic transection and presence of suture closure of the pancreatic duct did not affect drain output. In conclusion, the amount and amylase concentration of postsurgical drainage after distal pancreatectomy for chronic pancreatitis is dependent on markers of renal dysfunction, malnutrition, biliary disease, and possibly inflammation. These factors, if medically reversible, should be addressed in patients who are candidates for distal pancreatectomy for chronic pancreatitis.

Keywords Distal pancreatectomy · Fistula · Leak · Chronic pancreatitis

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T. Schnelldorfer · D. B. Adams (✉)
Department of Surgery, Medical University of South Carolina,
96 Jonathan Lucas Street, P. O. Box 250613,
Charleston, SC 29425, USA
e-mail: adamsdav@musc.edu

P. D. Mauldin
Department of Pharmacy and Clinical Science,
Medical University of South Carolina,
Charleston, SC, USA

D. N. Lewin
Department of Pathology and Laboratory Medicine,
Medical University of South Carolina,
Charleston, SC, USA

Introduction

Distal pancreatectomy for chronic pancreatitis is utilized in patients with left-sided pancreatic duct disruption or a mass suspicious for malignancy. It also has a limited role in management of intractable pain in patients with left-sided disease only. One of the most common complications after distal pancreatectomy is fistulous drainage from the pancreatic remnant. From 0 to 7% of patients undergoing distal pancreatectomy for chronic pancreatitis have been reported to develop this complication.[1–4] Debate continues about the definition of a postoperative pancreatic fistula. Most centers would agree that it is dependent on the amount of drainage from the surgical or radiologic placed drains, the amylase concentration in the drainage, and the time the fistula occurs.[5] Pancreatic fistula after distal pancreatectomy can be associated with other complications indirectly leading to increased hospital cost [6].

Reports about factors influencing the development of pancreatic fistulas after distal pancreatectomy are scarce.

Hence, identification of patients who are at high risk for pancreatic fistula after this operation is often made subjectively and not based on evidence. The aim of this study was to evaluate clinical factors associated with the amount of drainage and amylase concentration from drains placed near the pancreatic remnant.

Patients and Methods

The records of 91 consecutive patients who underwent distal pancreatectomy for chronic pancreatitis at the Department of Surgery, Medical University of South Carolina, from 1995 through 2003 were retrospectively reviewed and analyzed. The study group consisted of 44 women and 47 men with a median age of 45 years (range 18 to 73 years). Morbidity and mortality included complications during operation, hospitalization, or within 30 days of discharge after operation. Pancreatic fistula was defined as drainage of more than 50 ml/day of amylase-rich fluid after postoperative day (POD) 6. Fluid was considered amylase-rich if the amylase level was greater than threefold the normal serum value. The operative specimen were reevaluated by a single pathologist and microscopically classified according to the degree of fibrosis. The extent and distribution of fibrosis was graded from 0 to 12 utilizing a previously established scoring system.[7] The daily output from surgical drains located at the pancreatic resection site or a percutaneous drain placed postoperatively under radiologic guidance was evaluated. Average daily drain volume between POD 2 and 20 was calculated by dividing the total volume of drained fluid during POD 2 through 20 by the number of days fluid was collected (ml/day). Drain amylase concentration was analyzed selectively. If more than one drain amylase concentration was available, the higher level was used for analysis.

Data is reported as percentage or median±SE unless otherwise specified. Pearson correlation coefficients (Prob>$|r|$ under H_0: Rho=0) were used for univariate analysis. P values <0.05 were considered significant. A backward selection process was used for the multivariate analysis utilizing SAS version 9.0 computer software (SAS Institute, Inc., Cary, NC, USA). Variables at the 0.10 level were included in the multivariate model for proper specification; however, significance was determined at the 0.05 level.

Results

The primary indications for operative treatment in the 91 patients included pancreatic duct disruption in 57 patients, intractable pain with disease isolated to the body and tail of the pancreas in 26 patients, and a mass suspicious for malignancy in 8 patients. Out of the 57 patients with pancreatic duct disruption there were 41 cases with a pseudocyst refractory to endoscopic and radiologic percutaneous management, 28 cases with a disconnected pancreatic tail, and 7 cases with a pancreatic fistula. In 10 patients with evidence of duct disruption, intraoperative findings of focal midbody necrosis were made in proximity to the duct leakage. All patients had a postoperative diagnosis of chronic pancreatitis confirmed by histological evaluation. Fibrosis scores of the distal pancreatectomy specimen showed a mean score of 9±0.3 (range 1 to12). Ten patients had a history of focal acute necrotizing pancreatitis on average of 13±4.4 months previously. The incidence of preoperatively evaluated clinical features typical for chronic pancreatitis is listed in Table 1.

Ninety-one patients underwent open distal pancreatectomy, including splenectomy in 78. Average operative time was 145±5.8 min. Average estimated blood loss per case was 800±126.3 ml. Perioperative blood transfusion was necessary in 49% of patients. All patients had a surgical drain placed in the left upper quadrant in proximity to the transection site of the pancreatic remnant. Perioperative complications were observed in 29% of patients and are listed in Table 2. Two deaths occurred after distal pancreatectomy: one because of a cerebral stroke and the other in a patient who died at home of unknown cause shortly after an uncomplicated hospital stay. Average hospital length of stay was 8±1.4 days. During this stay 13% of patients required a stay in the intensive care unit for an average of 4±0.9 days. The surgical drain was removed on an average of 7±0.6 days after operation. In 74 patients the drain was removed during their hospital stay (median

Table 1 Preoperative Disease-related Characteristics in the 91 Study Patients

Characteristic	Percent/Mean±SE
Risk factors for chronic pancreatitis	
Alcohol	34%
Idiopathic	23%
Gallstones	19%
Pancreas divisum	11%
Autoimmune	5%
Posttraumatic	2%
Familial	1%
Hyperparathyroidism	1%
Duration of symptoms	12±5.0months
Long-term need for prescription narcotics	54%
Insulin dependency	18%
Need for pancreatic enzyme replacement	33%
Nutritional status	
Body mass index	26.1±0.5
Serum albumin level	3.3±0.1g/dl

Table 2 Perioperative Complications after Distal Pancreatectomy for Chronic Pancreatitis (Multiple Complications per Patient Possible)

Complications	Percent
Morbidity	29
Intraabdominal complications	22
Intraabdominal abscess	16
Pancreatic leak	5
Delayed gastric emptying	2
Postoperative hemorrhage	2
Extraabdominal complications	10
Wound infection	2
Respiratory failure	3
Pneumonia	2
Urinary tract infection	2
Sepsis from nonabdominal source	1
Gastrointestinal bleed	1
Acute renal failure	1
Cardiac arrhythmia	1
Pulmonary embolus	1
Cerebral stroke	1
Mortality	2

6±0.4 days). Seventeen patients went home with the drain and had the drain removed at clinic follow-up (median 20± 0.4 days) including two of the five patients with a postoperative pancreatic fistula. Five patients required radiologic placement of percutaneous drains for a pancreatic fistula (two patients), abscess in the left upper quadrant (one patient), or both (two patients). Only 2 of the 15 patients with an intraabdominal abscess met the criteria for a pancreatic fistula. Highest drain volume was 90±32.0 ml on POD 0 and decreased to 30±13.2 ml on POD 2 (Fig. 1). Thereafter it slowly decreased but did not change significantly to 10±22.9 ml on POD 10 and 5±34.9 ml on POD 20. Seventy-two patients had the drain output evaluated for amylase concentration on average on POD 4±0.3. The average drain amylase level was 161±5,680 IU/l.

One hundred thirty-seven pre- and intraoperative clinical variables were compared to the average daily drain volume between POD 2 and 20 and to the drain amylase concentration using Pearson correlation coefficients (Table 3). Variables significant at $p<0.10$ were included in a multivariate backward regression analysis model. Variables independently influencing the drain volume or drain amylase level are listed in Table 4. These included preoperative variables representing renal dysfunction, malnutrition, and obstruction within the right-sided/remnant pancreatic duct, as well as operative variables representing intraabdominal adhesions and biliary disease or inflammation. In addition, to ensure statistical accuracy, preanalyses were performed, separately analyzing a correlation between the 137 variables and individual daily drain volume between POD 2 and 6. Multivariate analysis beyond POD 6 was not performed because of the decrease in power. These preanalyses provided similar results and supported the model used.

The strongest predictors of increased drain volume and drain amylase level were surrogates of renal dysfunction. Elevated preoperative serum creatinine concentration were independently associated with drain output. The clinical diagnosis of chronic renal failure was significant only on univariate analysis. Preoperative malnutrition measured by serum albumin level and preoperative use of total parenteral nutrition correlated with an increase in drain volume and drain amylase concentration. Other surrogates of preoperative malnutrition such as a history of weight loss and body mass index did not affect drain output. Preoperative ERCP was performed in all patients. Twenty-four patients had a known mild stricture of the dominant duct within the head of pancreas. Nineteen patients had known ductal dilation and eight patients had pancreatic stones in the pancreatic remnant. In patients with preoperative radiographic evidence of downstream ductal obstruction, a dilated pancreatic duct was the only finding that influenced the amount of

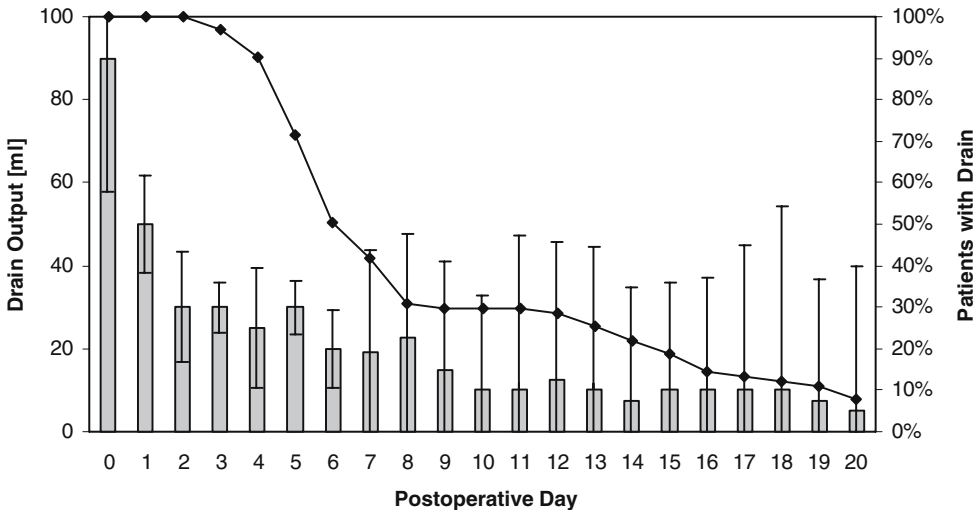

Figure 1 Median output from surgical or percutaneous drains± SE (column) and percentage of patients with drain in place (line).

Table 3 Univariate Model Comparing Pre- and Intraoperative Variables With Drain Volume and Drain Amylase Concentration

Variables	Effect on Drain Volume	*p* value	Effect on Amylase Level	*p* Value
Renal function				
Serum creatinine	↑	0.006	↑	<0.001
Blood urea nitrogen	↑	0.03	↑	0.05
History of chronic renal failure	–	–	↑	0.02
Nutrition				
Serum albumin	↓	0.004	–	–
Serum protein	↓	0.01	–	–
Preoperative anorexia	↑	0.05	–	–
Operative technique and operative trauma				
Transection of pancreas with stapler	↑	0.04	–	–
Number of operative drains	↑	0.02	–	–
Synchronous cholecystectomy	↑	0.03	–	–
History of intraabdominal operation	↓	0.04	–	–
Preoperative pancreatic duct changes				
Right-sided main pancreatic duct dilation	↑	0.04	–	–
Irregular main pancreatic duct	↓	0.03	–	–
Main pancreatic duct cutoff	↑	0.03	–	–
Others				
American Society of Anesthetists score	↑	0.04	–	–
Serum alkaline phosphatase	↑	0.008	↑	<0.001

The effect on drain output (increase ↑, decrease ↓) is shown for variables with a p value <0.05.

postoperative drainage but it did not affect the amylase level. Consistency of the pancreatic gland determined by its degree of fibrosis did not correlate with drain volume or amylase level. This applied for intralobular, perilobular, and overall degree of fibrosis. Inflammation in proximity to the transection site seen with midbody necrosis did not alter drainage output. The neck of pancreas was transected in 63 cases using electrocauterization, in 26 cases using a linear vascular stapling device, and in 2 cases using a scalpel. On univariate analysis the technique used to divide the gland showed an increase in the amount of drain output when stapling devices were used. This difference was not sustained after multivariate analysis. In the 65 cases of pancreatic transection with electrocauterization or scalpel, the capsule of the transection site was reapproximated with or without duct closure 45 times and in 23 patients the pancreatic duct was closed with or without capsule reapproximation. The technique utilized to close the duct did not correlate with the volume from the surgical drains nor their amylase level. Prior abdominal operations decreased the drain output volume. If cholecystectomy was performed at the same time or preoperative serum alkaline phosphatase levels were elevated, the amount of drainage was measurable higher. Simultaneous splenectomy and sinistral portal hypertension did not influence the amount of drainage. Postoperative prophylactic use of octreotide

Table 4 Multivariate Backward Stepwise Regression Model Comparing Drain Volume or Drain Amylase Concentration with Clinical Variables Showing a p Value <0.1 on Univariate Pearson Correlation

	Effect on Drain Volume	Effect on Amylase Level	*t* Value	*p* Value
Variables affecting drain volume, n=91, R^2=0.40				
Serum creatinine	↑		3.05	0.004
History of intraabdominal operation	↓		−2.68	0.01
Right-sided main pancreatic duct dilation	↑		2.65	0.01
Synchronous cholecystectomy	↑		2.53	0.02
Serum albumin	↓		−2.19	0.04
Variable affecting drain amylase level, n=72, R^2=0.40				
Serum creatinine		↑	8.55	<0.001
Blood urea nitrogen		↓	−3.43	0.001
Preoperative parenteral nutrition		↑	2.56	0.01
Serum alkaline phosphatase		↑	2.51	0.01

Listed are variables with p value <0.05 only.

was administered in 54% of patients from POD 0 till discharge. Octreotide use did not significantly alter drain volume or amylase concentration. The multivariate analysis model was controlled for the prophylactic use of octreotide.

Because postoperative variables could potentially represent an effect rather than a cause of drain output, with the exception of octreotide, they were not included in the multivariate model. On univariate analysis, high drain output and high drain amylase level were associated with an increase in incidence of postoperative intraabdominal abscesses (volume $p=0.02$, amylase $p=0.03$). Drain volume and amylase level also influenced the incidence of delayed gastric emptying measured by the time after operation regular diet was tolerated (volume $p<0.001$, amylase $p<0.001$). This was independent of postoperative ileus because the onset of bowel sounds and passage of bowel movements or flatus was not affected. Hospital length of stay was prolonged in patients with increased drain volume ($p<0.001$) but not with increased drain amylase concentration ($p>0.05$). Incidence of stay in the intensive care unit and need for rehospitalization during the first postoperative year was not influenced by drain output.

Discussion

After distal pancreatectomy, most pancreatic fistulas represent "transient fistulas," indicating that the leak does not affect the patient's overall clinical status and does not increase the admission rate to intensive care unit or delay hospital discharge. This is different from "clinically significant fistulas" seen with pancreaticoenteric anastomoses. These fistulas differ because of the potential activation of pancreatic proenzymes by enteric or biliary enterokinase, bacterial contamination from intestinal content, and inflammation from bile. After distal pancreatectomy the drainage of plain pancreatic juice and wound secretion have less adverse effects and, as demonstrated in this study, is less commonly associated with local infections. Medical treatment and potential manipulation of existing drains or percutaneous placement of additional drains can usually resolve any complications from undrained fluid collections and abscesses. This explains why none of the patients required operative intervention for a pancreatic fistula and why drain output did not affect ICU stay. Presuming adequate drainage, all fistulas after distal pancreatectomy should resolve eventually. The practice at this institution is to manage clinically stable patients with a controlled transient fistula in an outpatient setting. Out of the 17 patients who were discharged to home with a drain in place, only 2 met the criteria applied to define a pancreatic fistula. In the remainder, this reflects the operating surgeon's decision that the patient was clinically suitable for discharge, but the amount of drainage was felt to be too high for safe removal. Despite this practice, drain volume was associated with an increase in the length of hospital stay. This was most likely not because of the drain output itself, but rather the effect of drain volume on the incidence of abscess formation and delayed gastric emptying.

This study demonstrated that drain volume or drain amylase concentration independently correlated with markers of renal dysfunction, malnutrition, downstream pancreatic duct obstruction, intraabdominal adhesions, and biliary disease. Some of these variables could also be interpreted as markers of inflammation because low serum albumin levels and the presence of biliary disease can be signs of increased intraabdominal inflammation and tissue edema. The accuracy of drainage volume as a surrogate for pancreatic drainage is affected by postoperative hemorrhage, wound and peritoneal secretions, peritoneal absorption of fluid, and the effectiveness of the drain to evacuate the wound cavity. This causes drain volume alone to be an unreliable marker for pancreatic fistula. Drain amylase concentration is influenced by dilution and degree of exocrine insufficiency limiting its application as a single surrogate. Using drain volume in combination with drain amylase concentration improved accuracy, and although not perfect, is clinically the most practical surrogate. In this study, variables that altered both drain volume and drain amylase concentration were related to renal dysfunction, malnutrition, biliary disease, and possibly inflammation. Out of those variables, preoperative renal dysfunction was the strongest predictor of a pancreatic fistula formation. This is confirmed by a recent observational study evaluating risk factors for pancreatic fistula formation after pancreatic resection (87% pancreatic head resection, 12% distal pancreatectomy, and 1% segmental resection) for malignant and benign disease,[8] where multivariate analysis showed that out of 21 variables, preoperative serum creatinine level was the only independent risk factor for pancreatic fistula. Patients with a creatinine level above norm had 2.8 times increased risk of developing a pancreatic fistula. Increased fistula risk was ascribed to impaired wound healing in patients with renal dysfunction. A decrease in wound healing would also explain the increase in drain volume and amylase concentration found in the current study for patients with malnutrition and inflammation.

Pancreatic fibrosis was always thought to affect the incidence of pancreatic fistula. After pancreaticoduodenectomy observational studies identified texture of the gland remnant or degree of fibrosis of the gland as independent predictors for incidence of pancreatic fistula.[9,10] For left-sided resection this correlates with findings that the postoperative pancreatic fistula rate is higher in patients

undergoing distal pancreatectomy for malignancy, who have a presumed healthy gland within the remnant, vs patients undergoing distal pancreatectomy for chronic pancreatitis, who will have some degree of fibrosis in the remnant. After resection for malignancy the fistula rate is 17 to 28% compared to 0 to 7% for chronic pancreatitis.[1–4,11–13] To the best of our knowledge no data existed before this study examining the correlation between the degree of pancreatic fibrosis and fistula rate after distal pancreatectomy. This study showed clearly no correlation between the degree of parenchymal fibrosis and the amount of drain output or drain amylase concentration. A potential explanation for this discrepancy to what is thought to be common understanding is that most studies do not control for pancreatic inflammation. An atrophic pancreas with high degree of fibrosis often has little inflammation compared to a soft and edematous pancreas, which can present with acute inflammation.

Studies evaluating surgical technique utilized in pancreatic remnant closure after distal pancreatectomy suggested that staple closure might be slightly superior to suture closure in preventing postoperative fistula formation.[14] This data is difficult to interpret because of small case numbers, heterogeneity of the population, and lack of randomization in most trials. In our experience staple closure seems to be more suitable for small glands, which can be sufficiently mobilized enough to apply the device. Mobility of the tail often correlates with absence of peripancreatic inflammation, which itself could predict a lower fistula rate. This study suggests that the type of remnant closure did not independently affect drain output. Because of the retrospective nature of the study, this result might be biased by the surgeon's intraoperative decision to handle the closure in a manner most suitable for the individual situation.

The pure presence of a mild to moderate stricture within the head of pancreas did not affect drain output. If the stricture was obstructive, identified by dilation of the main pancreatic duct within the head, the drain volume was increased. Drain amylase concentration was not affected. This observation correlates with a small sample size observational study describing a trend toward decreased frequency of fistula after distal pancreatectomy when prophylactic pancreatic stents were used.[15]

Whether perioperative prophylaxis with octreotide alters postoperative pancreatic fistula rate after distal pancreatectomy remains undetermined. Three previous high-volume randomized multicenter trials assessing the effect of octreotide or vapreotide on fistula rate after pancreatic resection for multiple etiologies provided subgroup analysis of data for distal pancreatectomy. Two showed no difference in incidence of postoperative fistulas and one showed a significant decrease in fistula rate after treatment with octreotide.[16–18] For patients undergoing resection for chronic pancreatitis, octreotide seems to have a lesser effect on fistula prevention compared to patients with malignancy.[19,20] A similar finding was made in the current study where prophylactic octreotide use did not impact on drain output.

Conclusion

The amount of postsurgical drainage and drain amylase concentration after distal pancreatectomy for chronic pancreatitis is dependent on markers of renal dysfunction, malnutrition, biliary disease, and perhaps inflammation. These factors should be identified and whenever possible ameliorated in patients who are candidates for distal pancreatectomy for chronic pancreatitis.

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Decreasing Pancreatic Leak After Distal Pancreatectomy: Saline-coupled Radiofrequency Ablation in a Porcine Model

Mark J. Truty · Mark D. Sawyer · Florencia G. Que

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Abstract Despite marked improvements in pancreatic surgery, the high incidence and morbidity of pancreatic leak after resection has remained unchanged. The objective of this study was to evaluate the role of saline-coupled radiofrequency ablation (TissueLink) as an alternative to traditional methods of stump closure in an animal model of distal pancreatectomy. Forty swine were randomized after pancreatic transection and remnant stump was either oversewn in a traditional fashion (control) or treated with the device alone (TissueLink). Animals were killed and necropsied at 3 or 5 weeks postoperatively. Primary endpoints were the development of a pancreatic fistula defined as dye extravasation from the remnant duct, presence of undrained amylase-rich fluid collections/abscess, and greater than threefold drain/serum amylase after the third postoperative day. The incidence of pancreatic leak in the TissueLink group was 5.5 vs 42% in the control group ($p=0.01$). There were no differences in operative time or other clinical parameters measured. Histologic analysis of the remnant pancreatic stumps confirmed our results. These data support our hypothesis that saline-coupled radiofrequency ablation leads to obliteration of ducts with a resultant decrease in pancreatic leak and subsequent complications. This technology may play a substantial role in preventing this dreaded complication in the clinical setting.

Keywords Distal pancreatectomy · Pancreatic fistula · Pancreatic leak · Saline-coupled radiofrequency ablation

This study was presented at the Pancreas Club on May 21, 2006 and at the Society for Surgery of the Alimentary Tract during Digestive Disease Week on May 22, 2006 in Los Angeles, CA, USA, as a poster presentation.

M. J. Truty · F. G. Que (✉)
Department of Surgery, Mayo Clinic College of Medicine,
200 First Street SW, Mayo West 12,
Rochester, MN 55905, USA
e-mail: que.florencia@mayo.edu

M. J. Truty
e-mail: truty.mark@mayo.edu

M. D. Sawyer
Division of Trauma Critical Care and General Surgery,
Mayo Clinic College of Medicine,
Rochester, MN, USA

F. G. Que
Division of Gastrointestinal and General Surgery,
Mayo Clinic College of Medicine,
Rochester, MN, USA

Introduction

Whereas the mortality for pancreatic resection has decreased considerably over the last several decades, complications secondary to leakage from the pancreatic stump and anastomosis have remained relatively unchanged. The reported incidence after distal pancreatectomy is highly variable and in some studies as high as 60%, although more recent studies have reported the incidence as approximately 20–30%.[1–4] This wide range reflects the variability and stringency in the definition used for pancreatic leak, from asymptomatic elevated amylase levels in surgical drains to demonstrated ductal disruption or collections requiring interventions such as drainage or surgery.

The difficulty in preventing pancreatic leak after distal pancreatectomy has resulted in various methodologies to close the pancreatic stump; however, no method has been clearly proven to be superior (Table 1). A recent meta-analysis examining the various published methods for closure of the pancreatic remnant after distal pancreatectomy found insufficient data to draw any firm conclusions on the optimal technique of stump closure.[4] Thus, novel

Table 1 Various Methods and Techniques Used in Closure of Remnant After Distal Pancreatectomy

Methods
Traditional oversew
Duct ligation
Stapled closure
Enteric drainage
Omental plug
Serosal patch
Proximal duct stenting
Various sutures
Mesh overlay
Octreotide
Fibrin glue
Duct occlusion
Ultrasonic dissector/scalpel
Sealing devices
RF ablation

techniques and technologies are a logical direction in which to search for improvement.

Radiofrequency (RF) energy has been successfully employed in clinical medicine, perhaps most notably in tumor ablation. In recent years, a surgical device employing saline-coupled RF energy (TissueLink Medical Inc., Dover, NH, USA) has been developed for dissection, hemostatic sealing, and coagulation before renal and hepatic parenchymal transection.[5–13] Part of its efficacy is related to collagen shrinkage and fusion, which can completely obliterate the lumens of small vessels and ducts, thus making its use in distal pancreatectomy attractive by obviating the need for sutures, glues, or sealants on the remnant stump.[6] The device uses RF energy from a standard electrosurgical generator, which is concentrated near the tip and conducted through continuous low-volume saline irrigation and then into tissue where it is converted into heat (Fig. 1). The saline facilitates energy transfer between the device–tissue interface, maintaining contact with tissue and dispersing thermal energy, thus allowing tissue coagulation at a greater depth compared with standard electrical coagulation, and may protect against charring and eschar formation by providing evaporative surface cooling and limiting temperatures to 100°C.

We hypothesized that saline-coupled RF ablation (TissueLink) of the pancreatic stump would lead to a decrease in leak rates after distal pancreatectomy when compared to a traditional method of surgical stump closure. Our hypothesis was that this decrease would be secondary to the obliterative effect of RF energy on small ductal structures. We designed a prospective, randomized trial utilizing a porcine model of distal pancreatectomy to test our hypothesis.

Materials and Methods

Preoperative

All aspects of this study were performed as part of an animal research protocol according to institution guidelines and reviewed and approved by the Institutional Animal Care and Use Committee, Mayo Clinic College of Medicine. Preoperative and anesthesia care was provided by fully trained veterinary staff members. Forty domestic swine (*Sus scrofulus*) were used in this study. The animals were fasted overnight before the procedure. We used a combination of tiletamine and zolazepam/xylazine (4–7 mg/kg IM/1–2 mg/kg IM) for initial intramuscular sedation. Intravenous access was obtained by way of marginal ear vein cannulation using large gauge venous catheters. Atropine (0.05 mg/kg IV) was given 5 min before intubation to help dry secretions and the animals were endotracheally intubated in ventral recumbency and mechanically ventilated. Animals were maintained on isoflurane (1–2% vaporizer) and oxygen mixture. The stomach was decompressed by way of an orogastric tube. Perioperative fluids consisted of plasmalyte (balanced salt solution) at 10 cc/kg. Oxygen saturation and heart rate were monitored using pulse oximetry placed on the ear. Prophylactic doses of antibiotic (cefazolin 40 mg/kg IV) were administered 30 min before incision. All surgical procedures were done under sterile conditions in a fully equipped large animal operating room. The animals were secured supine on the operating table and sterile drapes were applied after a betadine preparation of the abdomen.

Intraoperative

All surgical procedures were performed by two of the authors (Truty and Que). A midline laparotomy using

Figure 1 TissueLink device used in study (DS3.5C™). Inset shows saline dripping from tip thought to improve tissue coupling and heating.

scalpel and electrosurgical dissection was used for intra-abdominal access extending from the xiphoid to the umbilicus. After initial exploration, the splenic vessels were clamped, ligated, and tied with 2-0 silk sutures and the spleen removed. The pancreas was then identified and the distal end mobilized with dissection of its retroperitoneal attachments (Fig. 2a). Approximately 8 cm of the distal pancreas was then transected with electrocautery and the specimen removed (Fig. 2b). After pancreatic transection, the animals were then randomized into either a control group (n=20) in which the remnant was oversewn with horizontal type mattress sutures, or TissueLink group (n=20) in which the remnant was treated with the DS3.5™ device alone for an ablation depth (thickness) of approximately 5 mm using electrosurgical generator settings of 100 W and a saline drip rate of 1 cc per/s (Fig. 2c,d). Completeness of TissueLink treatment was assessed visually and by direct palpation as the treated stump becomes pale, stiff, and rubbery. After stump management a 6.3-mm Jackson Pratt drain was then placed in the resection bed and brought out the animal's flank through a 3-mm stab wound, and the proximal end tunneled subcutaneously to the dorsum of the animal and connected to a collection chamber. The fascia was then closed with 0 Vicryl in a running fashion and the skin and subcutaneous tissue closed with 4-0 Vicryl subcuticular running sutures. A large transparent water-proof dressing was then applied to the incision and the animal was dressed in a garment to prevent chewing on the drains and to protect the incision.

Postoperative

After the procedure the animals were recovered in a monitored setting for several hours and then extubated when clinically indicated. Once ambulating they were housed in individual large animal facilities at our institution. Prophylactic doses of antibiotics (cefazolin 500 mg IV or cephalexin 500 mg PO) were administered for the first 5 days postoperatively. All animals were given ad libitum water only for the first 24 h and subsequently fed twice daily with pig chow thereafter. All animals were inspected twice daily by both the veterinary team and the authors in a blinded fashion to assess for fever, food intake, lethargy, emesis, narcotic need, anorexia, and bowel function in an effort to identify any suspicious clinical signs of pancreatic leak or sepsis. All animals received buprenorphine (0.03–0.05 mg/kg IM, IV q6–12 h) for postoperative analgesia on the first two postoperative days and then on an as needed basis. Drain output and quality was quantified daily for the first 10 postoperative days and samples snap frozen in liquid nitrogen for subsequent amylase determination at the conclusion of the study. Serum amylase levels were drawn both before initial incision and at subsequent necropsy.

Necropsy

Half of the animals in each group underwent necropsy in a randomized manner at 3 and 5 weeks after the initial

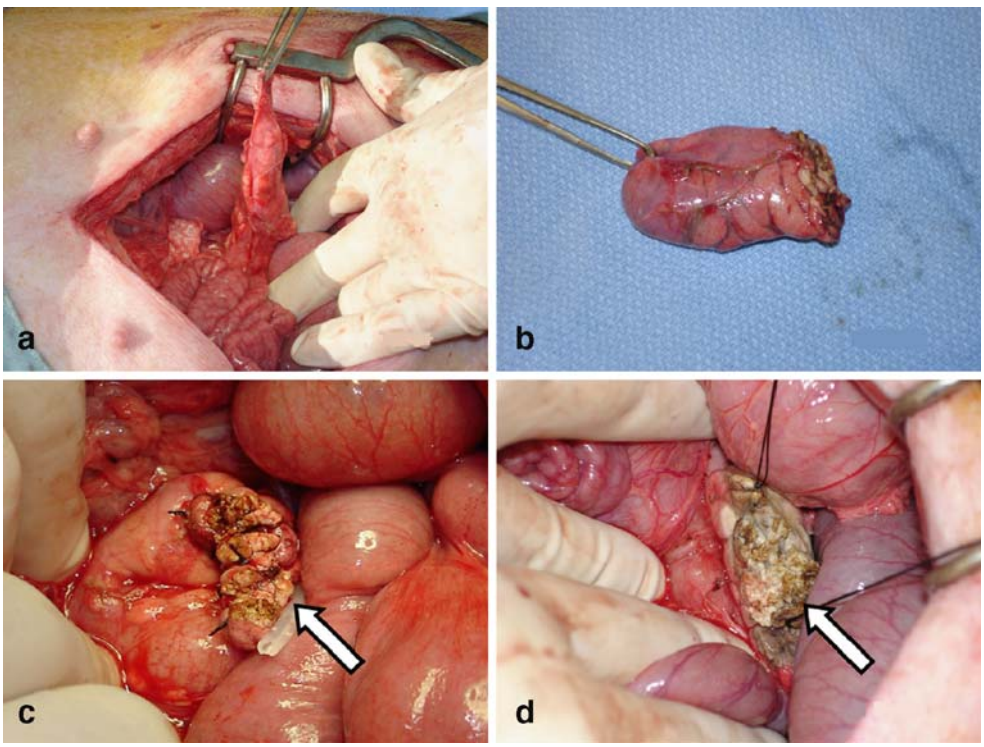

Figure 2 **a** Distal pancreas mobilized; **b** resected distal pancreas specimen; **c** control oversewn remnant; and **d** TissueLink treated remnant.

procedure to account for any late complications. At the time of necropsy all animals were sedated with tiletamine and zolazepam (4–7 mg/kg IM) and killed with a commercial euthanasia solution. At necropsy, all animals underwent exploratory laparotomy and the peritoneal cavity was assessed for excessive adhesions and any undrained fluid collections/abscess and peritoneal fluid samples sent for amylase determination and culture. The pancreatic resection beds were then assessed and photographed. In each animal, a duodenotomy was performed and the ampulla cannulated with a 21-gauge angiocatheter. A 1:10 dilution of methylene blue dye was then injected into the pancreatic duct in a retrograde fashion to assess for macroscopic dye extravasation from the pancreatic stump, which was our surrogate for a macroscopic leak (Fig. 3a,b). The remnant stumps were then carefully dissected, removed, and placed in 10% formalin for subsequent histopathologic analysis by a pathologist blinded to the group.

Amylase Measurements

Drain fluid was collected daily for the first 10 postoperative days or until the drains were malfunctioning because of animal manipulation. Fluid was snap frozen in liquid nitrogen, stored at −80°C until the completion of the study, and subsequently thawed and sent for amylase determination using a well-established standardized institutional protocol to measure α-amylase activity in the sample using a colorimetric rate reaction. Similar methodologies were used for serum amylase activity drawn from animals preoperatively and before necropsy. All measurements were performed by the Division of Clinical Core Laboratory Services, Mayo Clinic College of Medicine.

Histopathologic Analysis

Excision of the remaining pancreatic stump was performed in all animals at necropsy. Sections were performed at the level of the oversewn edge for the control animals and at the level of the interface of treatment for the TissueLink animals to a depth of approximately 2 cm of normal pancreas. Sections were imbedded in paraffin and cut at 5 μm and stained with hematoxylin and eosin. Slides were then reviewed by a pathologist blinded to the group.

Statistical Analysis

Factors were summarized as either percentages or means and standard deviations. Categorical variables were compared between groups using Fisher's exact test. Comparisons between groups for continuous variables were carried out using Wilcoxon rank-sum tests. Computerized statistical analysis was performed using SAS version 9.0 software.

Outcomes

The primary outcomes studied in our experiment were the development of a postoperative pancreatic leak or surgical complications defined as (1) macroscopic leak (evidence of dye extravasation from the pancreatic stump), (2) any

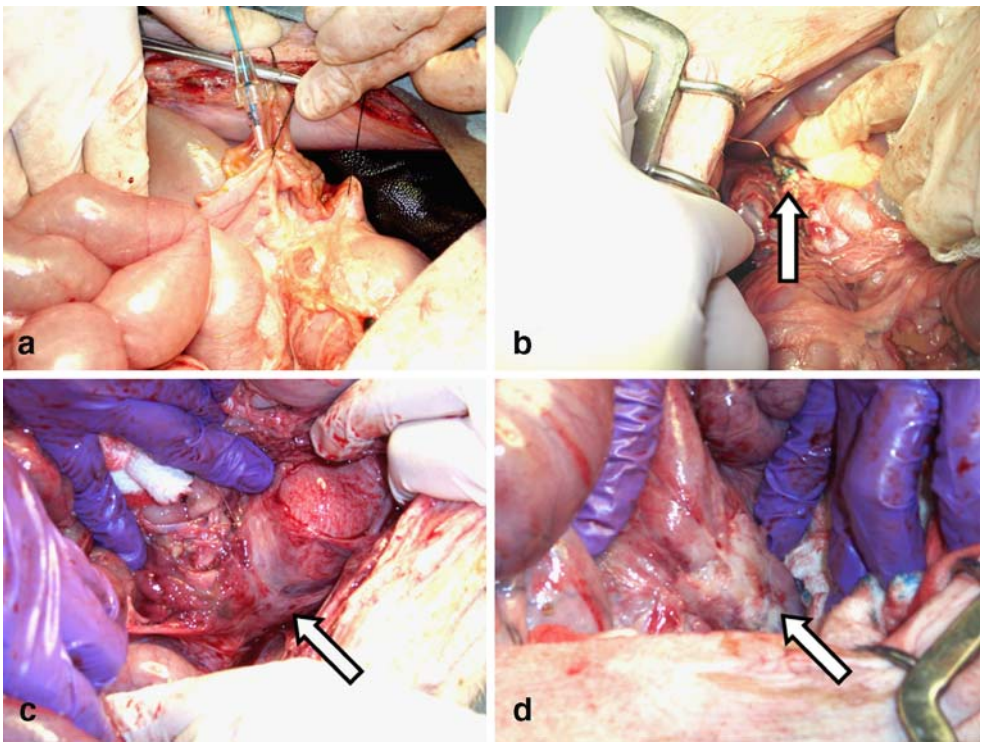

Figure 3 **a** At necropsy duodenotomy is performed and ampulla is cannulated with angiocatheter and methylene blue dye injected; **b** methylene blue dye is found extravasating from stump-positive for macroscopic leak; **c** control gross resection bed; and **d** TissueLink gross resection bed.

undrained amylase-rich fluid collections/abscess, and (3) greater than threefold drain/serum amylase after the third postoperative day (the definition of a biochemical leak according to the recent International Study Group on Pancreatic Fistula guidelines.[14] Secondary outcomes were operative time, wound infection, total daily drain output, and other perioperative clinical parameters (anorexia, emesis, lethargy, and narcotic need).

Results

There were no deviations from protocol as described in the methodology. There was one intraoperative death (TissueLink group) because of uncontrolled hemorrhage during splenectomy before pancreatic transection and treatment; otherwise, all animals survived the intraoperative procedures. Two animals (one in each group) were killed after 1 week to assess early histological effects of either treatment. In total 37 animals (19 control, 18 TissueLink) concluded the study and were used for outcome analysis.

The total rate of pancreatic leak and/or complications thereof in the TissueLink group was 5.5 vs 42% in the control group (p=0.01). These complications included: macroscopic dye extravasation (control 3, TissueLink 0) and undrained amylase-rich fluid collections/abscess (control 5, TissueLink 1) (Fig. 4). None of the animals had more than one of these findings at necropsy. We were unable to find a statistically significant difference in the postoperative drain amylase concentrations between groups at any of the postoperative days; however, when plotted against three times serum amylase values, which in this species of swine was approximately 6,200 IU, nearly all the animals in the control group had levels above this arbitrary definition of a biochemical leak (Fig. 5). Furthermore, all the animals that developed either macroscopic dye extravasation or had evidence of undrained amylase-rich fluid collections/abscess had drain amylase levels far in excess of three times

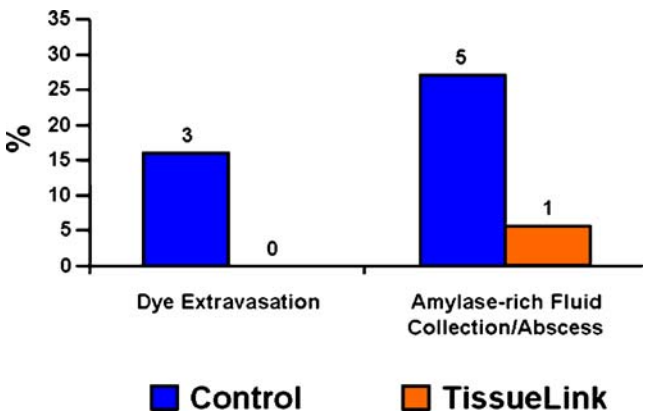

Figure 4 Pancreatic complications in control and TissueLink animals (p<0.01).

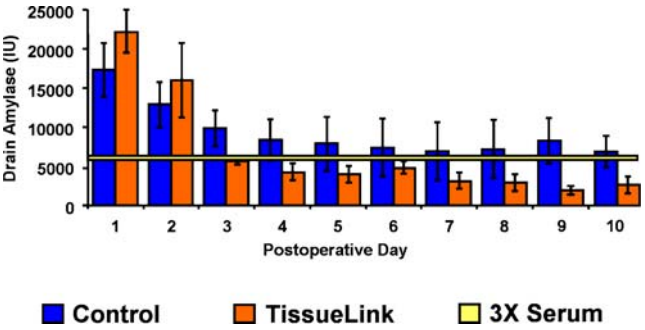

Figure 5 Total average drain amylase activity in the control and TissueLink animals over time (p=0.06). Line designates ×3 serum (6,200 IU) definition of biochemical leak.

the normal serum. In contrast 17 of the 18 animals in the TissueLink group had drain amylase levels below this cutoff after the third postoperative day and the one animal that had levels higher than this developed a stump abscess. On gross examination the resection beds in the control group had a subjectively raw and inflammatory reaction at the pancreatic stump, whereas the TissueLink animals developed a well-defined fibrous cap or scar on the stump aside from the one animal that was found to have a stump abscess (Fig. 3c,d).

The range of operative time was 55 to 105 min and did not differ significantly between groups (control 77±7.5 min, TissueLink 79.5±10.5 min). There also were no differences in animal bodyweight, intraoperative fluids, total daily drain output, and no differences in clinical parameters such as lethargy, emesis, anorexia, and narcotic need between groups (Table 2). At necropsy 11 animals in the control group had evidence of wound infection and six animals in the TissueLink group, but this did not reach statistical significance. There were no bowel complications or other evidence of thermal injury in the TissueLink animals.

Table 2 Various Other Parameters Measured in the Control and TissueLink Animals

Variable	Control	TissueLink	p Value
Bodyweight	44.6±4.0 kg	43.4±2.8 kg	0.58
Intraoperative fluids	562.5±171.8 cc	553.3±109.3 cc	0.98
Operative time	77±7.5 min	79.5±10.5 min	0.42
Days of (per animal)			
Antibiotics	5.6	5.7	0.85
Analgesia	2.2	2.4	0.59
Anorexia	0.3	1.1	0.25
Emesis	0.8	1.2	0.37
Lethargy	0.3	0.4	0.92
Fever	0.1	0.1	1.00
Wound infection	11	6	0.14

On histopathologic examination of the two animals from each group that were killed at 1 week, we found a stump abscess and evidence of ductular proliferation in the control specimen, whereas the TissueLink specimen had evidence of complete coagulative necrosis with obliteration of both vascular and ductal structures (Fig. 6a,b). At 3 weeks the control group stumps had evidence of a fibrinopurulent interface with residual pancreatic ductules whereas the TissueLink group remnants had evidence of fibroplasia with minimal inflammatory infiltrate and obliteration of ductules (Fig. 6c,d). At 5 weeks nearly all control specimens showed evidence of chronic inflammation whereas the TissueLink group stumps had a clearly demarcated interface between normal acinar cells and a fibrous noninflammatory scar or cap (Fig. 6e,f). Furthermore, all control group animals had evidence of a pyogranulomatous inflammatory reaction and microabscess formation surrounding the mattress sutures used for reinforcement of the pancreatic stump.

Discussion

The development of postoperative leaks after distal pancreatectomy persists despite improvements in other areas of pancreatic surgery. These fistulae result in more serious complications such as infection, abscess, or sepsis. Leaks after distal pancreatectomy increase the cost of care and subsequent health care resource utilization. A recently published retrospective analysis determined that those patients who developed leak after distal pancreatectomy incur costs twice that of those without a leak. Furthermore, the authors concluded that it was justifiable on financial grounds to use new interventions for every patient

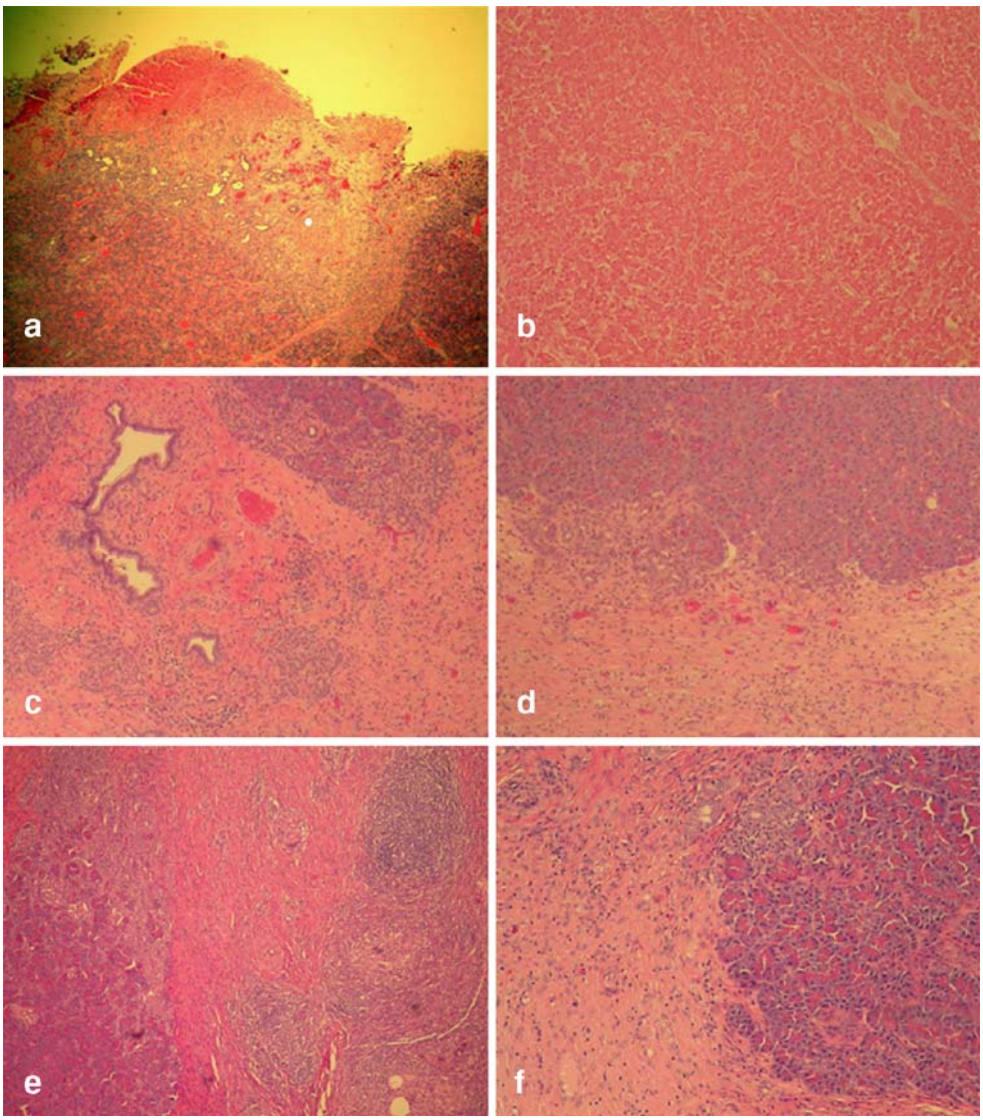

Figure 6 **a** Control at 1 week with stump abscess and ductular proliferation; **b** TissueLink at 1 week with complete coagulative necrosis and obliteration of ductal structures; **c** control at 3 weeks with fibrinopurulent interface and residual pancreatic ductules; **d** TissueLink at 3 weeks with fibroplasia and minimal inflammation; **e** control at 5 weeks with evidence of chronic inflammation; and **f** TissueLink at 5 weeks with clear interface of normal acinar cells and fibrous scar. All images are representative photomicrographs obtained from histopathologic slides.

undergoing distal pancreatectomy, even if those interventions modestly decreased the rate of postoperative pancreatic leak.[15]

To our knowledge, this is the first report of a randomized, prospective animal study using this RF technology for preventing leak after distal pancreatectomy. At the time of this publication there have been no reports of using TissueLink in distal pancreatic resection. However, it has been shown to be effective for dissection and resection of the uncinate process.[16] Other authors have also reported the role of this new technology in performing pancreaticoduodenectomy.[17] Historically, treatment of the main pancreatic duct was the primary focus in prevention of postoperative pancreatic leak. Fistula rates have been shown to be reduced significantly when the main pancreatic duct is identified and directly ligated after distal pancreatectomy, and most surgeons do attempt to identify the duct during closure of the remnant.[18] Our hypothesis instead focused on the microscopic pancreatic ducts, which are exposed on the cut surface of the gland (Fig. 7). We hypothesize that simple duct ligation and traditional oversewing of the gland with sutures does not completely seal the small ducts, leading to persistent extravasation of enzyme-rich pancreatic fluid, which bathes the ligated duct and leads to subsequent duct disruption and leak. Furthermore, the sutures themselves may cause tears within the pancreatic parenchyma and increase pancreatic leaks and provide a nidus of inflammation for development of infection and abscess formation. Studies examining risk factors for leak after pancreaticoduodenectomy reveal that the consistency and texture of the pancreas affects leak rate, with soft, nondiseased pancreata having a higher leak rate than fibrosed glands because of chronic inflammation.[19–21] New technologies such as TissueLink may obviate the need for sutures, as small vessels and ducts are more completely sealed, with a subsequent decrease in pancreatic fluid extravasation. Both the clinical and histological data from our study support this hypothesis. We have demonstrated that use of this RF device decreases leaks and complications compared to a traditional oversewing method in a porcine model, which is confirmed histologically in our pathology specimens that display minimal inflammatory reaction at the interface and subsequent fibrotic resolution in the TissueLink treated animals. In contrast, the control specimens displayed evidence of ongoing active inflammation. The presence of residual ductular proliferation in our control specimens is a common finding after pancreatic injury, and such trophic remodeling events may play a role in the development of persistent pancreatic fistula.[22] Our drain amylase determination did not reveal a statistically significant difference between groups; however, our study was not powered to detect such a difference. There was an obvious trend toward lower amylase concentrations in the TissueLink group, which could be clinically significant and perhaps would have been apparent if we had a larger sample size. The TissueLink group had mean drain amylase concentrations far below the current definition of a biochemical leak. In contrast, the swine in the control group, particularly all of those that developed a leak or a consequence thereof, had levels far in excess of this defining cutoff. One possible conclusion is that the development of leak is dependent on the overall level of enzyme-rich pancreatic fluid present at the resection bed. Thus, higher levels over a prolonged period of time may lead to main duct disruption and subsequent leak. Further studies would be necessary to support this hypothesis.

The extrapolation of our results to a human cohort is difficult as there are obvious species differences. Nonetheless we believe that our model is superior to previously published pilot studies in animal pancreatic surgery. The pig is an excellent approximation of the human pancreas both morphologically and physiologically. The pig pancreas has a capsule much like that of the human gland and its texture mimics that of a “soft” undiseased gland, thus making it an excellent model for high-risk pancreatic leak after pancreatic transection. Previous studies have examined various methods for preventing leak after distal pancreatectomy using canine models. The canine pancreas is morphologically and anatomically dissimilar to the human gland because it is multilobed and does not have a capsule. To our knowledge, our study is the only animal model of distal pancreatectomy utilizing a standard control in which the stump is oversewn in a traditional fashion, and appears to be the largest series performed in large animal pancreatic surgery.

There are some important technical considerations to keep in mind when using this device for pancreatic resection. Appropriate suction must be used when applying this device to tissues to minimize pooling of saline, which can dissipate the current applied and lead to lower tissue heating. In addition, there is a theoretical risk of associated thermal injury to nearby structures. Some authors speculate that the use of this device on the pancreas may in fact risk a higher postoperative pancreatic fistula risk because of associated necrosis of the remnant pancreas; however, when properly applied, the RF energy coagulates only to a depth of 3–5 mm. There have been concerns voiced about potential late complications with worries that the necrosed segment would slough and thus expose a raw pancreatic surface. For this reason, we chose to kill our animals at two time periods. There was one animal in the TissueLink group that developed a stump abscess after 5 weeks; however, we did not find a greater number of leaks or complications at the later time point with subset analysis. Previous studies have shown that pancreatic ischemia at watershed areas lead to increased fistula rates and anasto-

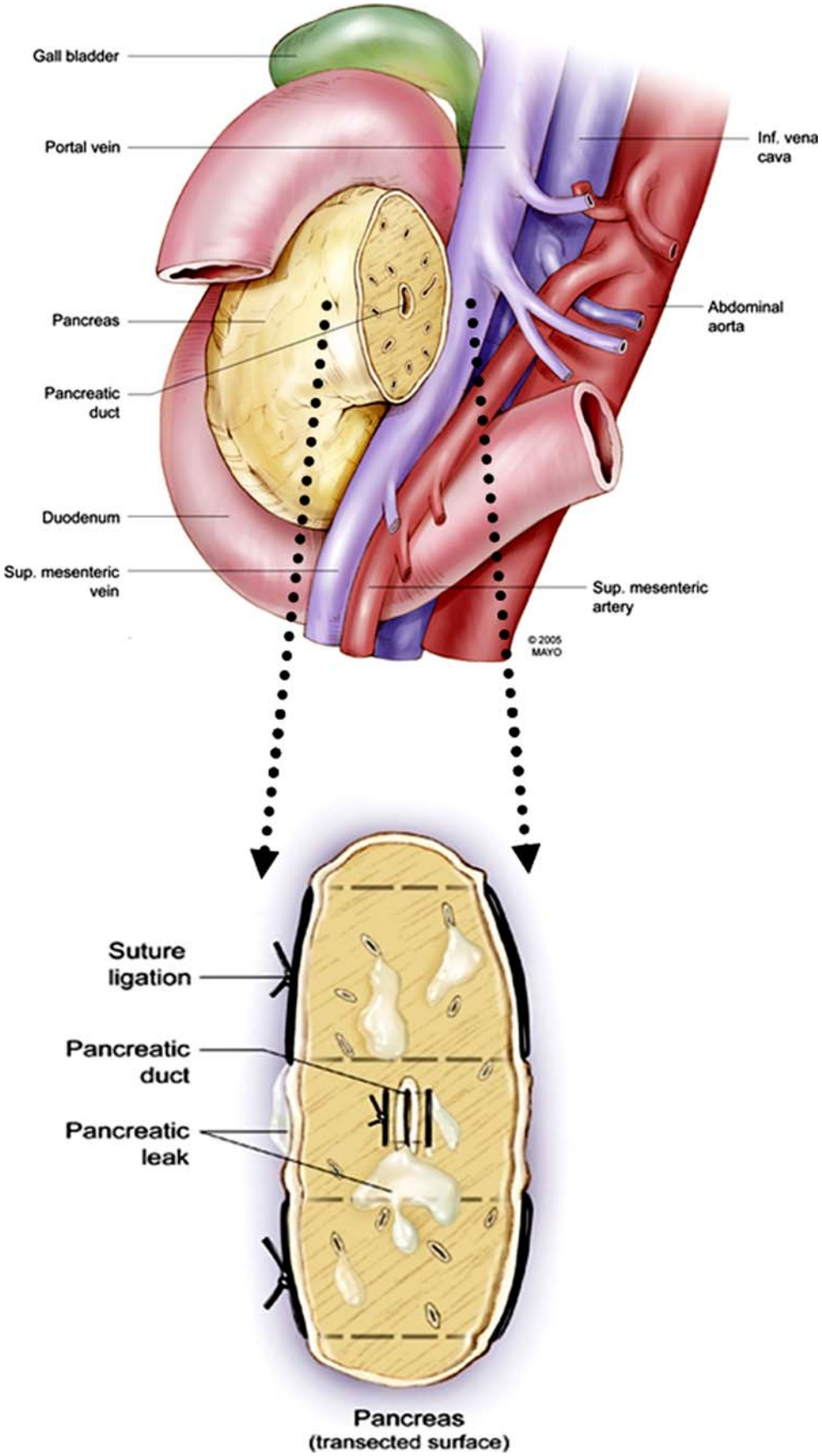

Figure 7 Our hypothesis demonstrating how traditional sutures may lead to ineffective microscopic duct sealing and parenchymal tears leading to pancreatic leak.

motic breakdown after pancreaticoduodenectomy.[23] Perhaps, coagulative tissue necrosis induced by RF energy might be less prone to complications compared with ischemic necrosis, but this remains speculative. Some authors have suggested using this device to "firm up" the peripheral edges of a soft pancreas so that it may hold sutures more securely before pancreaticoenteric anastomosis for pancreaticoduodenectomy; however, we cannot endorse this approach without appropriate pilot animal studies. In addition, the combined use of this device with sutures, in our opinion, would negate any possible benefit of the RF ablation as the sutures would risk parenchymal tears and may form a nidus of inflammation and subsequent infection as our control animals displayed on histologic examination. Similar suture microabscess findings have been reported in other studies as well.[24] Perhaps the marked inflammatory reaction induced by any sutures placed in the pancreas may be at least partially responsible for leak development because of its smoldering effect. Previous studies have looked at the type of suture and found that healing of the pancreas was not modified with different suture type and either absorbable or nonabsorbable was considered safe.[25] Furthermore, additional studies have looked at the durability of various suture materials in human pancreatic juice and bile and the only suture type to withstand the enzymatic activity of biliopancreatic fluid was polydioxanone, polypropylene, and silk.[26] Perhaps placing sutures in the pancreas may not be as benign as once believed. Stapled closure of the remnant stump eliminates the need for sutures and had promising results in decreasing pancreatic leak in early studies.[27,28] Subsequent studies have failed to show a difference between stapled vs sutured stumps, but many of those stapled stumps were oversewn as well.[29–32] In the previously mentioned meta-analysis there was a nonstatistically significant trend in favor of stapled treatment, thus there may be some real value in avoiding sutures in the pancreas.[4]

The TissueLink device has been used in both open and laparoscopic distal pancreatectomy as a way of pretreating the gland to decrease bleeding before transection by several pancreatic surgeons both at our institution and in several other centers (personal communication). We elected to first transect and then treat the stump in an effort to simulate a clinical scenario in which tumor margins must first be assessed intraoperatively by pathology. We cannot comment on whether pretreatment or treatment posttransection is more effective. In theory pretreatment may allow a safer and more bloodless transection in thicker glands; however, the confounding effect of the staples themselves within the coagulated gland has not been evaluated. Furthermore, many of these surgeons also oversew the stump with the same risks as previously discussed. One potential added benefit of this technology is of extending the resection margin by 3 to 5 mm in cancer patients by ablating any microscopically positive margins; however, the long-term oncologic utility of such benefit is yet to be determined.

Conclusions

It is evident that there is need for improvement in the prevention of postoperative pancreatic leak after pancreatic resection. The current techniques and methodologies have been unsuccessful in minimizing the incidence of this complication, which contributes to the majority of the morbidity and increases financial costs of pancreatic resection. Saline-couple RF ablation is a new modality that shows promise in limiting pancreatic leak after resection in our animal model. Based on our results, human trials would be a reasonable and logical next step toward assessing the clinical utility of the device in the pancreatic surgeon's armamentarium.

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Hyperbaric Oxygen Therapy Reduces Severity and Improves Survival in Severe Acute Pancreatitis

Mehrdad Nikfarjam · Christine M. Cuthbertson · Caterina Malcontenti-Wilson · Vijayaragavan Muralidharan · Ian Millar · Christopher Christophi

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Abstract Severe acute pancreatitis is characterized by pancreatic necrosis, resulting in local and systemic inflammation. Hyperbaric oxygen (HBO) therapy modulates inflammation, but has not been extensively studied in pancreatitis. This study investigates the effects of HBO in a rat model of severe acute pancreatitis. Sixty-four rats were induced with severe pancreatitis using 4% sodium taurocholate and randomized to HBO treatment or control. HBO was commenced 6 h after induction (100% oxygen at 2.5 atmospheres for 90 min) and continued every 12 h for a maximum of eight treatment episodes. Surviving animals were killed at 7 days. Severity of pancreatitis was graded macroscopically and microscopically. Lung edema was calculated using wet and dry lung weights. Macroscopic and microscopic severity scores (mean ± SE) of HBO-treated animals with pancreatitis (8.3±0.7; 9.6±0.4) were lower than those of controls (10.5±0.5; 11.1±0.4) (p=0.02 and p=0.03, respectively). The HBO-treated group had reduced pancreatic necrosis compared to controls (40±4% vs. 54±4%; p=0.003). There was no difference in pulmonary edema between the groups. Median survival in the HBO-treatment group was 51 h, compared to 26 h in controls. Day-7 survival was significantly improved in the HBO-treated animals compared to controls (40% vs. 27%; p=0.04). HBO therapy reduces overall severity, decreases the extent of necrosis, and improves survival in severe acute pancreatitis.

Keywords Severe acute pancreatitis · Necrosis · Hyperbaric oxygen therapy

Introduction

Acute pancreatitis is a common condition with an annual incidence ranging from 5 to 80 cases per 100,000 population.[1,2] Despite advances in the supportive management of this condition, the mortality rate of severe acute pancreatitis still approaches 30% in some series.[3] The key feature of severe disease is pancreatic tissue necrosis, leading to both local and systemic inflammatory responses.[4]

There is increasing evidence that microcirculatory alterations play a major role in the pathogenesis of acute pancreatitis.[5–12] Changes in vascular morphology and flow lead to decreased tissue oxygenation and worsening of pancreatitis severity. Apart from systemic antibiotics, administration of pharmacological agents does not significantly alter patient outcomes in severe acute pancreatitis.[13] Treatment has generally focused on modification of specific pathways involved in oxidative stress, ischemia reperfusion injury, and stabilization of the microcirculation.

A potential approach in the management of severe acute pancreatitis is the administration of hyperbaric oxygen (HBO). Beneficial effects of HBO therapy include increased tissue oxygenation, inhibition of ischemia reperfusion injury, and stimulation of angiogenesis.[14] HBO therapy also modifies neutrophil function, impairs bacterial replication, and has an overall antioxidant and antiedema effect.[14–16] Its potential role in modifying the pathophysiological effects of

M. Nikfarjam (✉) · C. M. Cuthbertson · C. Malcontenti-Wilson · V. Muralidharan · C. Christophi
Department of Surgery, University of Melbourne, Austin Hospital, Lance Townsend Building Level 8, Studley Rd, Heidelberg, Melbourne, Victoria 3084, Australia
e-mail: surgery-armc@unimelb.edu.au

I. Millar
Hyperbaric Unit, Alfred Hospital, Melbourne, Victoria, Australia

severe acute pancreatitis has not been fully elucidated. HBO therapy may simultaneously modify several pathways involved in the local and systemic inflammatory response and microcirculatory changes in acute pancreatitis and potentially reduce disease severity and mortality.[15,16]

The aim of this study is to evaluate the effects of HBO treatment on severity and mortality in a rat model of severe acute pancreatitis.

Methods

Animals

Experiments were conducted with the approval of the Austin Health Animal Ethics Committee. Male Wistar albino rats (280–320 g) were used. Animals were housed two per cage prior to surgery, with access to food and water ad libitum, and exposed to a 12-h light–dark cycle. Food was withdrawn from animals 12 h prior to the experiments.

Experimental Model of Severe Acute Pancreatitis

Rats were anesthetized by intraperitoneal injection of ketamine hydrochloride 100 mg/kg (Parke Davis, Melbourne, Victoria, Australia) and xylazine 10 mg/kg (Bayer, Melbourne, Victoria, Australia). Carprofen 5 mg/kg (Pfizer, Melbourne, Victoria, Australia) was administered subcutaneously for analgesia. Pancreatitis was induced by intraductal infusion of 4% sodium taurocholate (Sigma, Melbourne, Victoria, Australia) by modifications of methods previously described.[17]

A midline laparotomy was performed. A 27-gauge blunt needle was introduced into the distal end of the biliopancreatic duct through a duodenotomy. The proximal bile duct was temporarily occluded at the porta hepatis by a small vascular clamp. Sodium taurocholate (4% solution, 0.1 mL per 100 g) was infused at a pressure of 20 mm Hg controlled by a sphygmomanometer. At the end of the infusion, the needle was withdrawn and the clamps removed. The duodenum was closed with 6/0 prolene sutures and animals were hydrated by instillation of 5 mL 0.09% saline into the peritoneal cavity. Abdominal wounds were closed in two layers using 2/0 prolene sutures and animals recovered on heat pads.

Study Design

At the end of each operative session, animals with pancreatitis were randomly assigned to either HBO treatment or control. Four to eight animals were induced with pancreatitis in any one operative session. Thirty-two animals were allocated to each group.

HBO therapy [2.5 atmospheres (atm) 90 min] was commenced in the treatment group 6 h after induction of pancreatitis and administered every 12 h for a maximum of 4 days. Control animals received no treatment. Animals were assessed in a blinded manner at 6-h intervals following induction of pancreatitis based on an animal health scoring criteria and euthanized accordingly.[18] Animals classified as being in *very poor* health were killed immediately. Animals in *poor* health were administered 5 mL of 0.09% saline via subcutaneous injection when exhibiting signs of dehydration and carprofen 5 mg/kg subcutaneously for analgesia. Animals were then reassessed 2 h later and killed if there was no improvement in their condition. All animals surviving to day 7 were killed.

Blood was taken by tail vein bleed at 24 h to assess the early effects of HBO therapy compared to control and at day 7 in surviving animals. All HBO-treated animals at 24 h had completed two treatment sessions. The liver, pancreas, and lung were removed for analysis at the time of euthanasia in all animals following macroscopic assessment of disease severity.

HBO Administration

HBO was administered using a purpose-built animal hyperbaric chamber (Fink Engineering, Melbourne, Victoria, Australia) in assigned animals 6 h following induction of pancreatitis. Two to four animals were given HBO at one time. Treatment consisted of administration of 100% oxygen at 2.5 atm for 90 min, with a compression time of 10 min and a decompression time of 15 min. Protocols employed were according to recommendations used clinically for treatment of severe necrotizing infections.[14] HBO therapy was administered at 12-h intervals following initial treatment for a maximum of 4 days.

Assessment

Overall comparisons were made between HBO-treated and control animals with severe pancreatitis. Subgroup analysis of animals surviving to 7 days was performed.

Blood Tests

Serum was taken from animals for amylase and lipase measurement prior to induction of pancreatitis and at 24 h. Amylase and lipase was also measured again in those animals surviving to 7 days.

Severity Score

Laparotomy was performed on all animals at death or euthanasia. Macroscopic severity of pancreatitis was based

on a previously described scoring system.[19] Up to three points are allocated for each of ascites, extrapancreatic fat necrosis, pancreatic edema, hemorrhage, and necrosis. The maximum score by this method was.[15] Microscopic severity of pancreatitis was scored on hematoxylin and eosin (H&E)-stained sections by a scoring system modified from Yotsumoto et al.[20,21] The maximum possible score is 17 (Table 1). Sections in which no viable acinar tissue was seen were excluded from the comparison. All assessments were performed in a blinded manner, with the assessor unaware of the treatment group and the time of euthanasia or death.

Pancreatic Necrosis

Pancreatic tissue was sectioned longitudinally, formalin-fixed, and paraffin-embedded for histology. Cut sections were stained by H&E. Photomicrographs of the entire section were taken using an Olympus digital microscope (Coolscope, Nikon, Tokyo, Japan). A minimum of 12 images per pancreas were obtained from this micrograph at magnification ×100. These images were used for histological assessment using image analysis software and performed in a blinded manner (Image Pro-Plus version 4.5.1, Media Cybernetics, Bethesda, MD, USA). The area of pancreatic glandular tissue and pancreatic necrosis represented in each slide was measured. The percentage necrosis in each section was determined and an overall percentage of necrosis was determined using the calculation

$$N_{\text{total}}=[(N_1 \times A_1) + (N_2 \times A_2) + \ldots(N_n \times A_n)]/(A_1 + A_2 + \ldots A_n),$$

where N is the percentage necrosis and A is the area.

Table 1 Criteria for Microscopic Assessment of Pancreatitis Severity, Modified from Yotsumoto et al.[20,21]

Histological parameters	Score	Assessment
Edema	0	No edema
	1	Mild—interlobular septa expanded
	2	Moderate—interacinar septa expanded
	3	Severe—individual acini separated
Acinar necrosis	0	No necrosis
	3	Mild—<20%
	5	Moderate—21–50%
	7	Severe—>50%
Hemorrhage	0	No hemorrhage
	3	Mild—1–2 foci/slide
	5	Moderate—3–5 foci/slide
	7	Severe—>5 foci/slide

Lung Edema

The right lung of animals surviving to day 7 and those euthanized or found immediately after death were removed arbitrarily for analysis. A wet weight measurement of the lung, minus any connective tissue was taken using an analytical balance (AG204, DeltaRange®, Greifensee, Switzerland). The lung was then placed in containers and incubated in 37°C oven for 48 h. The desiccated lung tissue was reweighed and percentage fluid was calculated by the following formula:

$$\text{Relative water content}(\%) = (\text{wetweight} - \text{dryweight})/\text{dryweight} \times 100\%.$$

Survival

All animals surviving to 7 days postpancreatitis were considered to be long-term survivors. Animals were excluded from the study if there was a failure to induce pancreatitis based on changes noted in the pancreas at the time of induction or lack of elevations in amylase and lipase levels at 24 h compared to baseline. Animals were included in the study as censored data when the cause of death was unrelated to pancreatitis or treatment.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 11.5®, Chicago, IL, USA). All continuous variables and severity scores between control and treated animals were expressed as mean ± SEM but compared by the Mann–Whitney U test. Animal survival was determined using the Kaplan Meier method. Comparisons in survival were by the Breslow–Wilcoxon method, where losses occurring early are counted more heavily, and a constant hazard ratio is not assumed. A p value of less than 0.05 was considered statistically significant. All data are expressed as mean and SE, unless otherwise specified.

Animal numbers were based on calculations to achieve a power of 0.8 and p value of less than 0.05. Thirty-two animals were required in each group to detect a 30% improvement in survival at 7 days postoperatively from an expected survival of 20% in untreated animals.

Results

A total of 64 animals were used in this study. In two animals, acute pancreatitis was unable to be induced due to technical difficulties related to bile duct cannulation and one animal failed to recover following an anesthesia. These

animals were excluded from analysis. In three animals with established pancreatitis, the cause of death was related to operative complications and they were considered as censored data. Overall, euthanasia was implemented according to health scoring criteria in 40% of control animals with pancreatitis, compared to 36% in the HBO-treated group.

Blood Results

Baseline amylase and lipase levels in animals were 1,027±54 and 28±5 U/L, respectively. At 24 h, all animals induced with pancreatitis had significant elevations in serum amylase compared to baseline ($p<0.001$). The entire HBO group had undergone two treatment sessions by 24 h. Compared to controls, serum amylase (4,901±1,215 vs. 5,596±907 U/L $p=0.143$) and lipase levels (303±61 vs. 1,000±386 U/L, $p=0.089$) were not significantly different at 24 h. In surviving animals with pancreatitis, no differences were observed between HBO-treated and control groups at 7 days in serum amylase (3,981±1,444 vs. 8,996±3,705 U/L, $p=0.698$). There was also no statistically significant difference in serum lipase levels (418±199 vs. 1,505±524 U/L, $p=0.190$).

Severity Score

Pancreatic ascites, edema, necrosis, hemorrhage, and extrapancreatic fat necrosis were features of all animals induced with pancreatitis that did not survive beyond 72 h. Based on macroscopic scores, the overall severity of disease in the HBO-treated animals was significantly less than control animals with pancreatitis (8.3±0.7 vs. 10.5±0.5, $p=0.02$) (Fig. 1a). On histological examination, acute pancreatitis was characterized by necrosis, leukocyte infiltration, edema, and hemorrhage. The overall microscopic severity was also significantly reduced in animals undergoing HBO therapy, compared to the controls with pancreatitis (9.6±0.4 vs. 11.1±0.4, $p=0.03$) (Fig. 1b).

In surviving animals at 7 days, the macroscopic severity score of HBO-treated animals (4.8±0.5) was significantly less than that of controls with pancreatitis (8.1±0.5, $p=0.002$) (Fig. 1a). In animals surviving to 7 days, there was, however, no significant difference in the microscopic severity score (HBO: 10.0±0.8 vs. control: 11.8±0.9, $p=0.13$) (Fig. 1b).

Pancreatic Necrosis

Features of pancreatic acinar necrosis were prominent in all animals examined, confirming the development of severe necrotizing pancreatitis. Pancreatic hemorrhage was observed more commonly in animals not surviving beyond 72 h. Pancreatic acinar necrosis was observed throughout

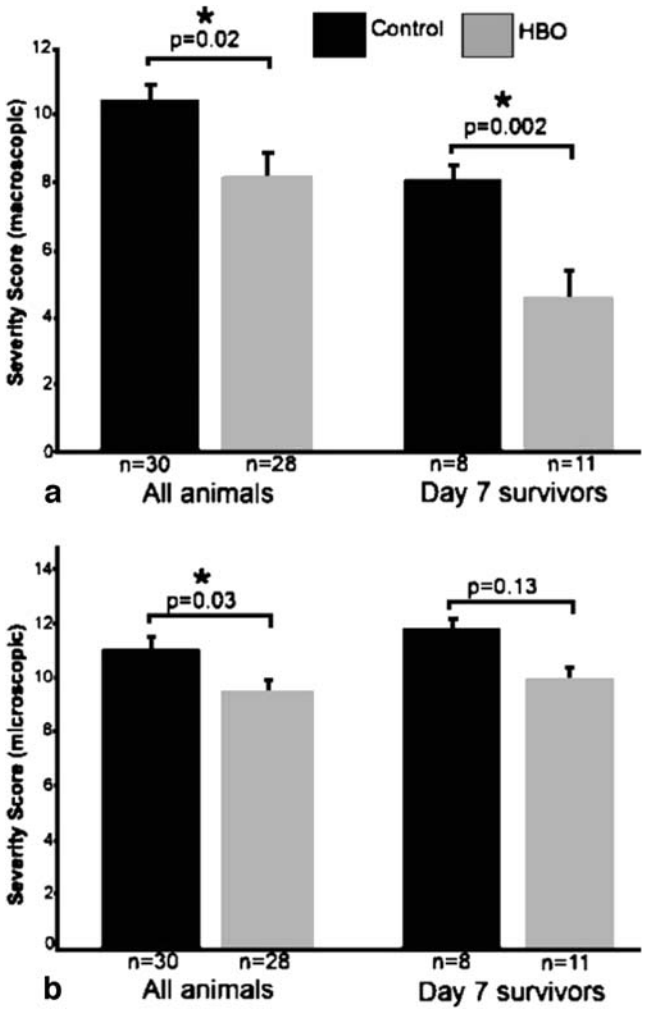

Figure 1 **a** Mean macroscopic pancreatitis severity score (as per Schulz et al.[19]), comparing control animals with pancreatitis to those with pancreatitis undergoing HBO therapy. Subgroup analysis of animals surviving to 7 days is shown. **b** Mean microscopic severity score of control vs. HBO-treatment groups (Mann–Whitney U test).

the gland at all time points. In both HBO and control groups, the head of the pancreas was affected significantly more than the tail (61±4 vs. 47±4%, $p<0.001$, paired samples T test). The overall pancreatic necrosis in HBO-treated animals was significantly less than the necrosis in control animals (40±4 vs. 54±4% $p=0.046$) (Fig. 2). In animals surviving to 7 days, the extent of pancreatic necrosis was similarly significantly lower in HBO-treated animals compared to controls with pancreatitis (46±6 vs. 70±7% $p=0.046$).

Lung Edema

Edema and pulmonary hemorrhage were observed on macroscopic assessment of lungs in some animals with pancreatitis. Overall, relative water content of the lungs in

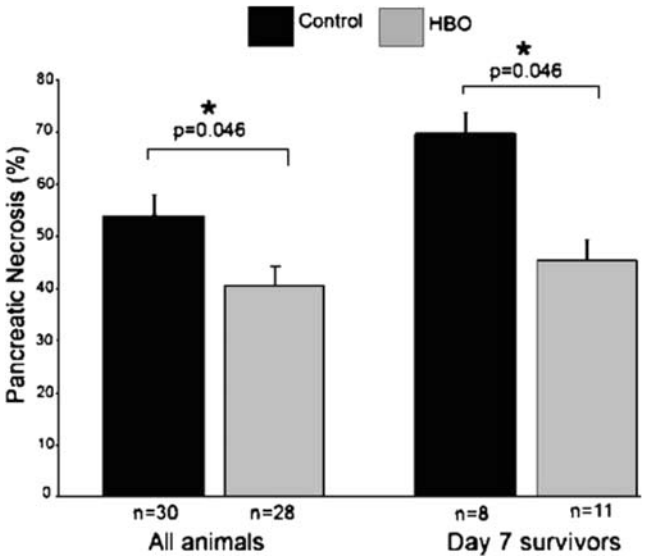

Figure 2 Mean pancreatic necrosis in control and HBO-treated animals. Subgroup analysis of animals surviving to 7 days is shown (Mann–Whitney U test).

HBO-treated animals was not significantly different to controls (76±1 vs. 76±1% p=0.737). Similarly, in animals surviving to 7 days, the relative water content in the HBO-treated group was not significantly different to control animals with pancreatitis (79±1 vs. 76±1% p=0.069).

Survival

One animal in the HBO group developed respiratory distress immediately following HBO therapy, 4 days post-disease onset, and died shortly thereafter. It was included as an uncensored death as part of the analysis. There was collapse and hemorrhage into both lungs and death was possibly the result of therapy or delayed complications of severe pancreatitis. HBO treatment was otherwise well tolerated without any apparent complications. The mortality rate in animals with severe pancreatitis was greatest within the first 72 h following induction of pancreatitis. In HBO-treated animals, the median and 7-day survival were 51± 44 h and 40±9%, respectively. In control animals the median and 7-day survival were 26±2 h and 27±8%, respectively. The overall survival was significantly greater in the HBO-treated animals than controls with pancreatitis (p=0.04) (Fig. 3).

Discussion

Acute pancreatitis is a common disorder with an incidences of 79.8 per 100,000 in the USA.[1] The incidence and etiology of the disease varies in different regions of the world, reflecting patterns of alcohol intake and gallstone prevalence.[1,22] Approximately 10 to 15% of patients have severe disease, with a fulminant course of pancreatic necrosis and multiorgan failure.[2,23] The mortality in this patient group generally ranges from 10 to 30%.[1,3,24] Antibiotics, early enteral feeding, and therapeutic endoscopy are advocated in the treatment of specific cases of severe pancreatitis. There is no single therapy to date, however, that consistently improves outcomes in this condition.[23]

The underlying processes involved in severe acute pancreatitis are complex and not fully elucidated. The critical initiating event appears to be activation of trypsinogen within pancreatic acinar cells and destruction of the duct and acinar cell cytoskeleton. This produces activation of an inflammatory cascade, mediated by various cytokines, immunocytes, and the complement system, leading to a systemic inflammatory response syndrome.[13] Pancreatic necrosis is the key feature in severe disease and results from impairment of the pancreatic microcirculation, free oxygen radical production, and ischemia reperfusion injury.[25–28] Inflammatory mediators play a critical role in the local and systemic manifestations of severe pancreatitis. These mediators include platelet activating factors, interleukins, bradykinin, and endothelins, all of which exacerbate microcirculatory disturbances within the pancreas, leading to hypoxia and tissue necrosis.[29,30]

Experimental studies in the treatment of severe pancreatitis generally include antagonists to specific mediators involved in the inflammatory process. IL-1β and TNF-α blocking commenced shortly following induction of pancreatitis reduces severity and improves survival in rat models.[30,31] In a study of 3% taurocholate and cerulein induced pancreatitis in rats, monoclonal antibody to TNF-α administered shortly following induction of disease ameliorated both parenchyma and fatty tissue necrosis caused by pancreatitis.[32] Antioxidant treatment in animal models of severe hemorrhagic pancreatitis has also shown promising results in reducing tissue damage and improving survival.[33] Anti-TNF-α and anti-IL-1β therapies in the clinical setting have failed to reproduce the same beneficial effects seen in experimental models.[34]

A potential alternative approach to modify the various pathophysiological pathways involved in severe pancreatitis is the administration of HBO. Beneficial effects of HBO therapy include increased tissue oxygenation by improved blood rheology and reduced shunting of blood from hypoperfused tissue.[14,35] The oxygen diffusion distance from the perfused capillaries is increased approximately fourfold with HBO administration.[36] HBO therapy can increase arterial oxygen tensions to 2,000 mm Hg and achieves partial pressure of oxygen in tissues in the order of 500 mm Hg.[37] Tissue oxygenation is increased to levels that are sufficient to support resting tissues without a contribution from hemoglobin, resulting in a reversal in

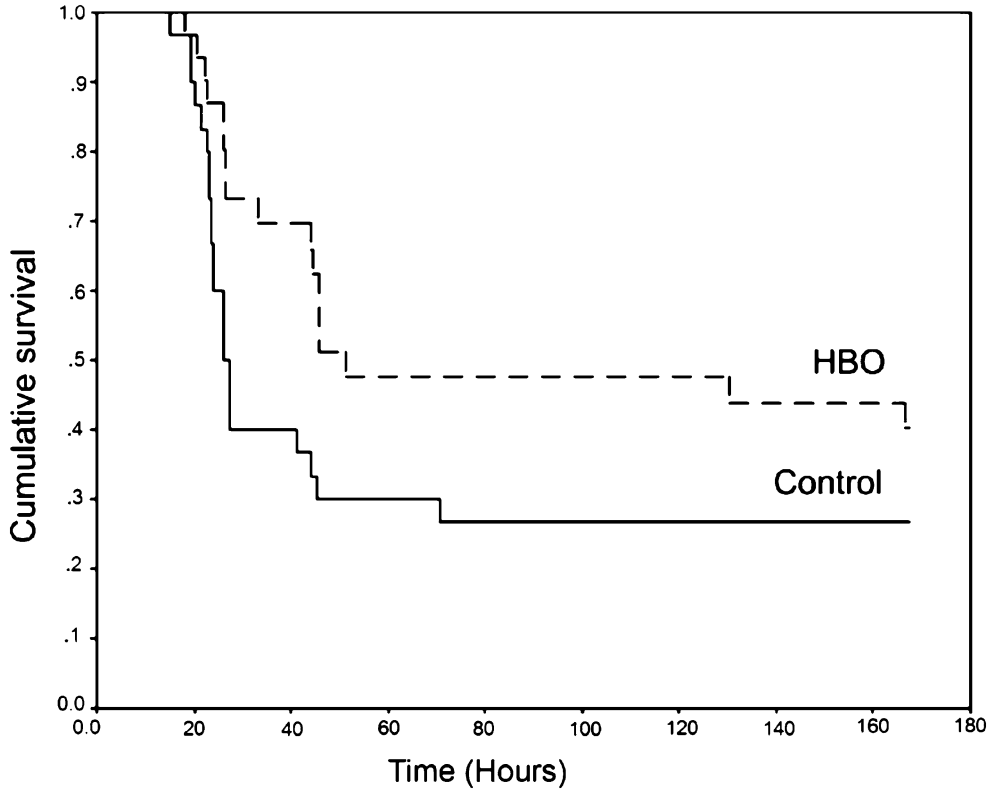

Figure 3 Kaplan Meier survival curve of rats with acute pancreatitis treated with HBO, compared to control (p=0.04, Breslow–Wilcoxon test).

hypoxia for the duration of the treatment and for a significant time following therapy.[38,39] It also inhibits ischemia reperfusion injury, stimulates collagen matrix formation needed for angiogenesis, modifies neutrophil function, and impairs bacterial replication and toxin production, with an overall antioxidant and antiedema effect.[15,16] HBO more specifically suppresses TNF-α and anti-IL-1β in response to lipopolysaccharide[40] and down-regulates ICAM-1 expression on endothelial cells to reduce polymorphonuclear leukocyte adhesion.[41] Its general anti-inflammatory effects are clearly demonstrated in "zymosan" models of shock, in which HBO attenuates the inflammatory response and reduces mortality.[42–44] All these features may significantly improve outcomes in severe pancreatitis.

Most available data pertaining to HBO and pancreatitis in a clinical setting are in the treatment of infective complications.[45] In a study of 12 patients with peripancreatic sepsis and abscess formation due to severe pancreatitis, there was significant clinical improvement, and surgical debridement was avoided in four of five patients assigned to HBO therapy (three to seven sessions) at 2.8 atm.[45] This was compared to seven patients with peripancreatic sepsis who had conventional treatment, with three deaths following surgical debridement. Although encouraging, the study was not randomized and the utility of HBO therapy in actually preventing complications was not defined.

In a rat model of severe pancreatitis, HBO therapy with either 40 or 100% oxygen up to 4 atm reduced the severity of pulmonary edema and improved pancreatic microcirculation.[46] HBO was well tolerated in treated animals and compared to normobaric hyperoxia and control. However, animals were not recovered following anesthesia to assess the impact of therapy on survival. In addition, the effect of only a single HBO treatment session on severe pancreatitis was tested. In a more recent study in rats with pancreatitis, HBO therapy for 48 h reduced histopathological findings (nonquantitative assessment) compared to controls with pancreatitis.[47] In two rat models of severe pancreatitis, oxidative injury was significantly reduced with HBO.[47,48] In a porcine model of severe acute necrotizing pancreatitis, HBO therapy combined with surgery resulted in improved survival. All animals without HBO died (five of five), while only two of five animals with HBO therapy died as a consequence of pancreatitis.[49] However, there are few studies to date with sufficient treated animal numbers to demonstrate a reduction in morbidity and mortality of severe acute pancreatitis with HBO therapy.

Our study was powered to detect a 30% reduction in mortality in severe pancreatitis following HBO therapy. The survival rate at day 7 in the HBO-treated animals in our study was 40% compared to 27% in control animals with pancreatitis, representing a 48% relative increase in survival (p=0.04). Features of the study design mean this survival advantage is relevant to the human situation. HBO treatment was commenced well after the induction of pancreatitis, when pancreatic injury and systemic inflammatory

effects were well established. Delays in presentation and diagnosis frequently occur in patients with pancreatitis. In addition, HBO therapy would rarely be available *immediately* upon patient presentation. We speculate that treatment at 6 h post severe pancreatitis in this model is more relevant to the clinical situation[50] than models where treatment is commenced immediately upon induction of pancreatitis.[46]

Our model produces significant pancreatitic necrosis in both HBO-treated and control animals (40±4 and 54±4%, respectively). There was a clear reduction in percentage necrosis in HBO-treated animals, both overall and at 7 days following therapy. Overall, there was a 14% absolute reduction in pancreatic necrosis in HBO-treated animals compared to controls (p=0.046), representing a 25% relative decrease in the extent of necrosis. When only animals surviving to 7 days were compared, the absolute reduction in necrosis was 24%, with a relative decrease of 34%. In this study, groups of animals were not killed at defined earlier time points for comparisons. The findings of our study contrast a previously published study, in which a single session of HBO failed to reduce the percentage necrosis following severe pancreatitis.[46] However, histological assessment in that study was at 9 h after a single HBO treatment session. Histological assessment in our study was performed at later time points, which may potentially explain the difference in detected effect.

In our study, there were also significant reductions in macroscopic severity grading of pancreatitis in HBO-treated animals compared to control animals with pancreatitis. Although the absolute changes in both microscopic and macroscopic pancreatitis severity following HBO treatment were small, it appeared to result in significant improvements in overall mortality. The microscopic scoring scale utilized was weighted toward acute features of pancreatitis, which may account for a nonsignificant difference in tissue taken at 7 days.

The pulmonary water content was similar between HBO-treated animals and controls overall and in those surviving to 7 days following therapy in our study. This is in contrast to a study by Chen et al.,[46] who reported reduced pulmonary edema following HBO treatment, assessed within 24 h of disease onset in a mild–moderate pancreatitis model. Our study was designed to determine differences in pulmonary edema at 7 days post disease onset. It is likely that the degree of pulmonary edema partly resolves by this time point. To demonstrate potential HBO pulmonary effects in severe acute pancreatitis, assessment of lung edema, histology, and blood gases at specified earlier time points is required.

There are some concerns regarding potential deterioration of respiratory function during HBO therapy.[51] In our study, one animal had potential respiratory complications related to HBO therapy. Treatment was uncomplicated in the remaining 31 animals. Based on published literature, intermittent therapy, ranging from 2 to 3 atm, generally has minimal pulmonary side effects.[52] However, careful monitoring of respiratory function, particularly in unwell patients, would appear prudent.

In conclusion, HBO therapy significantly reduces morbidity and mortality when administered to rats with established severe acute necrotizing pancreatitis. The exact mechanisms of HBO-induced reduction in mortality of severe pancreatitis require further investigation, but are almost certainly multifactorial.[14] Further evaluation of the action of HBO therapy in severe acute pancreatitis is warranted to improve treatment results, possibly by development of synergistic therapies.

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Glutamate-induced Calcium Transients in Rat Neurons of the Dorsal Motor Nucleus of the Vagus

John B. Ammori · Weizhen Zhang · Erika A. Newman · Michael W. Mulholland

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Abstract The dorsal motor nucleus of the vagus (DMNV) integrates peripheral and central signals and sends efferent output to the gastrointestinal system. Glutamate, the major excitatory neurotransmitter of the central nervous system, causes increases in intracellular calcium in DMNV neurons. The mechanisms by which glutamate activates calcium signaling in the DMNV were examined. DMNV neurons were isolated from neonatal rat brainstem using microdissection and enzymatic digestion. Exposure to glutamate caused intracellular Ca^{2+} increments in greater than 80% of cells. Removal of extracellular Ca^{2+} abolished intracellular Ca^{2+} transients. Kynurenic acid, a nonspecific glutamate receptor antagonist, abolished intracellular Ca^{2+} transients. Exposure to glutamate while blocking AMPA receptors with GYKI 52466 abolished the Ca^{2+} response. Exposure to (S)AMPA, an AMPA receptor agonist, caused intracellular Ca^{2+} increments in 97% of cells. Activation and antagonism of NMDA and kainate receptors produced no changes compared to control experiments. NiCl, a nonspecific Ca^{2+} channel blocker, abolished intracellular Ca^{2+} transients. Blocking T-type Ca^{2+} channels with mibefradil abolished the Ca^{2+} response in 76% of cells. Blockade of L-type and N-type Ca^{2+} channels did not affect the Ca^{2+} response. Glutamate mediates intracellular Ca^{2+} currents in DMNV neurons via the AMPA receptor and T-type Ca^{2+} channels, allowing influx of extracellular Ca^{2+}.

Keywords AMPA receptor · Calcium channel · Vagus nerve

Introduction

The dorsal vagal complex, located in the dorsal medulla, consists of three components: the area postrema, the nucleus of the solitary tract, and the dorsal motor nucleus of the vagus (DMNV). The area postrema abuts the fourth ventricle and detects chemical changes in the blood and cerebrospinal fluid. Through primary vagal afferents, gastrointestinal stimuli activate the nucleus of the solitary tract. The DMNV integrates both peripheral and central signals from several different sources including the area postrema, hypothalamus, and afferent neurons of the visceral organs, either directly or via interneurons in the nucleus of the solitary tract.[1–4] DMNV neurons provide the parasympathetic efferent outflow to the gastrointestinal system. The DMNV influences upper gastrointestinal activities such as swallowing, gut responses to distension, acidity, fat, and nutrient composition, and gallbladder contraction.[5–7] The functions of the DMNV are therefore linked to many areas of clinical relevance, including gastroesophageal reflux disease, dysphagia, emesis, functional dyspepsia, food intake, and irritable bowel syndrome.[8]

Glutamate is a major neurotransmitter of the central nervous system. Glutamate is integral to central processing of gastrointestinal reflex neurocircuitry in the dorsal vagal complex.[9] High levels of glutamate are present in labeled vagal afferent terminals and glutamate is suggested to be the main neurotransmitter released by vagal afferent terminals within the nucleus of the solitary tract.[10] Preganglionic neurons in the DMNV innervate the smooth muscle of the lower esophageal sphincter, stomach, small intestine, proximal colon, and gallbladder. Microinjection

J. B. Ammori · W. Zhang · E. A. Newman · M. W. Mulholland (✉)
Department of Surgery,
The University of Michigan Medical Center,
2101 Taubman Center, 1500 East Medical Center Drive,
Ann Arbor, Michigan 48109-0346, USA

of L-glutamate into the DMNV generally excites upper gastrointestinal functions.[6,8,11]

Glutamate acts through two families of receptors: metabotropic and ionotropic receptors.[12] Ionotropic glutamate receptors are ligand-gated ion channels, which are classified into three subtypes according to their most selective agonist: *N*-methyl-D-aspartic acid (NMDA), α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA), and kainate. The metabotropic receptors are G protein (guanine nucleotide-binding protein)-coupled receptors linked to second-messenger systems. Some are linked to phospholipase C, resulting in release of intracellular calcium, whereas others are negatively linked to adenylate cyclase.

Exposure of neurons to glutamate has been shown to increase the concentration of intracellular free Ca^{2+}.[13,14] The Ca^{2+} ion is an integral second messenger system, allowing transduction of extracellular signals into biologic responses. In neural systems, these responses include gene expression, apoptosis, intercellular communication, and synaptic neurotransmission.[15]

Recently, we described a method of serum-free culture of rat postnatal neurons derived from the DMNV.[16] Using in vitro culture of DMNV neurons, we investigated the mechanisms of glutamate-induced Ca^{2+} signaling.

Materials and Methods

Chemicals and Solutions

Fura-2-acetoxymethyl ester (fura-2-AM) was obtained from Molecular Probes (Eugene, OR). Hanks' balanced salt solution (HBSS) without phenol red, both with and without $CaCl_2$, neurobasal medium A, B27 supplement, L-glutamine, penicillin, and streptomycin were from Gibco (Grand Island, NY). βFGF was from Invitrogen (Carlsbad, CA). Poly-L-lysine, L-glutamate acid, *N*-methyl-D-aspartic acid (NMDA), (S)-α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA), (*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (ATPA), kynurenic acid, (+)-MK-801 hydrogen maleate, (2S,4R)-4-methylglutamic acid, GYKI 52466 hydrochloride, nifedipine, mibefradil dihydrochloride, and ω-conotoxin GVIA were purchased from Sigma-Aldrich (St. Louis, MO). Nickel (II) chloride hexahydrate ($NiCl_2$) was obtained from Acros Organics (Morris Plains, NJ). 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl] isothiourea mesylate (KB-R7943) was from Tocris Bioscience (Ellisville, MO).

In Vitro Culture of DMNV

Animal studies were approved by the University of Michigan Committee on Use and Care of Animals. DMNV neurons were isolated from 2- to 5-day old Sprague–Dawley rats (Harlan, Indianapolis, IN) in a manner previously described.[16] Rats were euthanized. The brainstem was rapidly removed, and chilled at 0°C in a dissection solution containing NaCl 138 mM, KCl 4 mM, $MgCl_2$ 1 mM, $CaCl_2$ 2 mM, glucose 20 mM, and HEPES 10 mM. Tissue blocks were prepared and sectioned transversely into 400-μm slices at the level of the obex using a Vibratome 3000 (Redding, CA). The DMNV area was identified under a dissecting microscope as the area immediately ventral to the nuclei of the solitary tract and dorsal to the XII nuclei. DMNV tissue was excised and then digested in an enzyme solution containing protease type XIV (0.6 mg/ml) and trypsin type I (0.4 mg/ml) at 32°C for 30–60 min. The tissue was then dissociated by gentle trituration with pipettes. Cells were plated onto poly-D-lysine-coated 25-mm slides in 35-mm culture dishes. Neurons were maintained at 37°C in an atmosphere of 5% CO_2 in serum-free culture media containing neurobasal medium A containing 2% B27 supplement, 2 mM glutamine, 1% penicillin and streptomycin, and 5 ng/ml βFGF. After 4 days, one-half of the medium was replaced and experiments were conducted at 7–21 days.

Cell preparation for imaging

Two micromoles per milliliter of the fluorescent dye fura-2-AM was added to neurons in fresh warm serum-free growth media and incubated at 37°C for 30–60 min experimentation. Loaded coverslips were washed and resuspended in HBSS. Coverslips were then placed in a perfusion chamber mounted on the stage of an inverted fluorescence microscope (Nikon). The rate of superfusion of buffer and reagents was kept constant at 1 ml/min. For Ca^{2+}-free conditions, HBSS without $CaCl_2$ was used.

$[Ca^{2+}]_i$ Measurements

A Nikon inverted microscope with a ×40 oil immersion objective and TILLvisION digital imaging system (TILL Photonics, CA) were used. Single-cell cytoplasmic calcium ($[Ca^{2+}]_i$) was determined from the ratio of fluorescence intensity of fura-2-AM at 340 and 380 nm, monitored by an intensified charge-couple device camera, and subsequently digitized. Background intensity at each emission wavelength was corrected. The system was used to obtain whole-field images. Boxes were placed on all cells of interest for simultaneous measurement of $[Ca^{2+}]_i$. A ratio pair was taken at every 1.5–3.0 s. The ratio of fluorescence intensities at 340 and 380 nm was plotted as the change in fluorescence ($\Delta F/F_0$), expressed as a percentage of the basal fluorescence ratio observed in the absence of stimulus. Cells were considered responsive if maximal $\Delta F/F_0$ was equal to or

greater than 10% from the baseline for each experimental condition.

Data Analysis

The software used was GraphPad Prism® from GraphPad Software Inc. (San Diego, CA). Results are expressed as mean± SEM. Data were analyzed using ANOVA and a Student's *t* test as appropriate. Significance was accepted as $P<0.05$.

Primary cell culture and maintenance techniques, preparation, media, and reagent vendors remained constant throughout all experiments. To exclude any potential contamination by glia cells, coverslips were superfused with KCl (55 mM) at the end of the experiments, and cells were excluded if intracellular Ca^{2+} did not increase.[17] In this study, *n* equals the number of neurons examined. At least four coverslips were used for each experimental condition.

Results

Superfusion of DMNV neurons with glutamate (10 μM) for 60 s caused intracellular calcium increments in greater than 80% of cells ($n=223$). The increment in intracellular Ca^{2+} reached a peak $\Delta F/F_0$ (57±3%) within 60 s, followed by a subsequent decline to baseline (Fig. 1a). All neurons responded with a calcium transient simultaneously, indicating that the effects of glutamate are all direct without any responses because of propagation of the excitation through gap junctions or synapses that formed in culture. Glutamate produced dose-dependent increments in maximal change in fluorescence ($n=808$, $P<0.05$), over a range from 0.01 to 100 μM (Fig. 1b), and in the percentage of cells responding (Fig 1c). For the remaining experiments, 10 μM was chosen as a working concentration.

Repetitive exposure to glutamate did not produce progressive decrements in peak $\Delta F/F_0$ responses ($n=69$; Fig. 2a). Peak $\Delta F/F_0$ for the three exposures was 76.1± 6.2%, 63.3±4.8%, and 77.4±5.6%, respectively (Fig. 2b). To determine whether extracellular stores of Ca^{2+} are involved in the transients, DMNV cells were superfused with glutamate in a Ca^{2+}-free buffer. None of the cells tested responded when extracellular Ca^{2+} was removed ($n=115$; Fig. 3a). The glutamate-induced Ca^{2+} response quickly returned to baseline upon removal of extracellular Ca^{2+} ($n=92$; Fig. 3b).

The next series of experiments sought to delineate the subtype of ionotropic glutamate receptor responsible for the glutamate-induced intracellular Ca^{2+} signal in DMNV neurons. Kynurenic acid (1 mM), a nonspecific ionotropic glutamate receptor antagonist, inhibited the Ca^{2+} transient in all cells ($n=81$; Fig. 4). Exposure to (*S*)-α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) for 60 s produced dose-dependent increments in maximal change in fluorescence ($n=328$, $P<0.05$), over a range from 0.01 to 5 μM (Fig. 5b), and in the percentage of cells responding (Fig 5c). Conversely, after initial glutamate exposure, subsequent exposure to glutamate while specifically blocking AMPA receptors with the antagonist GYKI 52466 resulted in dose-dependent decreases in peak

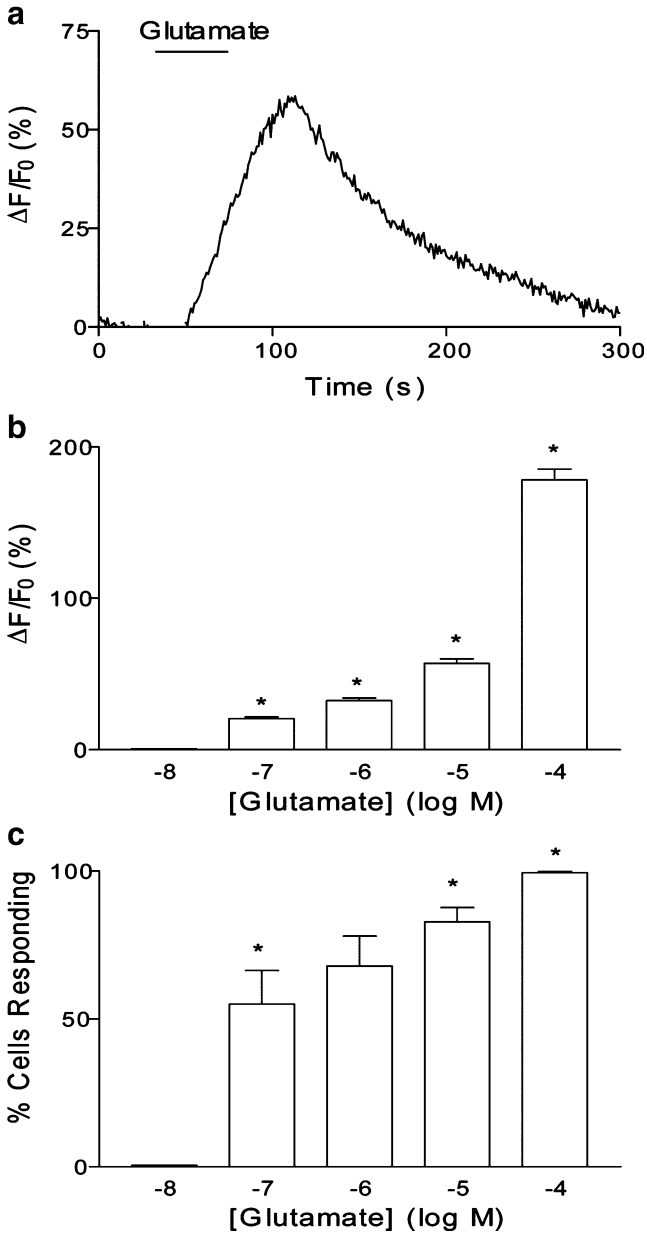

Figure 1 Dose-dependent response. A representative tracing of a single DMNV neuron responding to glutamate (10 μM) after perfusion for 60 s (a). The bar represents time of exposure. Cells were treated with glutamate over a concentration range of 10^{-8} to 10^{-4} M for 60 s ($n=110$, 164, 156, 223, and 155, respectively). Dose-dependent increases in averaged maximal changes in fluorescence ($\Delta F/F_0$) (b), and percentage of cells responding (c) were observed. Data represent mean±SEM. *$P<0.05$ versus lower dose.

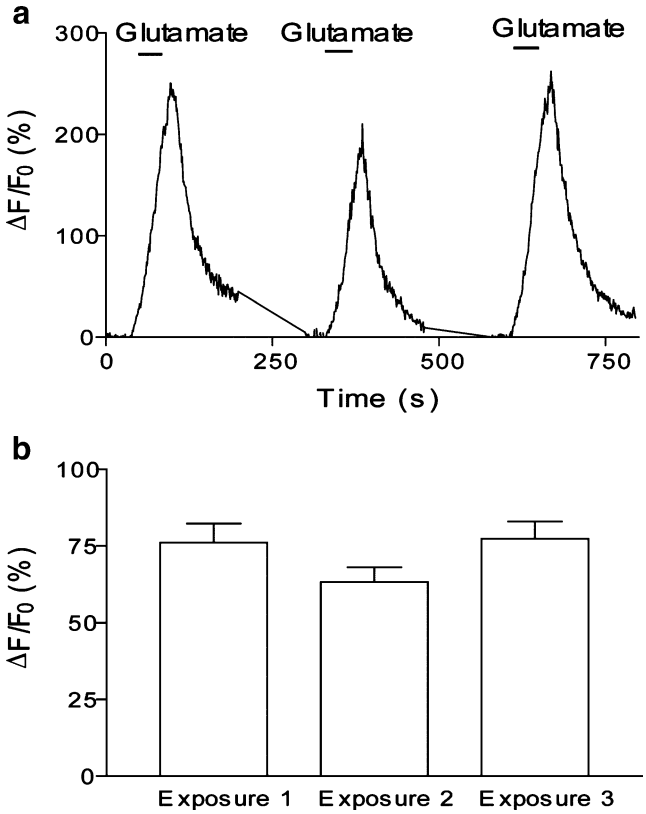

Figure 2 Repetitive exposure. A representative tracing of a single DMNV neuron responding to three sequential exposures to glutamate (10 μM) for 60 s (a). The bars represent time of exposure. Compiled data for all experiments (b). Data represent mean±SEM (n=69).

$\Delta F/F_0$ (Fig. 6b). As the concentration of GYKI 52466 was increased, a decreasing number of cells exhibited glutamate-induced intracellular Ca^{2+} responses (Fig. 6c). Exposure to *N*-methyl-D-aspartic acid (NMDA; 0.1, 1 and 10 μM) produced no intracellular calcium transients in any cell tested (n=158, 122, and 68, respectively). Even when magnesium was omitted from the extracellular medium and glycine (10 μM) added, NMDA induced no response. MK-801 (10 μM), a noncompetitive NMDA antagonist, abolished glutamate-induced Ca^{2+} signaling in only 1.4% of DMNV neurons (n=91) and produced no significant decrease to the glutamate-induced Ca^{2+} increments (76.9± 4.8% versus 62.5±3.5%). (*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (ATPA), a selective kainate receptor agonist, did not induce intracellular Ca^{2+} responses in any cells (n=129). (2*S*,4*R*)-4-methylglutamic acid (100 nM), a kainate receptor antagonist, did not abolish the calcium response or significantly change peak $\Delta F/F_0$ responses (100.3±4.4% versus 91.6±5.1%, n=88).

Because most AMPA receptors are Ca^{2+}-impermeable, and a functional association has been shown to voltage-gated Ca^{2+} channels [18,19], the next series of experiments sought to determine if any subtype of Ca^{2+} channels may be involved. The three major subtypes are T-type, L-type, and N-type Ca^{2+} channels. Superfusion of DMNV cells with $NiCl_2$ (3 mM), a nonspecific blocker of Ca^{2+} channels, abolished glutamate-induced intracellular Ca^{2+} transients in all cells (n=50; Fig 7). Mibefradil, a selective blocker of T-type calcium channels, was used at doses 1 μM, 10 μM, and 20 μM (n=71, 68, and 68, respectively; Fig. 8a). After initial glutamate exposure, subsequent exposure to glutamate while blocking T-type calcium channels with mibefradil resulted in a dose-dependent decrease in average peak $\Delta F/F_0$ (66.6±7.2%, 17.8±2.1%, 7.9±0.8%, respectively, Fig. 8b). As the concentration of mibefradil was increased, a decreasing percentage of cells exhibited a glutamate-induced intracellular Ca^{2+} response (76.0±11.3%, 59.0± 16.7%, 2.5±2.5%, respectively; Fig. 8c). After initial glutamate exposure, superfusion with nifedipine (1 μM), an L-type Ca^{2+} channel blocker, did not significantly change glutamate-induced peak $\Delta F/F_0$ (n=97). Likewise, conotoxin (1 μM), an N-type Ca^{2+} channel blocker,

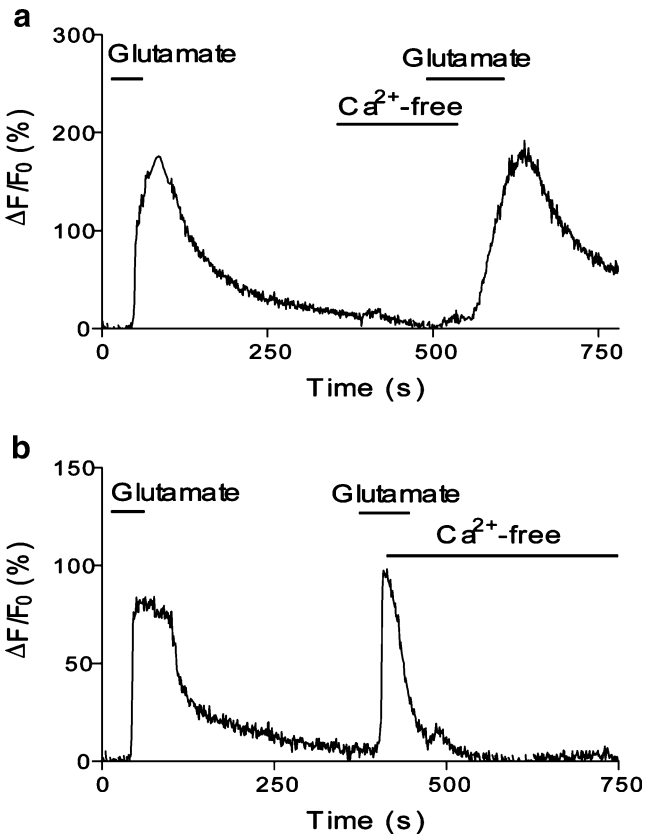

Figure 3 Removal of extracellular Ca^{2+}. Cells were superfused with glutamate for 60 s in a buffer containing Ca^{2+} and allowed to return to baseline $\Delta F/F_0$. Cells were then superfused in Ca^{2+}-free buffer for 160 s, with glutamate added during the last 60 s. Glutamate was continued for 60 more seconds as Ca^{2+} was added to the buffer (a). Cells were superfused with glutamate for 60 s in a buffer containing Ca^{2+} and allowed to return to baseline $\Delta F/F_0$. (b) Cells were then superfused with glutamate for 60 s, initially in a Ca^{2+}-containing buffer for 30 s, followed by a Ca^{2+}-free environment. The bars represent time of exposure.

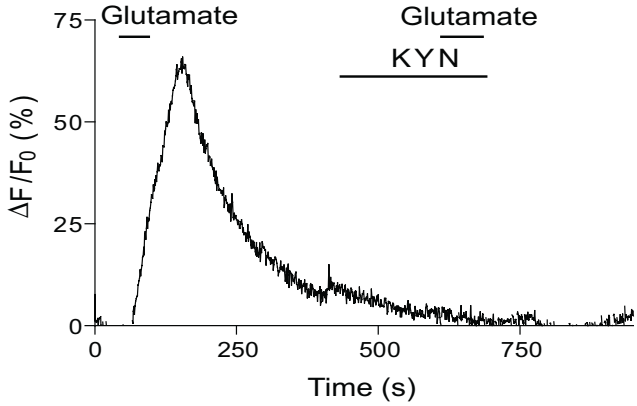

Figure 4 Effect of kynurenic acid (KYN). A representative cell is shown. Cells were superfused with glutamate for 60 s and allowed to return to baseline $\Delta F/F_0$. Cells were then pretreated with KYN for 300 s, followed by superfusion with glutamate in the presence of KYN for 60 s. The bars represent time of exposure.

produced no changes to the glutamate-induced Ca^{2+} increments (n=58).

AMPA receptors have been reported to increase intracellular Ca^{2+} by coupling with the Na^+/Ca^{2+} exchanger in rat hypothalamic neurons and astrocytes.[20,21] This action is caused by Na^+ influx through AMPA receptors driving reverse-mode operation of the Na^+/Ca^{2+} exchanger. To test the contribution of the Na^+/Ca^{2+} exchanger in DMNV cells, the noncompetitive specific inhibitor of the reverse-mode Na^+/Ca^{2+} exchanger, KB-R7943 (10 μM), was used. DMNV neurons were incubated with KB-R7943 for 10 min before a 60-s exposure to glutamate and KB-R7943. Blockade of the reverse-mode Na^+/Ca^{2+} exchanger produced no changes to the glutamate-induced Ca^{2+} increments (n=55; Fig. 9).

Discussion

The current study demonstrates that exposure of neurons of the DMNV to glutamate acts via the AMPA receptor and T-type Ca^{2+} channels, inducing an influx of extracellular Ca^{2+}. The findings that support this conclusion are fivefold: (1) glutamate produces increments in intracellular Ca^{2+} concentration, which are dose-dependent; (2) removal of extracellular Ca^{2+} abolishes the response; (3) blockade of AMPA receptors dose-dependently diminishes the Ca^{2+} response, whereas blockade of NDMA and kainate receptors has no effect; (4) exposure to AMPA produces dose-dependent increments in intracellular calcium response, but agonism of NMDA and kainate receptors do not; and (5) blockade of T-type Ca^{2+} receptors dose-dependently diminishes the Ca^{2+} response, whereas blockade of N-type and L-type channels has no effect.

Glutamate is a major neurotransmitter in the brain, and several studies have demonstrated its importance in the DMNV. Prior studies have used immunohistochemical staining of brainstem slices to show the presence of both AMPA and NMDA receptors in the DMNV.[22,23] Functionally, microinjection of glutamate into the DMNV has been shown to increase the strength of gallbladder contractility, alter gastric volume and contractility, and decrease lower esophageal sphincter pressure.[6,24,25] Injection of glutamate

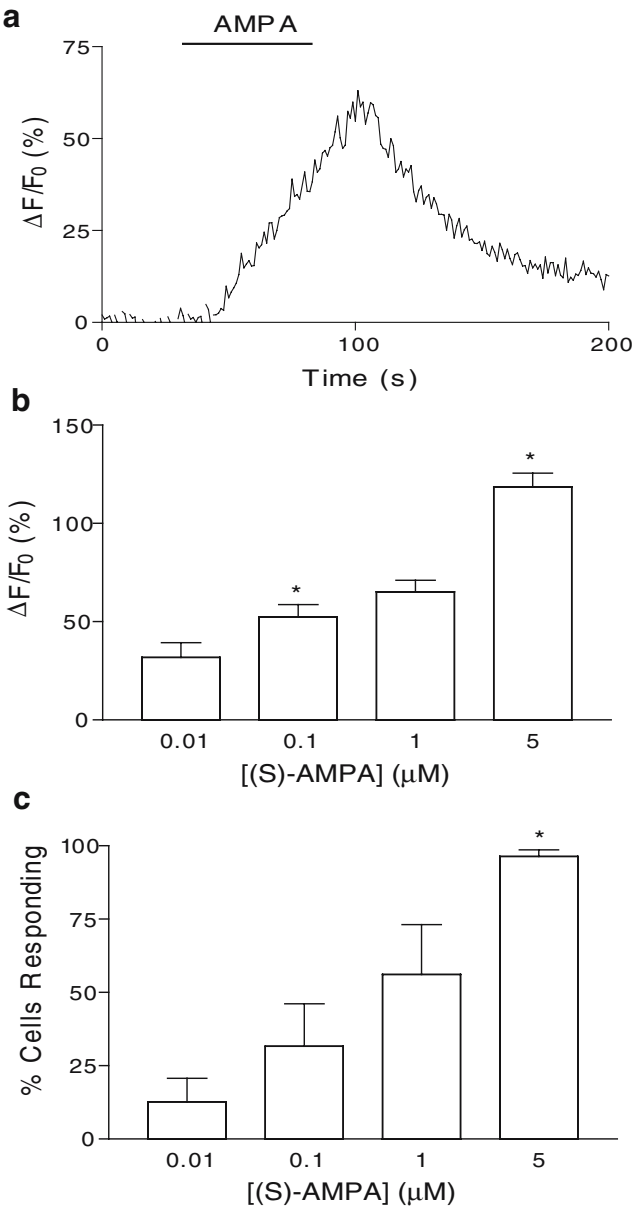

Figure 5 Effect of AMPA. A representative tracing of a single DMNV neuron responding to AMPA (5 μM) after perfusion for 60 s (a). The bar represents time of exposure. Cells were treated with AMPA over a concentration range of 0.01 to 5 μM for 60 s (n=86, 162, 102, and 78, respectively). Dose-dependent increases in averaged maximal changes in fluorescence ($\Delta F/F_0$) (b), and percentage of cells responding (c) was observed. Data represent mean±SEM. *P<0.05 versus lower dose.

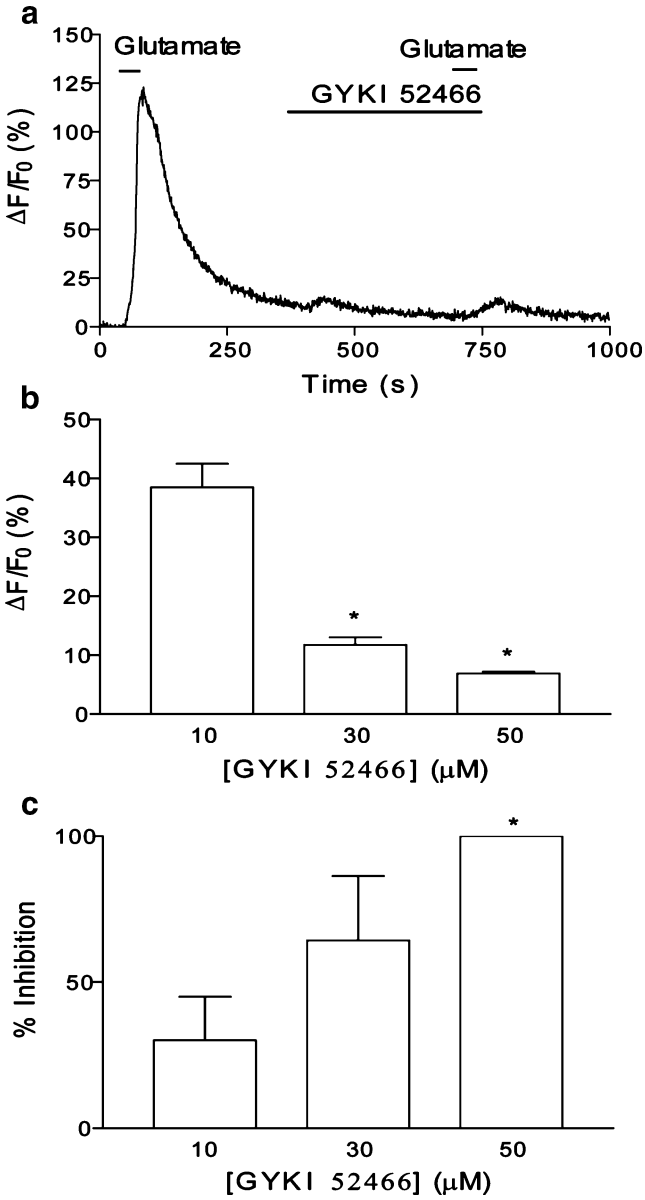

Figure 6 Effect of GYKI 52466. Cells were superfused with glutamate for 60 s and allowed to return to baseline $\Delta F/F_0$. Cells were then pretreated with GYKI 52466 for 300 s, followed by superfusion with glutamate in the presence of GYKI 52466 for 60 s. A representative tracing demonstrating inhibition of glutamate-induced calcium transients by GYKI 52466 (a). The bars represent time of exposure. Concentrations of 10, 30, and 50 μM were used (n=67, 64, and 43 respectively). Dose dependent decrease in averaged peak $\Delta F/F_0$ as GYKI 52466 concentration increased (b). The percentage of cells that initially responded to glutamate but were subsequently inhibited by GYKI 52466 increased in a dose-dependent manner (c). Data represent mean±SEM. *P<0.05 versus lower dose.

into the DMNV-induced responses in DMNV neurons similar to those caused by electrical stimulation of the lateral hypothalamus, an area important in gastrointestinal function and feeding behavior.[26] Similarly, stimulation of the paraventricular nucleus of the hypothalamus caused a depolarization of DMNV neurons, which was blocked by AMPA receptor inhibition.[27] A majority of neurons from the dorsal vagal complex that were activated by gastric balloon distention or intraduodenal infusion of either glucose or linolenic acid expressed AMPA and NMDA receptors.[22] Vago-vagal reflexes initiated by gastrointestinal distention have been shown to be mediated by glutamate; this response is attenuated by selective antagonism of AMPA, but not NMDA, receptors.[4,28] Blockade of non-NMDA receptors inhibited chemotherapy-induced emesis in ferrets, but blockade of NMDA receptors was not nearly as effective.[29,30]

NMDA receptors have been demonstrated in the DMNV immunohistochemically.[22] The present study suggests that these receptors do not operate by causing calcium flux. Prior studies have suggested that NMDA receptors in the DMNV act through a nitric oxide-cGMP pathway.[6]

AMPA receptors are the most abundant excitatory ligand-gated receptor channels in the central nervous system for fast synaptic transmission. They are composed of various combinations of four subunits (GluR1–4). Molecular diversity is further increased by alternative splicing and posttranscriptional nuclear RNA editing.[31] The majority of native AMPA receptors are heterooligomeric complexes containing GluR2.[32] This subunit has been immunohistochemically demonstrated in 70–100% of neurons in the DMNV.[23,33,34] Ca^{2+}-permeability is determined by the glutamine/arginine (Q/R) site, which undergoes RNA editing posttranscriptionally. Most AMPA receptors in the rat brain undergo editing to GluR2(R), which is Ca^{2+}-impermeable.[18,31,34,35] Unedited GluR2(Q) subunits are Ca^{2+}-permeable and have been described in numerous brain regions.[36–39] Ca^{2+}-permeable AMPA receptors lacking the GluR2 subunit have also been described.[32,40] Studies have also suggested that several cell types possess both Ca^{2+}-impermeable GluR2(R) and Ca^{2+}-permeable GluR2(Q).[37,39,41,42]

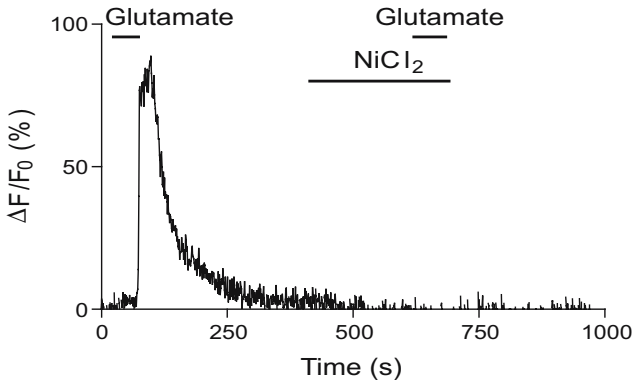

Figure 7 Effect of $NiCl_2$. A representative tracing is shown. Cells were superfused with glutamate for 60 s and allowed to return to baseline $\Delta F/F_0$. Cells were then pretreated with $NiCl_2$ for 300 s, followed by superfusion with glutamate in the presence of $NiCl_2$ for 60 s. The bars represent time of exposure.

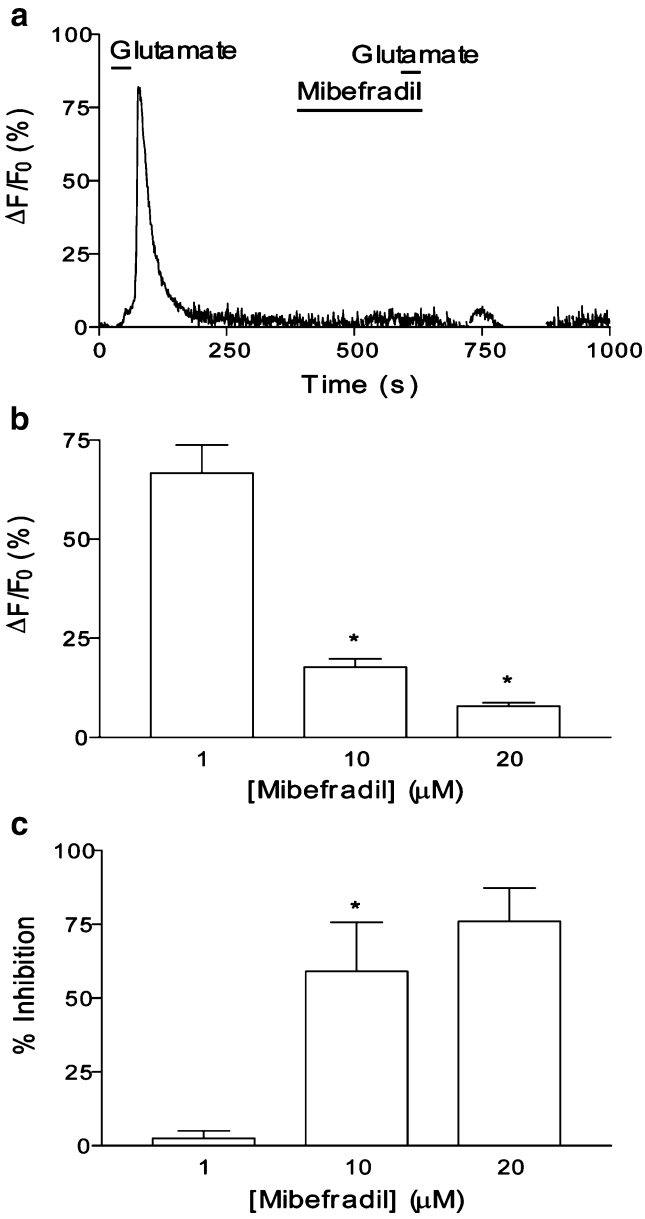

Figure 8 Effect of mibefradil. Cells were superfused with glutamate for 60 s and allowed to return to baseline $\Delta F/F_0$. Cells were then pretreated with mibefradil for 300 s, followed by superfusion with glutamate in the presence of mibefradil for 60 s. A representative tracing demonstrating inhibition of glutamate-induced calcium transients by mibefradil (a). The bars represent time of exposure. Dose-dependent decrease in averaged peak $\Delta F/F_0$ as mibefradil concentration increased (b). The percentage of cells that initially responded to glutamate but were subsequently inhibited by mibefradil increased in a dose-dependent manner (c). Data represent mean±SEM. *$P<0.05$ versus lower dose.

AMPA receptors generally have rapid and profound desensitization after glutamate stimulation.[43] This characteristic can be modified by a second common RNA edit at the arginine/glycine (R/G) site as well as co-assembly with auxiliary subunits.[44–46] Recently, there has been a suggestion that calcium flux is not solely determined by whole-cell relative Ca^{2+} permeability of AMPA receptors.[47] Coupling of AMPA receptors with the Na^+/Ca^{2+} exchanger has been demonstrated as a mechanism causing intracellular Ca^{2+} transients.[21] A recent study demonstrated the physical and functional association of AMPA receptors and voltage-gated Ca^{2+} channels in a complex with scaffolding proteins in brain tissue.[19] As prior studies have demonstrated predominantly GluR2-containing AMPA receptors in the DMNV, which are generally Ca^{2+}-impermeable and undergo rapid desensitization, the results of the present study suggest a functional association between AMPA receptors and T-type Ca^{2+} channels.

Two main classes of Ca^{2+} channels exist: high-voltage-activated (HVA) and low-voltage-activated (LVA). The HVA channels are further divided into N-type and L-type channels, whereas T-type channels are LVA. The latter channels are characterized by low-voltage activation, low unitary conductance, fast inactivation and slow deactivation kinetics, and strong steady-state inactivation at physiological resting potentials. T-type channels are found throughout the central nervous system including the hippocampus, amygdala, frontal cortex pyramids, CA1 pyramids, dorsal horn, and Purkinje.[48] These channels have been suggested

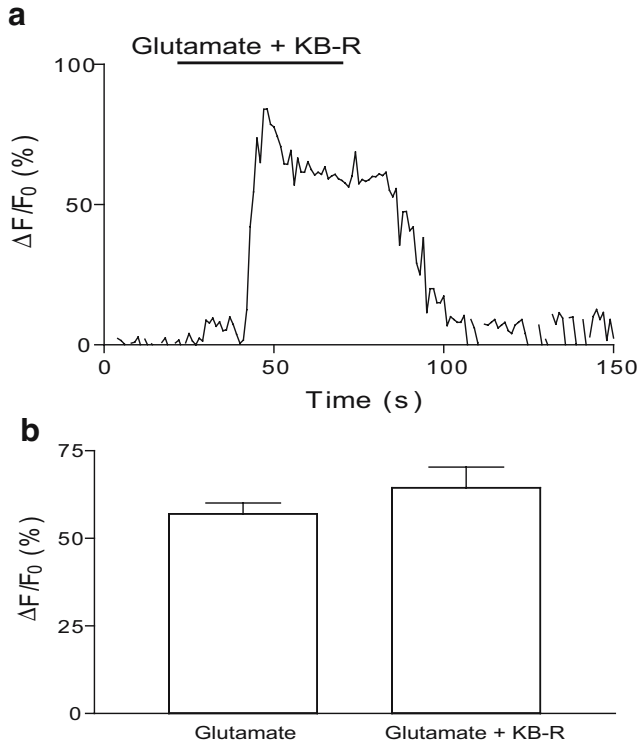

Figure 9 Effect of KB-R7943 (KB-R). Cells were incubated with KB-R7943 for 10 min before superfusion with glutamate in the presence of KB-R7943 for 60 s. A representative tracing (a). The bars represent time of exposure. No significant difference was seen in averaged peak $\Delta F/F_0$ ($n=55$) (b). Data represent mean±SEM.

to contribute to sleep, epilepsy, and neuronal pacemaker activity.[49] T-type channels are also present in developing neurons and play a role in proliferation and differentiation.[50] Neurotransmitter action on these channels has been mixed. For example, acetylcholine, serotonin, and substance P increase T-type Ca^{2+} currents in rat hippocampal neurons, hippocampal interneurons, and spinal horn neurons, respectively. Conversely, neuropeptide Y and somatostatin have been shown to inhibit T-type Ca^{2+} currents in rat dorsal root ganglia cells and pituitary somatotroph cells. In rat dorsal root ganglia neurons, $GABA_B$ receptor both activates and inhibits T-type Ca^{2+} channels depending on the concentration of agonist.[51] The results of the present study suggest that glutamate mediates intracellular Ca^{2+} currents via the AMPA receptor and T-type Ca^{2+} channels in DMNV neurons. Initial influx of Ca^{2+} via AMPA receptors may lead to activation of T-type channels in DMNV neurons. Alternatively, the AMPA receptors may be Ca^{2+}-impermeable, mediating Ca^{2+} influx through a signal activating T-type Ca^{2+} channels.

In vitro culture is useful to study the morphology, development, electrophysiology, neurochemistry, and pharmacology of DMNV neurons. However, there are also disadvantages. The temporal and environmentally controlled genetic changes are likely to be different in culture than in the brain. The influence of normal neuronal circuitry is lost in cell culture. The dorsal vagal complex is anatomically and functionally complex. DMNV neurons integrate signals from the nucleus of the solitary tract, area postrema, and hypothalamus and act as preganglionic efferents to the gut. A recent study showed that microinjection of glutamate into the nucleus of the solitary tract inhibited DMNV neurons, whereas injection into the DMNV was excitatory, indicating the importance of these connections under normal physiologic conditions.[4] Others have shown that glutamate does not induce noticeable effects on gastric motility and tone if GABAergic transmission from the nucleus of the solitary tract is inhibited.[52] Studies in cell culture cannot account for the normal in vivo interplay between these pathways. Furthermore, to achieve successful growth in culture, neonatal rats were used. Because T-type Ca^{2+} channels are important in development, it is possible that calcium transients from cells cultured from adult rats may be different.

There is active interest in glutamate as an integral neurotransmitter in normal and pathologic gastrointestinal processes. Advances in knowledge may be relevant to potential therapeutics in the treatment of gastroesophageal reflux disease, chemotherapy-induced emesis, and obesity. The results of the current study demonstrate that glutamate, acting through AMPA receptors and T-type Ca^{2+} channels, stimulates DMNV neurons by an influx of extracellular Ca^{2+}. Consideration of the physiological importance of a glutamate-induced Ca^{2+} signal is important in the complete understanding of the function and regulation of the DMNV, as well as in the development of future therapeutic targets.

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A Curative Resection Improves the Postoperative Survival Rate Even in Patients with Advanced Gallbladder Carcinoma

Masahiro Kai · Kazuo Chijiiwa · Jiro Ohuchida · Motoaki Nagano · Masahide Hiyoshi · Kazuhiro Kondo

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Abstract The aim of this study was to evaluate the results of our series of 90 operations for gallbladder carcinoma according to the Japanese Society of Biliary Surgery (JSBS) classification system and to clarify the appropriate surgical strategy for advanced gallbladder carcinoma based on the depth of primary tumor invasion and lymph node metastasis. Generally, only a surgical resection can achieve a prognostic improvement of the advanced gallbladder carcinoma. The survival of patients with this neoplasm depends strictly on the depth of histological primary tumor invasion and lymph node metastasis. A retrospective analysis was conducted on 90 patients from 1990 to 2004 who underwent a surgical resection of gallbladder carcinoma. The factors influencing survival were examined. Thirty-nine patients with palliative treatment (not resected cases), which was diagnosed as T3 or T4 by preoperative imagings, were also included in this study. The significance of the variables for survival was examined by the Kaplan–Meier method and the log-rank test followed by multivariate analyses using Cox's proportional hazard model. Portal invasion, lymph node metastasis, the surgical margin (+ vs. −) and the final curability (fCurA, B vs. C) were all found to be independent prognostic factors in the multivariate analysis. In pT2 gallbladder carcinoma, a better survival was achieved in an aggressive surgical approach, in order of a S4a+S5 hepatic resection, an extended cholecystectomy and a cholecystectomy. In pT3 and pT4, although radical extended surgery did not provide the opportunity for good survival even after lobectomy of the liver, the survival of patients with curative surgery was statistically better than in those without curative surgery. In addition, the nodal involvement of pN1 to pN2 was better than that with pN3. A S4a+S5 hepatectomy, therefore, appears to be adequate for the treatment of pT2 gallbladder carcinoma. Even in patients with pT3 and pT4 gallbladder carcinoma, long-term survival can be expected by an operation with a tumor-free surgical margin. The role of radical surgery, however, is considered to be limited in patients with pN3 lymph node metastasis.

Keywords Gallbladder carcinoma · Surgical resection · S4a+S5 hepatectomy · Prognostic factors

Gallbladder carcinomas, the most frequent form of bile duct cancer, is usually detected at an advanced stage because of the absence of specific symptoms and signs, despite recent progress in diagnostic modalities.[1] Recent advances in ultrasonography, computed tomography, magnetic resonance cholangiography, direct cholangiography, and endoscopic ultrasonography, however, have contributed to the increased detection of gallbladder carcinoma at a resectable stage.[1–3]

The prognosis in patients with early gallbladder carcinoma (tumors restricted to the mucosa or muscle layer) is associated with a 5-year survival rate ranging from 90% to 100%.[4–6] These lesions are most frequently diagnosed incidentally after a cholecystectomy for symptomatic diseases such as gallstones.[7] For the early gallbladder carcinoma including undiagnosed cases incidentally detected by macroscopic and microscopic examinations after open or laparoscopic cholecystectomy, most surgeons

M. Kai · K. Chijiiwa (✉) · J. Ohuchida · M. Nagano · M. Hiyoshi · K. Kondo
Department of Surgical Oncology and Regulation of Organ Function, Miyazaki University School of Medicine,
5200 Kihara, Kiyotake,
Miyazaki 889-1692, Japan
e-mail: kazuochi@med.miyazaki-u.ac.jp

agree that such patients do not require any surgery other than a simple cholecystectomy because no lymph node metastasis is evident.[4,8–11]

On the other hand, advanced gallbladder carcinoma is characterized by a very poor survival rate, and its surgical treatment ramains a matter of controversy. Recent papers from Japan have reported that radical surgery for advanced gallbladder carcinoma improve survival[12–14]; however, most Western authors have so far failed to establish any benefit in patients with advanced gallbladder carcinoma.[15,16]

It is well-known that a surgical resection provides the only hope for a long-term survival of patients with gallbladder carcinoma. An improvement in the outcome after a resection for the gallbladder carcinoma may thus depend on the selection of appropriate surgical strategies for advanced lesions. The depth of primary tumor invasion (pT) and lymph node metastasis (pN) have all been reported to be significant prognostic factors.[3,12,17–21]

Because pT can now be diagnosed either pre- or intraoperatively,[5,21,22] the choice of surgical procedure should thus be evaluated based on pT especially for advanced carcinoma greater than pT2. The aim of this study was to evaluate the results of curative resections for the advanced gallbladder carcinoma and to clarify the optimal surgical strategies, especially for the advanced gallbladder carcinoma.

Patients and Methods

Ninety patients with gallbladder carcinoma who had undergone a surgical resection from January 1990 to December 2004 at our University Hospital were retrospectively analyzed. They included 41 men and 49 women with a mean age of 65 years old (range 36–89 years). Thirty-nine patients with palliative treatment (not resected cases), diagnosed as T3 or T4 by preoperative imagings, were also included in this study. pT, pN, and the final stage (fStage) was examined based on the Japanese Society of Biliary Surgery (JSBS) classification system.[23]

The depth of primary tumor invasion (pT) was classified into the following four groups: pT1, tumors restricted to the mucosa or muscle layer, a lack of direct invasion of the liver and hepatoduodenal ligament, and also no invasion of the portal veins and hepatic arteries; pT2, tumors invading the perimuscular connective tissue, no extension beyond the serosa or into the liver parenchyma and no invasion of the hepatoduodenal ligament, portal veins, and hepatic arteries; pT3, tumors perforating the serosa (visceral peritoneum) and/or directly invades liver, extension 5 mm in depth or less into the liver, and/or invading the right margin of the hepatoduodenal ligament, but not the left margin, without vascular (portal vein and hepatic artery) invasion; pT4, tumors extending more than 5 mm into the liver parenchyma and/or invades the left margin of the hepatoduodenal ligament and/or invading the portal veins or hepatic arteries. Lymph node metastasis (pN) was classified as follows: pN0, no regional lymph node metastasis; pN1, metastasis in cystic duct and/or pericholedochal node; pN2, metastasis in the hepatoduodenal ligament except pN1 and/or posterosuperior pancreas head and/or along the common hepatic artery; pN3, metastasis in the peripancreatic (head except posterosuperior pancreatic), celiac, superior mesenteric, and/or paraaortic lymph node metastasis. fStage and

Table 1 Classification Systems for the Staging and Curability by the JSBS

Final Stage (fStage)								
	H0, P0, M(−)				H1, 2, 3, P1, 2, 3, M(+)			
	pN_0	pN_1	pN_2	pN_3				
pT_1	I	II	III	IVa				
pT_2	II	III	III	IVa				
pT_3	III	III	IVa	IVb			IVb	
pT_4	IVa	IVa	IVb	IVb				
Final curability (fCur)								
	H	P	pN-D	pBM	pHM	pEM	M	Residual tumor
Final curability A (fCurA)	H0	P0	pN<D	pBM0	pHM0	pEM0	M(−)	(−)
Final curability B (fCurB)	H0	P0	pN=D	pBM1	pHM1	pEM1	M(−)	(−)
Final curability C (fCurC)	H1, 2, 3	P1, 2, 3	pN>D	pBM2	pHM2	pEM2	M(+)	(+)

H0 = no evidence of liver metastasis; H1 = metastasis limited to one lobe; H2 = a few metastases to both lobes; H3 = numerous metastases to both lobes; P0 = no evidence of peritoneal metastasis; P1 = metastasis to the peritoneum adjacent to extrahepatic bile ducts; P2 = a few metastases to the distant peritoneum; P3 = numerous metastases to the distant peritoneum; M = distant metastasis other than peritoneal and/or liver metastases; pN = histological lymph node metastasis; D = lymph node dissection; pBM = distal (cystic or bile duct) cut end; pHM = proximal (hepatic) cut end; pEM = dissected periductal structure; pBM0, pHM0, pEM0 = cancer-free margin of more than 5 mm in width; pBM1, pHM1, pEM1 = cancer-free margin of 5 mm or less in width; pBM2, pHM2, pEM2 = definite invasion of each surgical margin.

Table 2 Significant Prognostic Factors for the Patients with Gallbladder Carcinoma Based on Univariate and Multivariate Analyses*

Variables	Odds Ratio	95% Confidence Interval	*p* Value
Univariate analysis			
Tumor factor			
pT(1, 2 vs 3, 4)	4.808	2.488–9.346	0.0001
Serosal invasion (− vs +)	3.935	2.124–7.192	0.0001
pHinf (− vs +)	3.234	1.652–6.330	0.0006
pBinf(− vs +)	4.554	2.434–8.521	0.0001
pPV (− vs +)	12.14	3.230–45.63	0.0002
H (− vs +)	7.559	2.808–20.35	0.0001
Lymph node metastasis (− vs +)	5.458	2.769–10.76	0.0001
Operative factor			
Bile duct resecton except pT1(− vs +)	1.939	0.973–3.863	0.0598
Lymph node dissection except pT1 (D1 vs D2)	2.049	0.894–4.695	0.0902
Surgical margin (− vs +)	4.046	2.128–7.692	0.0001
Final curability (AB vs C)	5.650	2.994–10.64	0.0001
Multivariate analysis			
pPV(− vs +)	10.61	1.753–64.19	0.0101
Lymph node metastasis (− vs +)	3.247	1.504–7.010	0.0027
Surgical margin (− vs +)	12.98	1.030–166.7	0.0474
Final curability (AB vs C)	8.929	1.314–62.50	0.0252

*Cox's proportional hazard model; pT = depth of primary tumor invasion; pHinf = direct invasion of the liver; pBinf = invasion of the hepatoduodenal ligament; pPV = invasion of the portal veins; H = liver metastasis; D1 = complete dissection of group 1 lymph nodes; D2 = complete dissection of group 1 and 2 lymph nodes; Final curability A, B, C = refer to Table 1.

final curability (fCur) were classified according to the final histopathological diagnosis. A curative resection was defined as a complete removal of the cancer cells with negative histological margins without the presence of any residual tumor. The classification of fStage and fCur according to the JSBS system is shown in Table 1.

The significance of the variables for survival was examined by the Kaplan–Meier method and the log-rank test followed by multivariate analyses using the Cox's proportional hazard model. A probability value of less than 0.05 was considered to be significant.

Results

Univariate and Multivariate Analyses of the Prognostic Factors

Significant prognostic factors in the univariate analysis of the tumor factors were primary tumor invasion (pT), serosal invasion, direct invasion of the liver, invasion of the hepatoduodenal ligament, portal vein invasion, liver metastasis, and lymph node metastasis. Regarding surgical factors, the surgical margin (negative vs. positive) and final

Table 3 Histopathological Findings of Gallbladder Carcinoma Depending on the Depth of Primary Tumor Invasion (pT)

	pT1	pT2	pT3	pT4
Number of patients	17	34	9	30
Lymphatic invasion (ly) (%)	0	26/32(81)	6/8(75)	24/25(96)
Venous invasion (v) (%)	0	17/32(53)	8/8(100)	20/25(80)
Perineural invasion (pN) (%)	0	9/28(32)	4/8(50)	20/24(83)
Presence of lymph node metastasis (%)	0	16/34(47)	5/8(62)	20/29(69)
pN1	0	8/34(24)	1/8(13)	5/29(17)
pN2	0	7/34(21)	2/8(25)	10/29(34)
pN3	0	1/34(3)	2/8(25)	5/29(17)
Liver metastasis (H) (%)	0	1/34(3)	1/9(11)	5/30(17)
Peritoneal dissemination (P) (%)	0	0	0	2/30(7)
fStage I/II/III/IVa/IVb	17/0/0/0/0	0/17/15/1/1	0/0/4/2/3	0/0/0/12/18
Positive of surgical margin (%)	0	1/34(3)	3/9(33)	14/30(47)
Final curability C(fCurC) (%)	0	2/34(6)	4/9(44)	19/30(63)

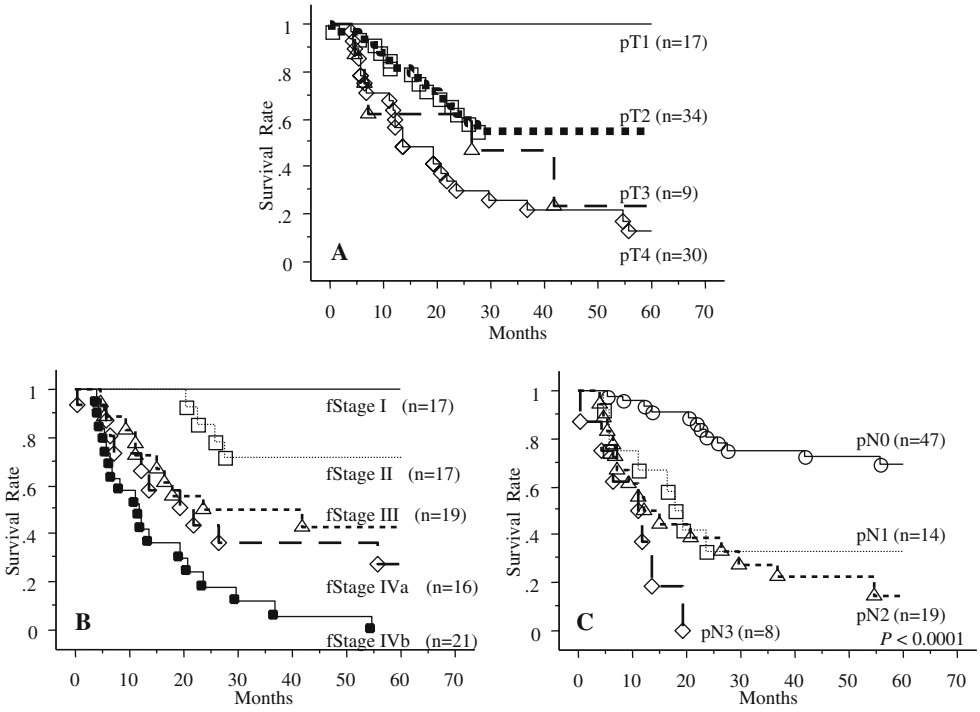

Figure 1 (A) The postoperative survival rate depending on the primary tumor invasion (pT) in patients with gallbladder carcinoma. (B) The postoperative survival rate depending on the final stage (fStage) in patients with gallbladder carcinoma. The classification of the final stage is shown in Table 1. (C) The postoperative survival rate depending on the extent of lymph node metastasis in patients with gallbladder carcinoma ($p<0.0001$, log-rank test).

curability (fCurA, B vs. fCurC) were significant prognostic factors by the univariate analysis. Bile duct resection and the degree of lymph node dissection (D1 vs. D2), except pT1 gallbladder carcinomas, had no significant effect on the postoperative survival. The absence of portal invasion (odds ratio [OR] 10.61, $p=0.0101$), the absence of lymph node metastasis (OR 3.247, $p=0.0027$), surgical margin (− vs. +, OR 12.98, $p=0.0474$), final curability (fCurA, B vs. C, OR 8.929, $p=0.0252$) were all independent prognostic factors based on a multivariate analysis (Table 2).

The Histopathological Findings of Gallbladder Carcinoma Depending on Primary Tumor Invasion (pT)

The number of patients were: pT1, 17; pT2, 34; pT3, 9; pT4, 30. Early gallbladder carcinoma (pT1) was 17 cases (19%) and advanced disease (pT2 to pT4) was 73 cases (81%).

The incidences of lymphatic (ly), venous (v), perineural (pn) invasions, lymph node metastasis, liver metastasis, and peritoneal dissemination, and also the distribution of the final stage, positive rate of surgical margin, and final curability depending on the pT classification are shown in Table 3. All of these factors were absent in pT1 gallbladder carcinoma. In the pT2 gallbladder carcinoma, the rates of ly, v, and pn were 81%, 53%, and 32%, respectively. In the pT3 and pT4, these rates were higher than those of pT2; the rates of ly, v, and pn were 75%, 100%, and 50%, respectively, in pT3 and 96%, 80%, and 83%, respectively, in pT4. The rates of lymph node metastasis were 47% in pT2, 62% in pT3, and 69% in pT4. Even in pT2, pN2 lymph node metastasis was seen in 21% of cases. In pT3 and pT4, the rates of pN2 and pN3 lymph node metastases were both 25% in pT3, and 34% and 17%, respectively, in pT4. The rate of a noncurative resection (fCurC) was 6% in pT2, 44% in pT3, and 63% in pT4.

From lymph node metastasis, 47 patients were classified as pN0, 14 as pN1, 19 as pN2, and 8 as pN3. The number of fStage I, II, III, IVa, and IVb patients was 17, 17, 19, 15 and 22, respectively.

The 5-year survival rates of the gallbladder carcinoma in patients with pT1, pT2, pT3, and pT4 were 100%, 58%,

Table 4 Surgical Procedures According to the pT Classification in the Patients with Gallbladder Carcinoma

Surgical Procedure	Cx	Ext. Cx	S4a+S5	Hx
pT1	11	4	2	–
pT2	9	21	4	–
pT3	3	6	–	–
pT4	3	7	16	4

Cx; Cholecystectomy, Ext. Cx; Extended Cholecystectomy, S4a+S5; anatomic resection of liver segment 5 and of the lower part of segment 4 together with the gallbladder, Hx; Hepatic lobectomy

Table 5 Bile Duct Resection in Patients with Gallbladder Carcinoma

Bile Duct Resection	BDR+(with PD)	BDR-
pT1	6 (1)	11
pT2	17 (2)	17
pT3	5 (1)	4
pT4	25 (3)	5

BDR = Bile duct resection, PD = pancreaticoduodenectomy

23%, and 12%, respectively (Fig. 1A). Those with pN0, pN1, pN2, and pN3 were 70%, 32%, 14%, and 0%, respectively (Fig. 1B), and with fStage I, II, III, IVa, and IVb were 100%, 77%, 42%, 28%, and 0%, respectively (Fig. 1C).

The Role of Surgery for Advanced Gallbladder Carcinoma

Because all 17 patients with pT1, irrespective of the surgical procedure, were alive without recurrence, the role of surgery was thus examined in the patients with pT2, pT3, and pT4 gallbladder carcinoma. The surgical procedures according to the pT classification are shown in Tables 4 and 5.

Of the 34 patients with pT2, a cholecystectomy was applied to nine, a resection of the gallbladder liver bed (extended cholecystectomy) to 21, a resection of the segments 4a and 5 of the liver to four. The postoperative 5-year survival rates after each procedure in the patients with pT2 gallbladder carcinoma are shown in Fig. 2A. A better survival was achieved in order of a S4a+S5 hepatic resection, an extended cholecystectomy, and a cholecystectomy. A cholecystectomy showed a significantly shorter survival than an extended cholecystectomy and a S4a+S5 hepatic resection (p=0.0481). According to the degree of lymph node dissection in pT2 gallbladder carcinoma except for one patient with pN3 lymph node metastasis, the patients with D1 (dissection of N1 lymph nodes) or more lymph node dissections statistically showed a longer survival than the patients with no dissection of the regional lymph nodes, but there was no statistically significant difference between a D1 and a D2 (dissection of N1 and N2 lymph nodes, respectively; Fig. 2B). A bile duct resection in pT2 gallbladder carcinoma had no significant effect on the postoperative survival (p=0.6694).

Of the 39 patients with pT3 or pT4 gallbladder carcinomas, a cholecystectomy was performed in six patients, an extended cholecystectomy in 13, a resection of the segments S4a and S5 of the liver in 16, and a hepatic lobectomy in four. In spite of the retrospective nature and the heterogenicity of the surgical approaches employed, the postoperative survival of the patients with pT3 or pT4 carcinoma who underwent curative surgery (fCurA+B) was significantly better than that in those undergoing fCurC (Fig. 3A). In addition, even if the fCurC was applied to pT3 or pT4 gallbladder carcinomas, its survival rate was significantly better than that in the nonresected patients (diagnosed as T3 and T4 by preoperative imaging; Fig. 3A). The clinicopathological features of pT3 and pT4 gallbladder carcinoma according to the cases of fCurA+B, fCurC, and not resected tumors are shown in Table 6. The backgrounds in these three groups were different. The incidences of peritoneal dissemination, distant metastasis, and pT4 were significantly higher in the nonresected group than those in the fCurC group. In pT3 and pT4, the survival of patients with nodal involvement of pN1 to pN2 was better than that in those with pN3 (Fig. 3B). A surgical resection provided no survival benefit for the patients with pN3 lymph node metastasis. The survival of the patients with pN3 lymph node metastasis was not better than that of the nonresected patients.

Discussion

The depth of primary tumor invasion (pT) and lymph node metastasis (pN) are critical prognostic factors in patients with gallbladder carcinoma.[3,12,17–21] To select an appropriate procedure either before or during an operation,

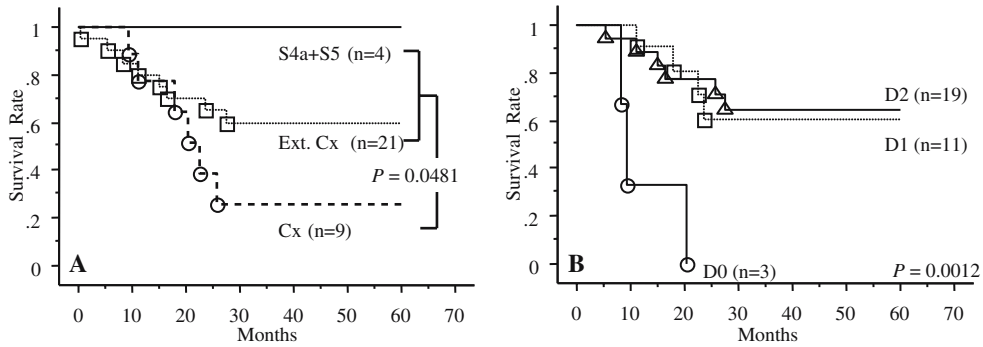

Figure 2 (A) The postoperative survival rate depending on the surgical procedure in patients with pT2 gallbladder carcinoma. S4a+S5; S4a+S5 hepatic resection (anatomic resection of the segment 5 and of the lower part of segment 4 of the liver together with the gallbladder, Ext. Cx; an extended cholecystectomy (resection of the gallbladder together with the gallbladder bed of the liver 2 cm or more in depth), Cx; cholecystectomy. A cholecystectomy was a significantly shorter survival than either an extended cholecystectomy or a S4a+S5 hepatic resection (p=0.0481, log-rank test). (B) The postoperative survival rate depending on the extent of lymph node dissection in patients with pT2 gallbladder carcinoma except for one patient with pN3 lymph node metastasis (p=0.0012, log-rank test). D0; No dissection of the regional lymph nodes, D1; a complete dissection of group 1 lymph node, D2; complete dissection of group 1 and 2 lymph nodes.

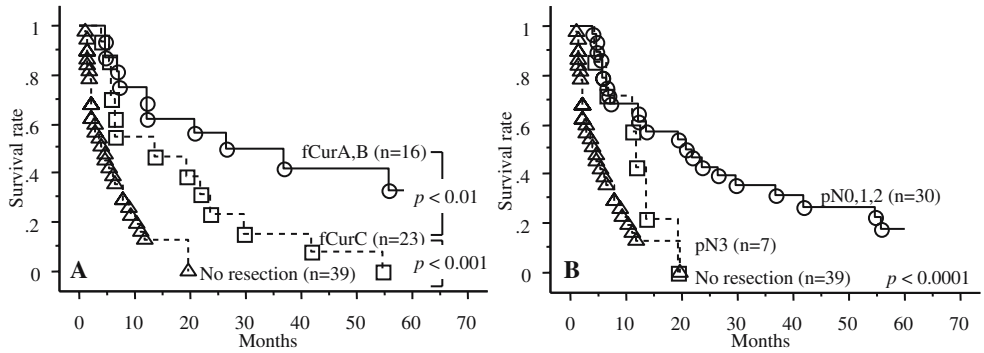

Figure 3 (A) The postoperative survival rate depending on the final curability in patients with pT3 and pT4 gallbladder carcinoma ($p<$ 0.0001, log-rank test). The classification of final curability (fCurA, B and C) is shown in Table 1. (B) The postoperative survival rate depending on the extent of lymph node metastasis in patients with pT3 and pT4 gallbladder carcinoma ($p<0.0001$, log-rank test).

such factors as the depth of primary tumor invasion (pT) and lymph node metastasis (pN) are extremely useful variables, because the primary tumor with or without the gross invasion of adjacent structures, some sites of metastasis and lymph node metastasis can be recognized by preoperative imaging techniques, endoscopic ultrasonography, and computed tomography, and operative ultrasonography.[24] The optimal surgical procedure according to the depth of primary tumor invasion, especially regarding the extent of a hepatic resection, bile duct resection, and radical lymph node dissection still remains controversial.[14,18,25–27]

A surgical resection is effective for tumors limited to the gallbladder wall, which are defined as pT1, whereas the surgical treatment for advanced lesions, especially pT3 or pT4, remains unsatisfactory. An aggressive approach is thus required to resect advanced carcinomas. Extensive surgery has been reported to result in a long-term survival in patients with advanced gallbladder carcinoma.[12–14,25] On the other hand, Donohue et al.[28] reported the patient outcome to be not demonstrably affected by aggressive therapy for the advanced gallbladder carcinoma, and a multicenter study has reported no long-term survivors among the patients with node-positive disease.[27] Cubertafond et al.[29] reported that no patients with T3 and T4 (based on the staging criteria of the American Joint Committee on Cancer) gallbladder carcinoma survived more than 36 months after an extended cholecystectomy.

We and some authors have thus proposed surgical management for pT2 gallbladder carcinoma.[3,12,17–21,25] In patients with pT2 gallbladder carcinoma treated with a simple cholecystectomy alone, postoperative survival remains pessimistic.[19,30,31] We have proposed an extended cholecystectomy or more aggressive surgery, a S4a+S5 hepatic resection or extended right lobectomy of the liver, for patients with pT2 gallbladder carcinoma.[3,17,19–21] A simple cholecystectomy is not adequate for pT2 gallbladder carcinoma, because the rate of lymph node metastasis is 47%, with a 21% rate of pN2-node-positive in our surgical series; moreover, some studies heve reported that even in the absence of hepatic invasion, metastases to the liver (mainly the segment 4a and 5) are possible.[32] In our study, the survival of patients with a simple cholecystectomy showed a poorer prognosis than that of an extended cholecystectomy and a S4a+S5 hepatic resection, which confirmed the previous result.[21] In the present study, the

Table 6 Clinicopathological Features of pT3 and pT4 Gallbladder Carcinoma Include Not Resected Cases Diagnosed as T3 or T4 by Several Types of Imaging

Number of Patients	fCurA, B 16	fCurC 23	Not Resected 39
Presence of lymph node metastasis(%)	9/16/(57)^{NS*}	16/21(76)^{NS}	24/30(80)
(p)N1(%)	2/16(13)	4/21(19)	4/30(13)
(p)N2(%)	7/16(44)	5/21(24)	6/30(20)
(p)N3(%)	0†	7/21(33)^{NS}	14/30(47)
Liver metastasis (H)(%)	0†	6/23(26)^{NS}	19/38(50)
Peritoneal dissemination (P)(%)	0^{NS}	2/23(9)‡	12/23(52)
Distant Metastasis (%)	0^{NS}	0†	9/39(23)
(p)T3/(p)T4	5/11^{NS}	4/19†	1/38
(f)Stage III/IVa/IVb	3/8/5†	1/6/16^{NS}	0/5/34

An asterisk indicates significance (†, $p<0.05$; ‡ $p<0.01$) of the difference compared with fCurAB and fCurC or fCurC and not resected group.
*NS = not significant

pT2 patients with a D1 (dissection in cystic duct and pericholedochal lymph node) or greater lymph node dissection showed a statistically prolonged survival in comparison to that of patients without lymph node dissection (D0), but no statistically significant difference was seen with or without resection of the extrahepatic bile duct. Based on the results, preserving the bile duct seems preferable. However, a lymph node dissection in the hepatoduodenal ligament without bile duct resection is difficult to perform while keeping the blood supply. Therefore, to perform a complete dissection of the lymph node in the hepatoduodenal ligament, we believe that the combined resection of the common bile duct is necessary and suitable for the pT2 gallbladder carcinoma.

For patients with pT3 and pT4 gallbladder carcinomas, a curative resection is the only chance of a long-term survival. Fong et al.[33] have reported that radical resection offers significantly greater chance of long-term survival than the less radical procedures or nonoperative therapy in selected patients even with advanced gallbladder carcinoma. Taner et al.[34] also have presented that radical surgical resection can improve patient survival. Lymph node metastasis is common, and in our surgical series the rate of lymph node metastasis was 62% in pT3 tumors and 69% in pT4 tumors. In the pT3 or pT4 gallbladder carcinomas, the survival of patients with curative surgery (fCurA+B) was significantly better than that in those with no curative resection (fCurC). In addition, the survival in patients with nodal involvement of pN1 to pN2 was better than that in those with pN3. The role of radical surgery cannot be concluded from the present study because the backgrounds were different, but seems to be limited in patients with pN3 lymph node metastasis. Although the clinical course of pT3 and pT4 gallbladder carcinoma remains dismal, a curative resection of tumor (fCurA+B), excluding cases with pN3 lymph node metastasis, statistically prolonged the survival in comparison to those without curative resection (fCurC) and also in those with no resection of the tumor. We propose that even in the patients with pT3 or pT4 gallbladder carcinoma without pN3 lymph node metastasis, a long-term survival can be expected by surgery with a tumor-free surgical margin. We believe that pN3 lymph node metastasis, especially para-aortic lesions, are thus not indicated for a curative resection.

Recently, an extended right hepatic lobectomy with a pancreaticoduodenectomy has been advocated for advanced gallbladder carcinomas such as pT3 or pT4 tumors because of the frequent presence of lymph node metastasis posterior to the head of the pancreas and along the root of the mesentery.[35–37] However, the perioperative mortality rate is high and few patients have shown any survival advantage after such extensive surgery.[36–38] Therefore, the indications for this radical procedure remain limited.

Conclusion

In conclusion, a S4a+S5 hepatic resection, lymph node dissection, and bile duct resection for a complete lymph node dissection of hepatoduodenal ligament are therefore mandatory for pT2 gallbladder carcinoma. Although the clinical course of pT3 or pT4 gallbladder carcinoma remains dismal, long-term survival can be expected by a curative surgery with a tumor-free surgical margin. Survival depends on the ability to achieve a curative resection, including a hepatectomy and lymph node dissection in patients with local extended tumors according to the depth of primary tumor invasion. In addition, a multidisciplinary approach including radiochemotherapy should be considered to prevent either local recurrence or hepatic metastasis after a surgical resection for patients with pT3 or pT4 gallbladder carcinoma.

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Modified Jejunoileal Bypass Surgery with Biliary Diversion for Morbid Obesity and Changes in Liver Histology During Follow-up

Iraj Fazel · Akram Pourshams · Shahin Merat · Roya Hemayati · Masoud Sotoudeh · Reza Malekzadeh

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Abstract
Background and aims Bariatric surgery is the most effective treatment for morbid obesity. The classic procedure, jejunoileal bypass, has many complications including rapid progress of liver disease. The senior author (I.F.) has developed a modification of jejunoileal bypass, which we believe overcomes many of the shortcomings of the classic procedure.
Methods Consecutive patients referring for bariatric surgery were included. A modified jejunoileal bypass in which the defunctionalized limb is eliminated by anastomosing its ends to the gall bladder and cecum was performed. Liver biopsies were taken during operation and at a mean of 16 months later. The patients were followed for 5 years.
Results Forty-three patients were enrolled. The mean value of weight and body mass index (BMI) fell from 128 kg and 46 kg/m^2 before operation to 85 kg and 31 kg/m^2 at 5 years, respectively ($p<0.001$). There was no significant change in the degree of liver steatosis and necroinflammation. The mean liver fibrosis score increased from 0.1 to 0.9 ($p=0.015$). No sign of advanced liver disease was observed during the 5-year follow-up.
Conclusion The modified jejunoileal bypass is very effective in inducing and maintaining weight loss for 5 years and does not lead to hepatic failure or rapid progression of liver disease.

Keyword Obesity · Morbid · Bariatric Surgery · Jejunoileal bypass · Fatty Liver · Liver cirrhosis

Introduction

Obesity is an important health problem whose incidence is increasing rapidly in most parts of the world. Iran is not an exception, and obesity has reached epidemic proportions with more than 25% of the population affected and over 60% overweight.[1,2]

I. Fazel (✉)
Department of Surgery, Medical School,
Shahid Beheshti University of Medical Sciences,
P.O. Box 19395/4655, Tehran, Iran
e-mail: fazel@ams.ac.ir

A. Pourshams · S. Merat · R. Hemayati · M. Sotoudeh · R. Malekzadeh
Digestive Disease Research Center,
Shariati Hospital, Medical Sciences/University of Tehran,
Tehran, Iran

But for morbid obesity, defined as body mass index (BMI) greater than 40 kg/m^2, nonsurgical treatments are rarely effective.[3] Recent studies have shown that the weight loss induced by bariatric surgery normalizes the metabolic abnormalities associated with obesity and hepatic steatosis, including hepatic insulin sensitivity, and decreases the hepatic expression of factors involved in the progression of liver disease.[4,5]

Bariatric surgical procedures can be divided into restrictive and malabsorptive procedures. Combinations of these two techniques are also commonly used. Restrictive procedures are generally simpler to perform, but their effectiveness is not as prominent or as long-lasting as malabsorptive procedures. Malabsorptive procedures are highly effective in reducing weight but carry a considerable risk of metabolic complications.[6–8] Rapid progression of liver disease and even end-stage liver disease has been reported.

The classic malabsorptive procedure is jejunoileal bypass, which is now seldom practiced. The procedure involves dividing the proximal jejunum and anastomosing the proximal end to the distal ileum. In this procedure, the

long-bypassed segment of the jejunum forms a defunctionalized limb occasionally referred to as a "blind loop". Stasis in the defunctionalized limb leads to bacterial overgrowth whose products are directly delivered to the liver via the portal vein. It is believed that this bacterial overgrowth is responsible for many of the serious metabolic complications associated with this procedure. Furthermore, the disturbance of bile metabolism and delivery of excessive bile into the colon is a cause for intractable diarrhea, which is also a frequent complication. Advanced liver disease is one of the grave complications of jejunoileal bypass and reversal of the procedure is indicated if this complication occurs.[9–11] Unfortunately, despite reversal, liver disease may persist or even continue to progress in many patients.[12,13]

There are many modifications of the classic jejunoileal bypass procedure, which attempt to reduce complications mainly by eliminating the defunctionalized limb. Here we present a new modification with a long-term follow-up of patients focusing on liver histology. In our modification, the defunctionalized limb has been eliminated and bile metabolism and recirculation has been improved by anastomosing the redundant jejunal loop between the gall bladder and cecum.

Methods

Patients

Consecutive patients referring for bariatric surgery during a 21-year period from 1982 to 2002 were included. Patients were included if they were between 10 and 65 years old, had a BMI of 35 or greater, had failed at least 6 months of nonsurgical treatment, and were committed to long-term follow-up. Exclusion criteria included active alcohol or substance abuse, advanced concomitant disease severe enough to preclude surgery, lack of support from family, psychological problems including suicidal ideation, and of course, not consenting to the procedure.

Operative Procedure

All procedures were performed by a single surgeon (I.F.). The procedure is a modified jejunoileal bypass (MJB) originally developed by one of the authors (I.F.). The entire length of the small bowel is measured and divided at two points: 15 cm distal to the Treitz ligament, and 45 cm proximal to the ileocecal valve. The bowel continuity is established with end-to-end anastomosis of the proximal 15 cm of the jejunum to the distal 45 cm of the terminal ileum. Finally, the proximal end of the bypassed segment is anastomosed to the gall bladder, and the distal end to the cecum (Fig. 1). Gall stones were removed if found. Appendectomy and liver biopsy was done in all patients.

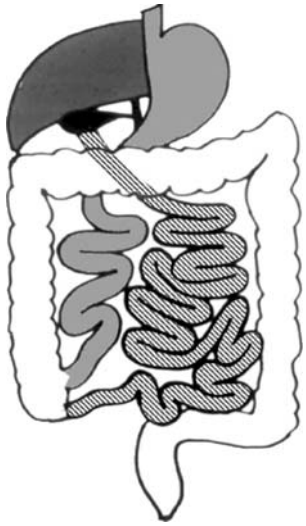

Figure 1 Anatomy of the gut after modified jejunoileal bypass.

Patient Follow-up

Patients were followed by clinical observation and detailed laboratory tests every 3 months. They all received vitamins and mineral supplements. Measurements were recorded and analyzed at post operative months 0, 3, 12, 36, and 60. A second liver biopsy was performed after 1 year or later in patients who agreed.

Pathologic Scoring of Liver Histology

The degree of necroinflammation and fibrosis was scored according to the modified hepatitis activity index (HAI) proposed by Ishak et al. in which necroinflammation is scored between 0 and 18 and hepatic fibrosis is scored between 0 and 614. The degree of steatosis was scored according to the percentage of hepatocytes having fat droplets (Table 1).

All histologic scorings were made by a single pathologist who was unaware of the clinical characteristics of the patients.

Results

Fifty-two patients referred for bariatric surgery during the study period. Nine patients chose other types of operation

Table 1 Scoring of Hepatic Steatosis

Percent of hepatocytes containing fat droplets	Steatosis score
0	0
1–25	1
26–50	2
51–75	3
76–100	4

and 43 patients underwent MJB. The initial characteristics of patients who underwent MJB are given in Table 2.

Surgical Success

One patient, the oldest one in the series, developed mechanical bowel obstruction caused by technical reasons and was reoperated. She developed renal and cardiopulmonary failure and passed away a few days after the reoperation.

Reversal of Procedure

A reversal procedure was performed on six patients (14%) during the follow-up period. One patient developed acute abdominal pain 6 months after MJB. She was found to have a 20-cm-long segmental gangrene of jejunum and as no etiology was found, the bypass was reversed. The reason for reversal in the other patients was severe uncontrollable osteomalacia in one, severe weakness without weight loss in one (in spite of parenteral vitamin supplementation), and severe continuing weight loss (protein-calorie malnutrition) in three. All patients undergoing reversal regained their preoperation weight within 6 months.

Changes in Weight and BMI

The patients were followed up for 5 years. Measurements were made at months 0, 3, 12, 36, and 60. The mean (±SD) value of weight and BMI fell from 128 (±22) kg and 46 (±7) kg/m^2 before operation to 85 (±20) kg and 31 (±7) kg/m^2, respectively, at month 60. The changes in weight and BMI are charted in Fig. 2 and Table 3. The decreases in weight and BMI were significant ($p<0.001$) at each measurement compared to the previous one. The exceptions are measurements at month 60, which show a small increase compared to month 36. This Increase is not statistically significant ($p=0.3$ for both weight and BMI).

Table 2 Initial Characteristics of 43 Patients Undergoing Modified Jejunoileal Bypass

Characteristics	Values
Age (years, mean ± SD)	35±10
Sex (M/F)	11/32
Weight (kg, mean ± SD)	128±22
Height (cm, mean ± SD)	167±8
BMI (kg/m^2, mean ± SD)	46±7
Hgb (g/dL, mean ± SD)	14.5±1.8
AST (IU/L, mean ± SD)	35±21
ALT (IU/L, mean ± SD)	34±18
Fasting blood sugar (mg/dL, mean ± SD)	120±42
Triglycerides (mg/dL, mean ± SD)	167±76
Total cholesterol (mg/dL, mean ± SD)	213±72

Preoperative Liver Histology

Needle biopsy of the liver was performed in all 43 patients undergoing MJB during the procedure. However, only 32 samples were adequate for histologic examination. Only one patient had a fibrosis score (stage) of one, all other patients were zero. There was no significant correlation between HAI and degree of steatosis.

Postoperative Liver Biopsy

Only 14 patients consented to the second liver biopsy. The second biopsy was performed at a mean of 16.0±0.5 months after operation. The fibrosis score was zero in three patients, one in 10 patients, and two in one patient.

For 13 patients, liver biopsies were available before and after operation. Among this group, the mean HAI score had changed from 2.2 to 3.3 ($p=0.37$), the mean steatosis score from 1.5 to 1.6 ($p=0.99$), and the mean fibrosis score from 0.1 to 0.9 ($p=0.015$)

Changes in Alanine Aminotransferase (ALT)

The changes in alanine aminotransferase (ALT) from operation till 60 months postop are charted in Fig. 3. Although a trend for increased ALT is seen at month 3 and thereafter a trend for decreased ALT is seen, none of these trends reach statistical significance.

Metabolic Profile

Twenty percent of patients were hyperglycemic (fetal bovine serum [FBS]>115) before operation and 5 were on oral hypoglycemic agents. All these patients had normal FBS 1 year after operation and were off oral hypoglycemic agents. Thirty percent had hyperlipidemia with elevated triglyceride (TG) and/or low-density lipoprotein (LDL) cholesterol; all showed improvement after surgery.

Clinical Follow-up

All patients who did not undergo reversal operation (36 patients) were satisfied with the operation and the degree of weight loss. The most common postoperative complains were easy fatigability and flatulence.

None of the patients developed nephrolithiasis, cololelithiasis, megaloblastic anemia, or symptoms of the “blind loop syndrome” during the 5-year follow-up.

No liver failure was observed. Prothrombin time, platelet count, and albumin levels were normal in all cases and no splenomegaly was seen. In follow-up endoscopies, no patient had esophageal varices or portal hypertensive gastropathy.

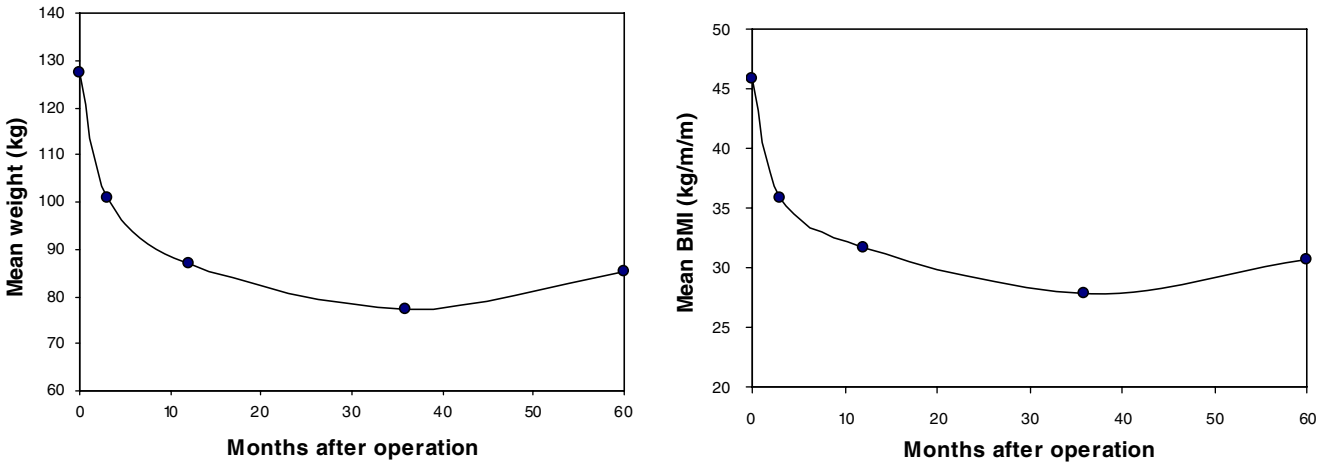

Figure 2 Changes in weight and BMI from operation till 5 years postoperation.

The mean number of bowel movements within 2 months post operation was 6.5 per day. The diarrhea was well controlled in all cases with 1–2 tablets of diphenoxylate per day. The mean number of bowel movements fell to 3–4 per day during further follow-up. Except for the patients who underwent reversal, no patient was admitted because of complications of surgery.

It is interesting to mention that two of our cases (5%) complained of constipation after the operation. They both had a preoperative history of anismus-type constipation also known as outlet obstruction constipation.

Discussion

Further progression of liver disease and even end-stage liver disease has been reported after malabsorptive bariatric surgery. There are various explanations for this finding. Rapid weight loss has always been associated with progression of steatohepatitis.[15–17] Deficiencies of protein and various nutrients such as vitamins and minerals have also been implicated. One of the widely accepted explanations is the implication of stasis and bacterial overgrowth in the defunctionalized limb produced during the operation. Many researchers believe the bacterial overgrowth results in portal endotoxinemia or endogenous ethanol production, which subsequently induces steatosis and steatohepatitis in a liver that may be sensitized by obesity.[18–20]

The classic malabsorptive procedure, jejunoileal bypass, produces a long defunctionalized limb. It is believed that stasis and bacterial overgrowth in this defunctionalized limb accounts for many serious complications seen with this procedure. There have been many modifications of this procedure to overcome this problem.[21] The biliopancreatic diversion (BPD), with or without the duodenal switch procedure, tries to overcome the defunctionalized limb problem by routing biliary and pancreatic secretions through the defunctionalized limb.[22,23] The flow of bile and pancreatic juice prevents stasis and bacterial overgrowth, but the length of intestine where bile, pancreatic juice, and food mix is very small. Thus, malabsorption of protein and lipid soluble vitamins is quite frequent.[24]

Table 3 Changes in Weight and BMI of Patients Undergoing Modified Jejunoileal Bypass

	N	Weight (kg) ± SD	BMI (kg/m^2) ± SD
Before operation	43	128±22	46±7
3 months post operation	37	101±18	36±5
12 months post operation	32	87±17	32±6
36 months post operation	36	77±14	28±5
60 months post operation	30	85±20	31±7

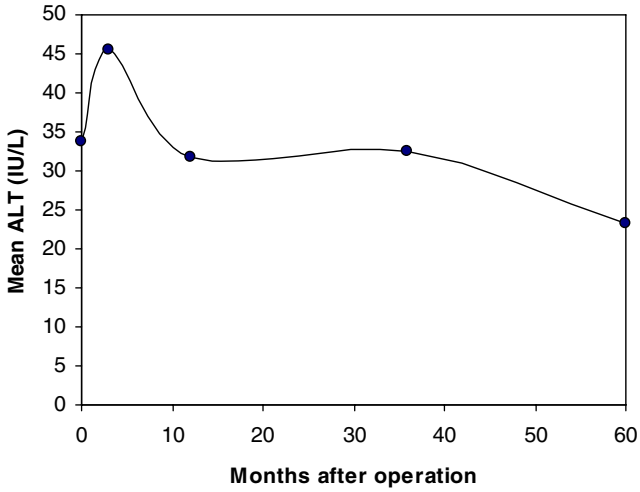

Figure 3 Changes in alanine aminotransferase (ALT) after modified jejunoileal bypass.

The modified bypass procedure we have used in this study connects the proximal end of the defunctionalized limb to the gall bladder and the distal end to the cecum. Thus, flow of bile from gall bladder to cecum through the formerly defunctionalized limb overcomes stasis. Furthermore, because of the wide opening between the gall bladder and the intestine, gall stones, common in the standard procedure, are not formed. Not all the bile secreted from the liver goes through the gall bladder and the extra loop. Some of the bile goes through the common bile duct to the duodenum and is available for the absorption of fat soluble vitamins. Furthermore, the pancreatic secretions are added to food at the normal site and are in contact with food as long as possible in the shortened intestine. The terminal ileum is preserved in the path of digested food, thus B12 deficiency and megaloblastic anemia is eliminated.

Another frequent complication observed in classic jejunoileal bypass is nephrolithiasis, which is believed to be caused by excess absorption of unbound oxalate from the gut. No case of nephrolithiasis was seen in our series. We believe that as bile metabolism and circulation is minimally disturbed in MJB, the amount of oxalurea is less marked.

The procedure is not very complicated to perform and we had only one mortality in our series (2%), which is similar to the mortality reported by others.[25]

In this study, we have shown that the MJB procedure is highly effective in reducing weight and maintaining weight loss for at least 5 years. The changes in liver histology as indicated by HAI and the steatosis score were not significant at a mean of 16 months after operation. Although the degree of fibrosis has significantly increased during this period, the magnitude of this advancement, 0.8 scores, is not large and of uncertain clinical significance. More important is the absence of rapid progression of liver disease or significant liver-related morbidity as seen in subjects undergoing classic jejunoileal bypass. Of course, longer follow-up is required to study the long-term effects on liver histology.

A similar result has been obtained by Stratopoulos et al.,[26] who studied patients losing weight after a restrictive procedure. In this study, 51 patients underwent first and second liver biopsies at an average of 18 months apart. Fibrosis improved in almost 50% of their patients, but 12% had increase in fibrosis. No patient had rapid progression of liver disease.

In a large study by Kral et al.,[27] which included 689 patients, 104 patients underwent a second liver biopsy after a mean of 41 months. Severe fibrosis (stages 3–5) decreased in 28 patients, whereas mild fibrosis (stages 1–2) appeared in 42. In general, however, the degree of fibrosis decreased over time. Even in the 11 patients having cirrhosis in the first biopsy, the fibrosis stage decreased from a mean of 5 to 3 and in seven patients cirrhotic nodules disappeared.

From our study and others it appears that some patients experience worsening of liver fibrosis by bariatric surgery. However, this worsening is generally mild and not clinically significant. In general, even severe liver disease improves. Considering the numerous improvements observed in other obesity comorbidities, the risk of mild increased fibrosis in some patients is well justified.[25]

Conclusion

The MJB is very effective in inducing sustained weight loss without causing clinically significant liver disease. We believe that many of the shortcomings and complications of classic jejunoileal bypass are overcome by this modification and MJB is a viable option for the morbidly obese patient, especially those willing to eat just as well after operation or those failing gastric bypass. Further study on this procedure is warranted.

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Impact of Splenectomy in Patients with Gastric Adenocarcinoma of the Cardia

Chikara Kunisaki · Hirochika Makino · Hirokazu Suwa · Tsutomu Sato · Takashi Oshima · Yasuhiko Nagano · Syoichi Fujii · Hirotoshi Akiyama · Masato Nomura · Yuichi Otsuka · Hidetaka A. Ono · Takashi Kosaka · Ryo Takagawa · Yasushi Ichikawa · Hiroshi Shimada

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Abstract Previous reports have suggested that splenectomy treatment of gastric carcinoma of the cardia results in poor patient outcome, but the reason for this is unclear. This study aimed to clarify the impact of splenectomy for gastric carcinoma patients. A total of 118 patients with gastric carcinoma of the cardia were enrolled in this study. The characteristics of patients with lymph node metastasis at the splenic hilum were determined, and the effects of lymph node dissection or splenectomy on postoperative morbidity, mortality, and pattern of recurrence were evaluated. Advanced tumors were common in patients with lymph node metastasis at the splenic hilum, Siewert type III, greater curvature sites, larger and deeper tumors, multiple metastatic lymph nodes, and high incidences of para-aortic lymph node metastasis frequently observed. The effectiveness of lymph node dissection of the splenic hilum was low and equal to that of dissection of the para-aortic lymph nodes. Postoperative morbidity, as represented by pancreatic fistula, was high following splenectomy or pancreaticosplenectomy, but patient mortality did not occur. Hematogenous metastasis was common, as well as peritoneal metastasis after curative gastrectomy. Splenectomy should be limited in those patients with gastric cardia tumors invading the spleen or with metastatic bulky lymph nodes extending to the spleen.

Keywords Gastric adenocarcinoma · Cardia · Splenectomy · Lymph node dissection

Introduction

Curative gastrectomy combined with complete removal of regional lymph nodes is a promising approach for the treatment of advanced gastric cancer. The conventional surgery for patients with gastric adenocarcinoma of the cardia is total gastrectomy with D2 lymphadenectomy (complete dissection of the first- and second-tier lymph nodes) accompanied by pancreaticosplenectomy or splenectomy in Japan, whereas D2 gastrectomy is not common in western countries. D2 gastrectomy is required for dissection of the lymph nodes at the splenic hilum and along the splenic artery, and numerous studies have examined the surgical efficacy and survival of patients with advanced gastric cancer of the cardia undergoing total gastrectomy and splenectomy.[1–7] Many of these suggest that prophylactic splenctomy is not effective for such patients as it results in poor survival and increased morbidity and mortality.

According to Japanese guidelines,[8] lymph nodes at the splenic hilum are included in the second tier, and therefore removed under D2 gastrectomy, in those patients with gastric cancer in the upper third of the stomach or the middle third to the upper third of the stomach or when the tumor occupies the entire stomach. However, the prophylactic removal of the spleen in these patients is a contentious issue.

C. Kunisaki (✉) · H. Makino · H. Suwa · T. Sato · T. Oshima · Y. Nagano · S. Fujii
Department of Surgery, Gastroenterological Center, Yokohama City University, 4-57, Urafune-cho, Minami-ku, Yokohama 232-0024, Japan
e-mail: s0714@med.yokohama-cu.ac.jp

H. Akiyama · M. Nomura · Y. Otsuka · H. A. Ono · T. Kosaka · R. Takagawa · Y. Ichikawa · H. Shimada
Department Gastrotenterological Surgery, Graduate School of Medicine, Yokohama City University, Yokohama, Japan

At our institution, para-aortic lymph node dissection is routinely employed for advanced gastric cancer in the upper third of the stomach.[9,10] We are therefore in a position to evaluate earlier reports that found a correlation between lymph node metastasis at the splenic hilum (second tier) and along the left-lateral side of the abdominal aorta (third tier) in patients with tumors in the cardia. In this study, the impact of splenectomy for gastric carcinoma of the cardia was assessed by evaluating patient characteristics, survival time, effect of lymph node dissection, and postoperative morbidity and mortality with and without lymph node metastasis at the splenic hilum. We also determined the reasons for the observed survival outcomes.

Materials and Methods

The study group consisted of 118 patients (91 males and 27 females) from the Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Japan, and affiliated institutions. The mean age of the patients ± standard deviation (SD) was 64.2±10.8 years. All subjects were preoperatively confirmed to have gastric adenocarcinoma by analyses of endoscopic biopsy specimens. All patients had tumors deeper than muscularis propria (advanced gastric cancer) and underwent D2 gastrectomy with lymph node dissection between April 1992 and March 2002. No patient received neoadjuvant chemotherapy. Data were retrieved from operative and pathological reports. Follow-up data were obtained from the outpatient clinical database.

The following clinicopathological variables were evaluated by experienced pathologists from each institution: gender (male or female); tumor site (Siewert type II, Siewert type III); macroscopic appearance (superficial, well-defined, ill-defined); tumor diameter (<50, ≥50 to <100, or ≥100 mm); histological type [differentiated (well differentiated, moderately differentiated, or papillary) or undifferentiated (poorly differentiated, signet-ring cell, or mucinous)]; lymph-node metastasis; and depth of invasion [T2 (muscularis propria, subserosa), T3 (serosa penetrated), T4 (adjacent organs)], lymphatic invasion, venous invasion, and stage according to the Japanese classification of gastric carcinoma (JGC).[8] Preoperative imaging studies were used to determine tumor location, macroscopic appearance, tumor diameter, depth of invasion, and lymph-node metastasis. Imaging studies were routinely performed following an upper gastrointestinal barium meal, endoscopic examination, and computed tomography (CT) scanning. Ultrasonography (US) of the abdomen and endoscopic US were also performed in 92 (78.0%) and 42 patients (35.6%), respectively. Tumor diameter and depth of invasion were measured by both endoscopic examination and a barium-swallow study. Depth of invasion was also identified by endoscopic US in some patients. The clinicopathological terminology in this article principally follows that of the JGC.

The Japanese Gastric Cancer Association has standardized lymph-node dissections for gastric cancer. In this study, D2 gastrectomy (complete dissection of the first- and second-tier lymph nodes) was performed in 42 patients, and superextended D3 gastrectomy (complete dissection of the first-, second-, and third-tier lymph nodes) was performed in the remaining 76 patients suspected of having metastatic para-aortic lymph nodes (stage IV) and tumors that had invaded deeper than the subserosa without any other distant metastasis, as assessed by preoperative imaging tools. We performed D3 gastrectomy only for symptomatic patients for whom curative resection would be expected by such superextended lymphadenectomy. All procedures were performed in accordance with the JGC. Of the 118 patients receiving curative gastrectomy, 68 underwent pancreaticosplenectomy and 50 underwent splenectomy. Prophylactic pancreaticosplenectomy was employed before December 2001, and prophylactic splenectomy after January 2002.

Adjuvant chemotherapy (oral 5-fluoropyrimidine) was administered to 27 of 40 patients (67.5%) with stage III cancer and in 14 of 16 patients (87.5%) with stage IV cancer. Patient follow-ups were carried out at the outpatient department according to our standard protocol (every 8–12 weeks for at least 5 years). At these appointments, a medical interview was conducted by the physician to review the progress and health of the patient. Subjects also underwent hematological examinations every 3 months, US or CT scans every 6 months, and chest radiography and endoscopic examinations every year. After 5 years, the follow-ups were continued on an annual basis. The mean follow-up duration was 57.2±44.5 months for all registered patients.

Efficacy of Lymph Node Dissection

We calculated the index for the efficacy of lymph node dissection as the incidence of metastasis to a region multiplied by the 5-year survival rate of patients with metastasis to that region and divided by 100.[11]

Definition of Pancreatic Fistula

Pancreatic fistula is defined if pus-filled dirty discharge with amylase concentration ≥1,000 U/L drained from the drain placed the peripancreatic stump for more than 7 days.[12]

Statistical Analysis

Data were analyzed using the SPSS statistical software program version 10.0 for Windows (SPSS, Chicago, IL,

Table 1 Characteristics of Patients Receiving Curative Gastrectomy With and Without Lymph Node Metastasis at the Splenic Hilum

	Lymph node metastasis at the splenic hilum		
	Positive (n=6)	Negative (n=112)	p value
Gender (male/female)	4/2	87/25	0.5316
Age (years) (mean ± SD)	56.3±10.7	64.7±10.7	0.0661
Tumor site			0.0088
Siewert type II	2	89	
Siewert type III	4	23	
Localization			0.0140
Lesser curvature predominant	2	87	
Greater curvature predominant	4	25	
Macroscopic appearance			0.9785
Superficial	2	42	
Well-defined	3	53	
Ill-defined	1	17	
Tumor diameter (mm)			0.0118
<50	1	45	
≥50 to <100	2	56	
≥100	3	11	
Depth of invasion			0.0822
T2 (MP, SS)	1	70	
T3 (SE)	4	34	
T4 (SI)	1	8	
Histological type			0.6760
Differentiated	4	65	
Undifferentiated	2	47	
Number of metastatic			0.0133
Lymph nodes	13.7±7.0	4.1±9.2	
Metastasis to lymph nodes along the distal splenic artery			0.1208
Presence	1	4	
Metastasis to para-aortic lymph nodes			0.0017
Presence	3	10	
Lymphatic invasion			0.2861
Presence	6	94	
Venous invasion			0.4031
Presence	5	75	
Stage			0.0006
IB/II/IIIA/IIIB/IV	0/0/0/3/3	21/41/27/10/13	

MP = muscularis propria, SS = subserosa, SE = serosa exposed, SI = adjacent organs

USA). Patient characteristics were compared using the two-tailed Fisher exact test or the Chi-square test with Yates correction. Quantitative variables were compared using the Student's t test and expressed as means ± SD. Survival curves were constructed using the Kaplan–Meier method and compared using the log-rank test. The probability (p) values were considered to be statistically significant at the <0.05 level.

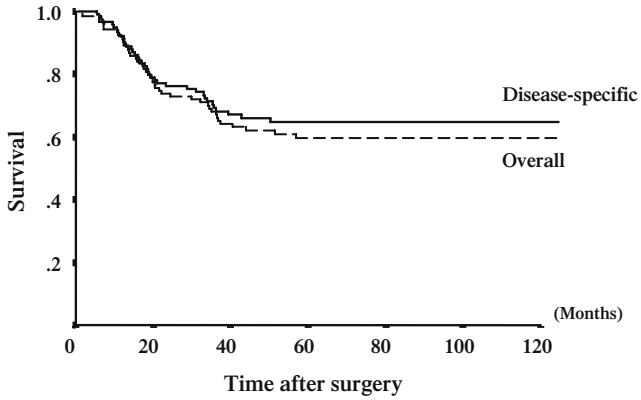

Figure 1 Overall and disease-specific survival.

Results

Characteristics of Patients with and without Lymph Node Metastasis at the Splenic Hilum

Focusing on the 131 patients who underwent curative resection, those with metastatic lymph nodes at the splenic hilum had more Siewert type III tumors, were more likely to locate at the greater curvature side, have larger tumors, suffered more metastasis to the para-aortic lymph nodes, and developed more advanced tumors than those patients who did not have metastatic lymph nodes at the splenic hilum (Table 1).

Patient Survival

In all 118 patients receiving curative gastrectomy, the 5-year overall survival was 60.3%, and disease-specific survival rate was 64.8% (Fig. 1). According to stage, the 5-year disease-specific survival rate was 89.1% (21 in stage IB), 80.2% (41) in stage II, 53.7% (40) in stage IIIA and B, and 25.0% (16) in stage IV (Fig. 2).

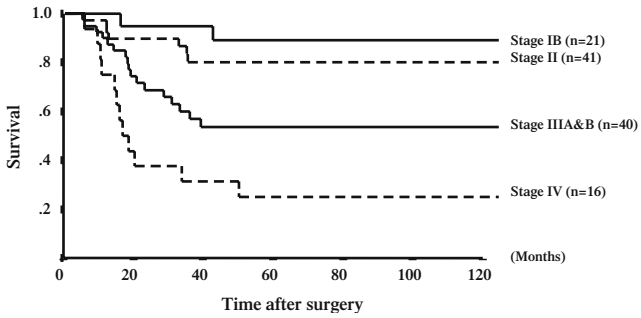

Figure 2 Patient survival according to tumor stage.

Table 2 Effect of Lymph Node Dissection According to the Incidence of Lymph Node Metastasis and 5-year Survival Rate

Site of lymph node	Incidence of lymph node metastasis	5-year disease-specific survival rate (%)	Index[a]
Right cardial	0.350	39.5	13.8
Left cardial	0.262	54.7	14.3
Along the lesser curvature	0.514	60.9	31.3
Along the short gastric vessels	0.061	80.0	4.87
Along the left gastroepiploic vessels	0.107	20.8	2.23
Along the right gastroepiploic vessels	0.092	48.6	4.46
Suprapyloric	0.087	50.0	4.35
Infrapyloric	0.031	0	–
Along the left gastric artery	0.200	34.3	6.86
Along the common hepatic artery	0.034	0	–
Around the celiac artery	0.130	33.3	4.34
Splenic hilum	0.073	33.3	2.44
Along the splenic artery (proximal side)	0.094	62.5	5.88
Along the splenic artery (distal side)	0.178	60.0	10.7
Lateral side of the abdominal aorta from the level of the celiac trunk to the inferior mesenteric artery	0.121	18.5	2.24

[a]Incidence of lymph node metastasis × 5-year disease-specific survival rate

Efficacy of Lymph Node Dissection

The highest efficacy index was obtained for dissection of lymph nodes along the lesser curvature, followed, in decreasing order, by the cardial lymph nodes and the lymph nodes along the splenic, left gastric arteries and short gastric vessels. Dissection of the left lateral para-aortic lymph nodes and lymph nodes at the splenic hilum did not have a high efficacy value (Table 2).

Postoperative Morbidity and Mortality

Of the 118 patients who underwent splenectomy or pancreticosplenectomy, pancreatic fistula was diagnosed in eight individuals (6.8%). Of these patients, an abscess cavity or enhancement of the pancreatic duct was seen in two patients. Anastomotic leakage was observed in seven patients, lymphorrhea in two patients, and respiratory complications in one patient (Table 3).

Cause of Death

After curative gastrectomy, recurrence was observed in 39 patients (33.1%) (17 hematogenous, 17 peritoneal, 5 lymphogenous). Mean follow-up time was 23.3±20.5 months and mean time to recurrence was 14.3±17.4 months in patients following recurrence. Otherwise, two patients died of other cancers (one from lung cancer, one from leukemia). Two patients died of cardiovascular disease, including an 88-year-old man who died from endocarditis within 30 days of the curative gastrectomy; one patient died from respiratory failure and another died in a traffic accident.

Discussion

The findings of this study suggest that splenectomy aimed at complete removal of the lymph nodes at the splenic hilum has little survival benefit in patients with tumors of the gastric cardia. This is probably due to the fact that patients with metastatic lymph nodes at the splenic hilum also have metastatic lymph nodes in the far regional lymph node areas such as the para-aortic lymph node. In addition, the incidence of morbidity such as pancreatic fistula after splenectomy, including pancreaticosplenectomy, is high. Therefore, we propose that splenectomy should be limited in patients with gastric cardia tumors or metastatic bulky lymph nodes extending to the spleen. Although the number of patients with metastatic lymph nodes at the splenic hilum is so small in this retrospective study, these results might suggest strategy for gastric cardia tumors.

Table 3 Postoperative Morbidity

Variable	Number of patients (%)
Pancreatic fistula	8 (6.8)
Anastomotic leakage	7 (5.9)
Lymphorrhea	2 (1.7)
Respiratory	1 (0.8)

In patients with lymph node metastasis at the splenic hilum, Siewert type III tumors and tumors located in the greater curvature were frequently observed. These findings may, however, depend on the regional lymphatic drainage from each site of the cardia.[13,14] Moreover, patients with advanced-stage tumors that were larger or deeper suffered from more lymph node metastasis in the splenic hilum than patients with tumors at an earlier stage. This supports the results of an earlier study in which patients with stage III or IV tumors developed lymph node metastasis in the splenic hilum.[15]

In our previous study into lymphatic flow from the stomach in patients with gastric cancer of the cardia, we reported that the high incidence of lymphatic flow to the left lateral side of the abdominal aorta equaled that to the splenic hilum.[16] As shown in the current study, there was a significant correlation of the incidence of lymph node metastasis in the splenic hilum and along the abdominal aorta. Patients with lymph node metastases in the splenic hilum suffered extensive metastases that also included the para-aortic lymph nodes. As a result, surgical outcomes are likely to be poor even after splenectomy or pancreaticosplenectomy, which contrasts with a previous report that argued for splenectomy to be performed in high-risk patients who have macroscopic lymph node involvement at the splenic hilum.[17]

Many earlier studies found no surgical benefit in lymph node dissection of the splenic hilum for tumors in the gastric cardia, but they did observe increased morbidity following splenectomy or pancreaticosplenectomy.[18–21] A prospective randomized clinical trial of patients with gastric adenocarcinoma of the cardia found no improvements in survival if splenectomy was performed after total gastrectomy. Moreover, in a subgroup analysis of two prospective multicenter randomized controlled trials comparing D1 gastrectomy and D2 gastrectomy, postoperative morbidity and mortality offset long-term survival in patients undergoing D2 gastrectomy with pancreaticosplenectomy.[22,23]

Patients receiving pancreaticosplenectomy or splenectomy sometimes suffer fatal postoperative morbidities such as pancreatic fistula. In the current study, we did not encounter any mortality, although the incidence of pancreatic fistula was high. However, the definition of pancreatic fistula is notably different between studies. In this study, we adopted the same definition with a previous randomized Japanese trial, and the incidence of pancreatic fistula in this study was equal to that of the previous report.[12]

This is the first study to discuss the impact of splenectomy from the viewpoint of the efficacy of lymph node dissection, which was calculated using the incidence of lymph node metastasis and 5-year survival rate.[11] As such, the index of lymph nodes at the splenic hilum was equal to that of lymph nodes along the lateral side of the abdominal aorta. As the para-aortic lymph nodes are defined as the third-tier group (N3) by the JGC, classification of lymph nodes at the splenic hilum may also be changed to N3. In our previous study, D3 gastrectomy did not contribute to long-term patient survival in all registered patients and only showed surgical benefits in patients with median-sized tumors (50–100 mm) or tumors of stage pN1 (number of metastatic lymph nodes: 1–6).[10] This suggests that even superextended D3 gastrectomy including splenectomy is not an adequate surgical treatment for larger tumors and multiple metastatic lymph nodes, which are particularly common in patients with lymph node metastasis at the splenic hilum.

In the current study, hematogenous recurrence was most frequently observed after curative gastrectomy in patients with tumors in the cardia. In general, peritoneal metastasis was the most common pattern of recurrence in patients with advanced gastric cancer, which supports our previous findings in a different population.[24] In patients with advanced and pN1 tumors, location of tumor (the upper third of the stomach) and venous invasion independently predicted hematogenous metastasis, for which an effective treatment is lacking. Although it is unclear why venous invasion and hematogenous metastasis are so frequent in these patients, anatomical differences between the cardia and the more distal region of the stomach might contribute to these results.

In conclusion, gastric cardia tumors with lymph node metastasis at the splenic hilum may not be suitable for surgical treatment, particularly because morbidity after total gastrectomy combined with splenectomy is high. Moreover, D3 gastrectomy should not be indicated for patients with gastric cardia tumors. Therefore, we propose that splenectomy should be limited in patients with gastric cardia tumors invading the spleen or in those with metastatic bulky lymph nodes extending to the spleen.

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Passage of "Colonic Cast" after Colorectal Surgery: Report of Four Cases and Review of the Literature

Sabri Erguney · Nihat Yavuz · Yeliz E. Ersoy · Serkan Teksoz · Dogan Selcuk · Gunduz Ogut · Gulen Dogusoy · Olcay Alver

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Abstract Anal passage of a full-thickness infarcted colonic segment (so-called "cast") not accompanied by any features of acute peritonitis is a very rare occurrence and may be the main advertising manifestation of acute colonic ischemia. Most of the reported cases of acute colonic ischemia are secondary to abdominal aortic aneurysms and ensuing inferior mesenteric artery thrombosis or to the repair of these aneurysms. The preceding events causing ischemia in other cases are Hartmann reversal, rectal resection and colonic J-pouch construction, and acute pancreatitis. In this article we present our experience on four cases of colonic cast passage, all of which developed subsequent to colorectal resection. Three of these casts are supposed to be mucosal and one is transmural. Generally, surgery is the rule and consists of the resection of the concerned ischemic segment. Every clinician should be aware of this form of presentation of bowel ischemia, not only following aneurysm surgery but also in the postoperative course of colorectal surgery.

Keywords Colonic cast · Colorectal surgery · Colonic ischemia

Introduction

Anal passage of a full-thickness infarcted colonic segment not accompanied by any features of acute peritonitis is a very rare occurrence. Necrosis, dissection, and passage of only inner layers of the bowel reflect a less severe ischemia. The discharge of such a necrotic bowel segment (so-called "cast") may be the main advertising manifestation of acute colonic ischemia. We present herein our experience on four cases of colonic cast passage, all of which developed subsequent to colorectal resection.

S. Erguney · N. Yavuz · Y. E. Ersoy (✉) · S. Teksoz · O. Alver
General Surgery Department, Cerrahpasa Medical Faculty,
Istanbul University,
Istanbul, Turkey
e-mail: yelizemineersoy@yahoo.com

D. Selcuk · G. Ogut
Radiology Department, Cerrahpasa Medical Faculty,
Istanbul University,
Istanbul, Turkey

G. Dogusoy
Pathology Department, Cerrahpasa Medical Faculty,
Istanbul University,
Istanbul, Turkey

Case 1

A 47-year-old man with a history of type II diabetes mellitus for the last 2 years was admitted with a rectal tumor 8 cm proximal to the anal verge. He underwent a low anterior resection combined with total mesorectal excision, and a low colorectal anastomosis was performed by Griffen–Knight technique using TA 55 Roticulator® and EEA 31® stapler. The inferior mesenteric artery (IMA) was ligated at its origin before it gave off the left colic branch. During the mobilization of the descending colon, a minor bleeding controlled by suture-ligation occurred in the mesocolon, so a complementary diverting right transverse loop colostomy to secure the anastomosis was estimated to be necessary. Pathologic examination was reported as T2N1 adenocarcinoma. The postoperative period was completely uneventful. The patient received chemoradiation therapy subsequently and digital dilatations were performed for a few times to resolve his slight anastomotic stricture. Six

months after the first operation, his colostomy was closed. Two months later, while he was only complaining of frequent stools presumably due to the anastomotic stricture and also to the loss of rectal ampulla, he described the passage of a 7-cm-long necrotic tissue (cast) per rectum. As he was far away from our clinic, the cast could neither be seen nor examined, and his colonoscopy report was learned 3 weeks later, which revealed no peculiarity except a slight anastomotic stricture treated by balloon dilatation. He is still doing well 13 months after this colonoscopic control.

Case 2

A 49-year-old male patient was hospitalized for a rectal tumor 15 cm proximal to the anal verge and a synchroneous tumor in his right kidney. He had a history of right hemicolectomy for colon cancer performed 20 years ago. After right radical nephrectomy and anterior rectal resection, colorectal anastomosis was performed manually. As the patency of his middle colic artery (whether tied or not during the previous right hemicolectomy) was unknown, the left colic branch of the IMA was preserved. Pathologic examination of the specimen revealed T2N0 adenocarcinoma developed on a villous adenoma and a renal cell carcinoma with no peripheral invasion. For 2 weeks, he suffered from a fever of unknown origin and diarrhea. Stool cultures did not show any abnormal growth and Clostridium toxin A and B were both negative. At the end of this period, he passed a 25-cm-long necrotic tube, which was proven histopathologically to be "a necrotic tissue associated with intense bacterial colonization." After the diarrhea and fever regressed, the patient was discharged. One month later, he presented to our Emergency Unit with complete colonic obstruction. Relaparotomy demonstrated that the remaining proximal left colon 25 cm in length had become stiff, the wall had thickened and contracted, and the small bowel proximal to it had extremely dilated. Because the colorectal anastomosis was completely obstructed, the diseased colonic segment was resected. Since the distal (rectal) stump was unidentifiable, it could not be closed. A Brooke ileostomy was carried out and an antiadhesive film (Sepramesh®) was placed on the rectal stump, which was left open. Histopathological examination of the specimen demonstrated "subacute ischemic colitis with mucosal and partially mural infarction." Five months later, he has been reoperated on to reestablish the intestinal continuity: the ileostomy was taken down and the ileum was anastomosed to the posterior wall of the rectal remnant by the use of a transanally applied EEA 28® stapler. Four months later, he again presented with acute anastomotic obstruction that could not be passed by colonoscope. A balloon dilatation was successfully performed and repeated three times in a period of 5 months, and he has been in good health since his last dilatation performed 27 months ago.

Case 3

A 70-year-old female was admitted to our hospital suffering from a right colon carcinoma with a 1-cm metastasis to hepatic segment 7. She had no history of previous illnesses or comorbidities. Laparo-assisted right hemicolectomy and hepatic metastasectomy was performed. Ileocolic, right colic and the right branch of the middle colic artery were transected using Ligasure®, and functional end-to-end ileotransverse anastomosis was done using a GIA 55® stapler. Pathological examination revealed a T2N1M1 adenocarcinoma. During the postoperative period, she developed distention, diarrhea, and mild pyrexia. Stool cultures, toxin A and B, all remained negative. Three weeks after the operation, she passed a 10-cm-long cast per rectum, the microscopic appearance of which was transmural necrosis of the colon. As all symptoms subsided, she was discharged after a venous port application for the subsequent chemotherapy. Five months later, she was hospitalized for abdominal distention and colicky abdominal pain. Barium enema revealed a stricture at the anastomotic site, and an abdominal CT scan showed dilated ileal loops and a hypodense lesion (metastasis) located in segment 5 of the liver. She was reoperated on and a limited ileocolic resection centered on the anastomosis was performed; afterwards, the continuity was established by a new side-to-side ileocolic anastomosis. Her hepatic metastasis was also resected. Pathological examination of the removed ileocolic segment revealed fibro-inflammatory adhesions and acute ischemic enteritis on the ileal side of the anastomosis. Her postoperative course was uneventful and she remains well 11 months later.

Case 4

A 71-year-old male was hospitalized for a sigmoid colon tumor. He had an antecedant of hypertension and moderate COPD. He was operated on and a sigmoid colon resection was performed after the IMA was ligated at its origin. An end-to-end colorectal anastomosis was carried out manually. Pathological examination demonstrated a T3NO tumor. Postoperatively, he developed an unexplained abdominal distention, diarrhea, and hyperpyrexia, without any associated acute abdominal sign. A right basal atelectasis was treated conservatively. One month after his operation, he passed a 40-cm cast per rectum (Fig. 1), which was reported histopathologically to be a transmural necrosis of the colon (Fig. 2). The clinical picture resolved and the patient was discharged. One week later he came back with hyperpyrexia and diarrhea. He presented a mild leucocytosis (WBC:

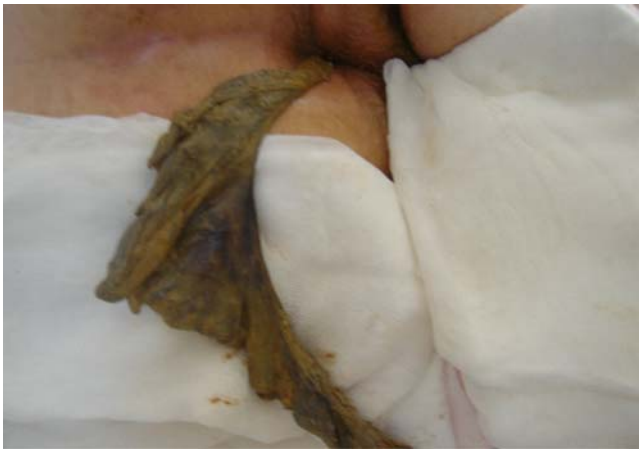

Figure 1 Transanal expulsion of the colonic cast.

10,000/mm^3), a high but (when compared to past values) decreased C-reactive protein (CRP) level (25 mg/L). Stool was negative for culture and toxin A and B. Colonoscopy showed complete destruction of the mucosa and scattered pseudopolips beginning at the level of the slightly narrowed anastomosis extending up to the level of left colonic flexura. Pathological examination of the colonoscopic biopsies revealed "ulcer basement at the depth of submucosa covered by granulation tissue and exudative material." Therefore, oral metronidazole was prescribed. After his clinical status improved he was discharged once again. Two months later, while he was still suffering from abdominal distention and diarrhea, a colonoscopy was attempted, but the anastomosis could not be overpassed and barium enema showed a nearly complete obstruction at this level with a long string-like narrowing above (Fig. 3). The abdominal CT and CT-angiography showed the narrowed ischemic colonic segment and underlying vascular interruption (Figs. 4 and 5). The patient was then reoperated: The descending colon was

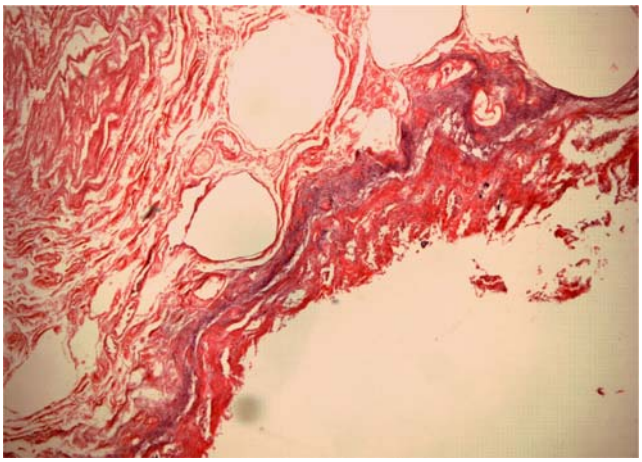

Figure 2 Microscopic appearance of the expulsed cast (×40). Transmural necrosis affecting all layers of the bowel wall, namely, the mucosa, submucosa, and muscularis propria, can be observed.

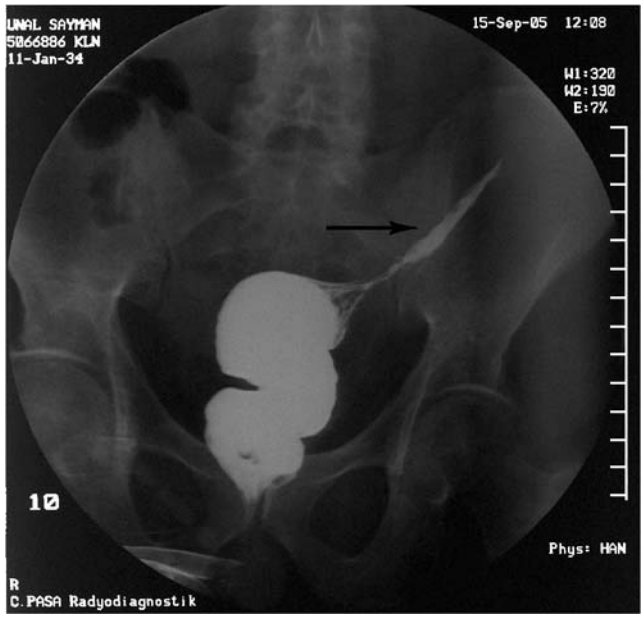

Figure 3 Barium enema showing tight stricture (*arrow*) beginning at the level of the rectosigmoideal junction.

unrecognizable from the level of the colorectal anastomosis up to the splenic flexura. The bed of the discharged colonic segment was unseparable from the posterolateral abdominal wall (Fig. 6). Just proximal to it, the whole transverse and ascending colon and the small bowel was extremely dilated. A subtotal colectomy was performed, and as the destroyed descending colon was unseparable from the neighboring posterolateral abdominal wall, it was left in place and simply its inner surface was curetted. Pathological examination of the distal margin of the specimen (Fig. 7), which is the transitional zone between healthy but dilated transverse

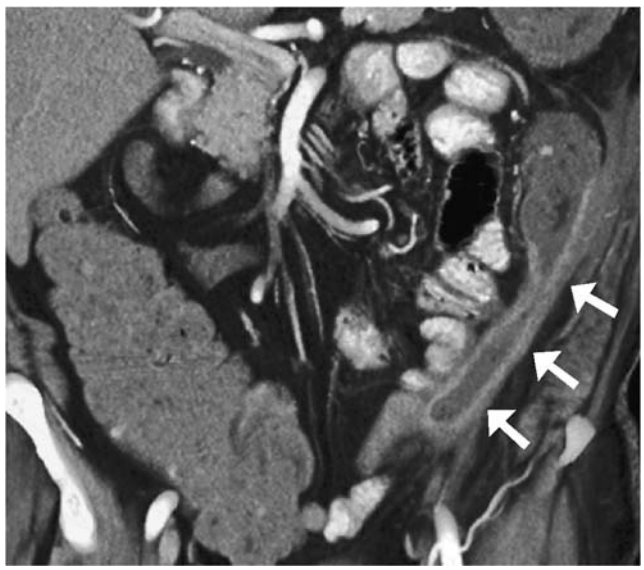

Figure 4 Coronal multiplanar reformatted multidetector CT image shows luminal narrowing, mural thickening, and contrast fixation in a long segment of the descending colon (*arrows*), indicating ischemia.

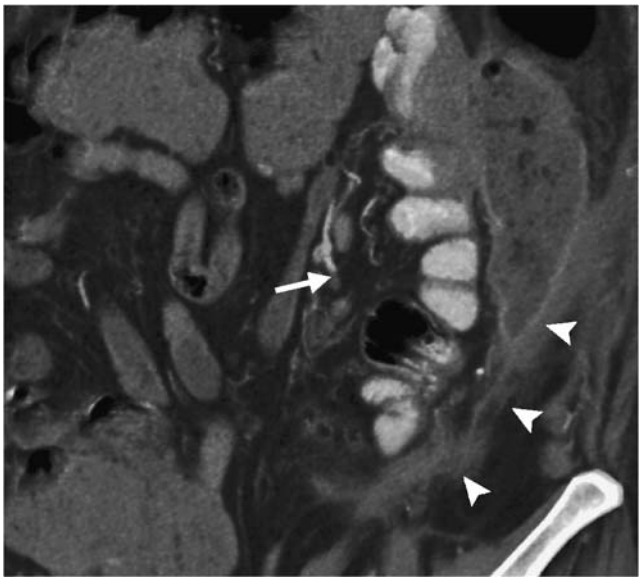

Figure 5 Coronal maximum intensity projection multidetector CT image reveals cut-off of a vascular branch deriving from a collateral of the middle colic artery, which is indicative of thrombosis (*arrow*). Note ischemic colonic segment (*arrowheads*) in the territory of this vessel.

colon and the discharged descending colon, showed acute ischemia associated with hemorrhagic transmural infarction (Fig. 8). A side-to-end ileorectal anastomosis established the intestinal continuity, and the patient has been symptom-free since this second operation performed 12 months ago.

Discussion

Acute colonic ischemia comprises a spectrum ranging from reversible colopathy (submucosal or intramural hemorrhage) to fulminant, universal colitis and frank gangrene.[1] Marston[2] classified them as transient, stricturing, and gangrenous

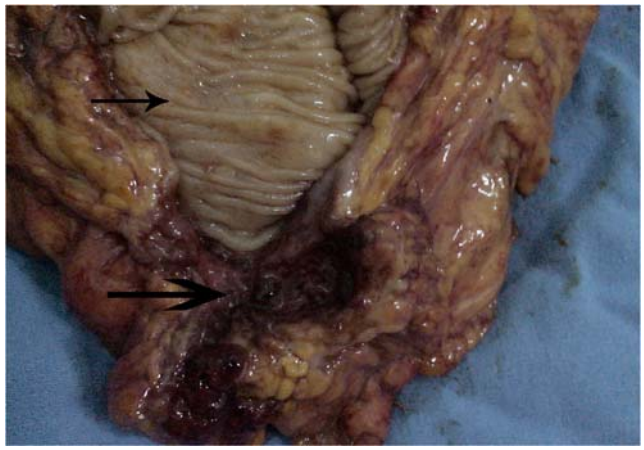

Figure 7 The photograph of the distal margin of the specimen showing the transitional zone (*thick arrow*) between the dilated transverse colon (*thin arrow*) and the destroyed descending colon (shown in Fig. 6).

forms according to the severity of ischemia. In the transient, mild form seen in more than 50% of all patients, only the mucosa and submucosa are affected. The symptoms are resolved with conservative measures and no long-term sequelae develop. The intermediate form involves a variable thickness of the muscularis propria in addition and leads to either stricturing of the involved segment or persistent colitis with symptoms of chronic obstruction. In the worst form of ischemia, which affects 10% to 20% of all patients, transmural gangrenous necrosis occurs, leading to peritonitis necessitating prompt surgical intervention.[3] This latter form universally presents with an abdominal catastrophe, but few patients have been reported to pass the infarcted colonic segment transanally, without such a devastating clinical picture, i.e., the development of peritonitis.[4–11]

This particular form of acute necrotizing ischemia, first reported by Speakman[5] in 1984, presents initially in a

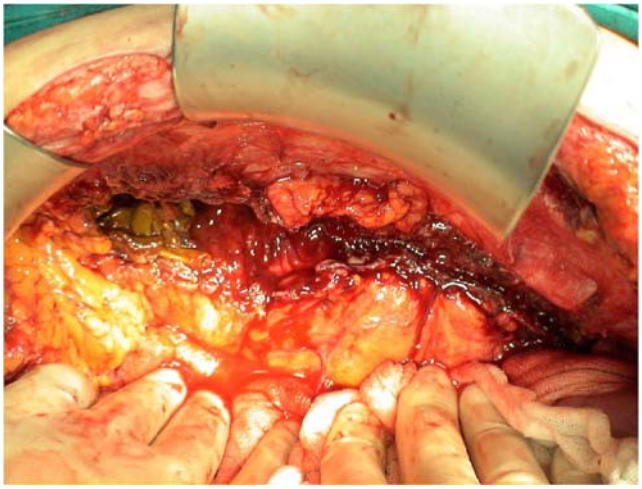

Figure 6 Intraoperative appearance of the "inflammatory tunnel" representing the bed of the discharged descending colon.

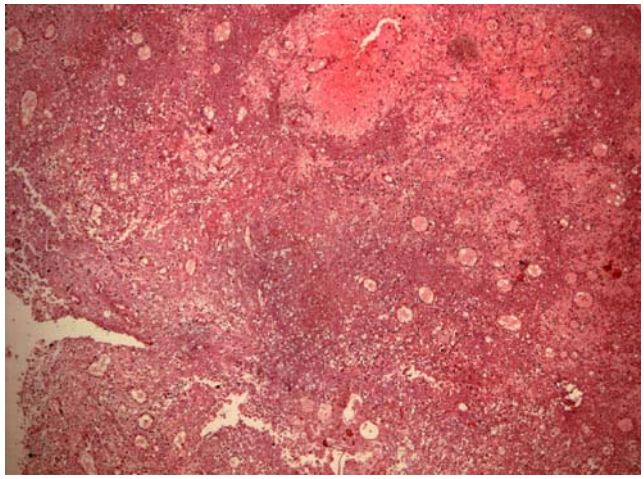

Figure 8 Microscopic view of the transitional zone (distal margin of the specimen corresponding to the most proximal part of the ischemic descending colon) demonstrating transmural hemorrhagic infarction (×40).

clinically insidious fashion, with systemic signs suggesting an inflammatory syndrome (anorexia, mild fever, tachycardia, weight loss, elevated WBC count, erythrocyte sedimentation rate, CRP, etc.), abdominal pain or simply discomfort, abdominal distention, and watery or bloody diarrhea. This period lasts for a few weeks and culminates pathognomonically in the transanal discharge of the necrotic colonic segment, seeming like "the airbladder of a fish,"[12] the so-called cast.

Most of these reported cases of acute colonic ischemia are secondary to abdominal aortic aneurysms and ensuing IMA thrombosis[6,7] or to the repair of these aneurysms.[5,8,10,11] The preceding event causing ischemia in the other three cases are Hartmann reversal,[4] rectal resection and colonic J-pouch construction,[9] and acute pancreatitis.[10] In all of these reported cases, the affected ischemic segment is situated in the left colon, whereas the rectum is utmost spared. The occurrence in the left colon may be explained by the fact that, in most of these cases, the triggering event directly concerned the IMA or its territory, i.e., the left colon.[4–7,9–11] However, in spite of the insufficiency of the marginal artery in the right colon reaching nearly 50% of the population and, moreover, a recent trend of increase in the occurrence of right-sided ischemia reflecting an increased incidence of nonocclusive ischemia, the absence of reports on right-sided casts in the literature is noteworthy.[3] The preservation of the rectum, at least of its outer layer, may be explained by the knowledge that its muscular layer is supplied by the middle rectal artery derived from the internal iliac artery.[2,7,13]

Case 4 of this article is the 10th reported example of such a transmural necrosis without the development of acute peritonitis. The ischemia in this case was presumably due to the sacrifice of the IMA while preserving the proximal segment of the left colon (Table 1).

A point that merits attention is that some case reports on this subject seem suspicious about the depth of ischemic necrosis. Sakanoue et al.[9] describe an "easily resected J-pouch," although it was "disintegrated and replaced by a tube of granulation tissue." Although in the literature this case is included in the "full thickness" group based solely on the examination of the cast, this does not seem true. In our experience, resection of segments with even partial thickness necrosis was always difficult, and in case 4, with a full-thickness cast of this series, the concerned segment consisted only of a "track" unseparable from neighboring structures, similar to descriptions made in the articles of Gregory and Barrett,[6] Phillips and Armitage,[7] and Beattie et al.[11]

The specimen of one patient in the article of Ardigo et al.[10] is macroscopically described as "thickened and revealing corkscrew-type serosal vessels." This description is in accordance with the picturesquely described "eel in rigor mortis"[2] similar to our observation in case 2 of this series, which corresponds to a partial-thickness cast, as confirmed by the pathological examination of the specimen. We suggest that the depth of the ischemic necrosis must be relied on the pathological examination of the specimen and not solely on that of the "cast." The case presented by Beattie et al.[11] and case 4 of the present study are examples to such an attitude. The presence of infarcted muscularis propria on the cast is not proof of full-thickness necrosis of the bowel wall. In fact, histological examination of the cast expulsed by case 3 of our study showed "transmural necrosis," whereas the examination of the specimen consisting of the ischemic ileocolic segment revealed only "acute ischemic enteritis on the ileal side of the anastomosis" but not an unidentifiable bowel wall.

In the literature, the treatment of acute large bowel ischemia presenting with the passage of a full-thickness cast has always been surgical, except in one patient reported by Ardigo et al.,[10] who probably might have required surgery if he had lived longer. Indications for surgery are mainly intractable diarrhea[4,7,10,11] and stricture.[4,9]

The initially reported cases of "colon cast" are marked by prompt interventions following the cast passage, presumably due to an enthusiastic desire to discover the pathology or to the fear of missing a lethal condition.[5,6] However, the need for urgent surgery was really arguable in two cases because of the development of generalized peritonitis and sepsis owing to free perforation of the "inflammatory tunnel" in one[11] and because of occurrence of an acute abdomen in the other.[8] Later, this interval lengthened up to 11 months depending on the patient's symptomatology and willingness to undergo surgery.[4] Foley et al.[4] recommend a limited period of conservative management during which the bowel is placed at rest and broad-spectrum antibiotics covering the fecal flora are prescribed to minimize the consequences of ischemia.[1–3] Colonoscopic dilatations may defer the intervention. However, the danger of performing dilatations, even with simple colonoscopy, in such a critical ground must be emphasized.[1,4,10]

Surgery, soon or late, is the rule and consists of the resection of the concerned ischemic segment. This intervention may be very atypical and cannot be qualified as a "true resection." As seen in case 4 of this series, the "colon's track"(inflammatory tunnel) may be partially or totally left in situ and may be laid open.[6,10,11] The rectal stump may too be left open, as it was in case 2 of this series.[6] Following resection, the decision of making a primary anastomosis or not must depend on general status and local abdominal conditions of the patient. It seems that the more the surgery is deferred and the more it becomes elective, the more the opportunity of performing primary anastomosis increases.

The expulsed "cast" may be composed only of necrotic mucosa more or less submucosa. This situation represents a less severe form of acute colonic ischemia, and it must be encountered far more then reported. Cases 2 and 3 and

Table 1 List of the Clinical and Pathologic Features of Patients who Passed a Colonic Cast

Author(ref)	Age, gender	Predisposing condition	Ischemic bowel segment, cast lenght	Depth of necrosis	Time to cast discharge	Time of intervention	Intervention
Speakman[5]	62, M	AAA repair	Sigmoid, NR	Full thickness	16 days	Immediate	Left hemicolectomy, Hartmann
Gregory[6]	64, M	AAA	Left colon, 96 cm	Full thickness	21 days	15 hours later	Transverse end colostomy (distal segment laid open)
Phillips[7]	55, M	AAA+IMA thrombosis	Left colon, rectal mucosa, 68 cm	Full thickness	14 days	2 months later	Total colectomy+ileoanal anastomosis+ileostomy
Sado[8]	67, M	AAA repair	Small and large bowel, 25 cm	Full thickness	?	?	Exploratory laparotomy
Sakanoue[9]	52, M	LAR(IMA preserved) +colonic J pouch-anal anastomosis	colonic pouch (sigmoid) NR	Full thickness	12 days	3 months later	Pouch resection, +coloanal anastomosis+ileostomy
Yoshiji [12]	76, F	Chronic constipation+colonic stenosis+atherosclerosis	Sigmoid, 15 cm	Mucosa-submucosa	2 days	No intervention	Conservative treatment, favourable
Ardigo[10]	85, M	AAA repair	Distal sigmoid-rectum, 26 cm	Full thickness	15 days	3 weeks later	Colostomy+Hartmann
Ardigo[10]	74, M	Acute pancreatitis	Sigmoid, 30 cm	Full thickness	19 days	Died before intervention	
Tsujimoto[14]	71, M	Use of an EMS device+atrial fibrillation+atherosclerosis	Sigmoid +rectum, NR	Mucosa-submucosa	2 days	No intervention	Conservative treatment, favorable
Foley[4]	67, F	Hartmann's reversal (IMA sacrified at first operation)+postop.hypotension	Descending colon, 21 cm	Full thickness	24 days	11 months later	Left colectomy, colorectal anastomosis
Beattie [11]	57, M	Endovascular AAA repair	Descending colon, 90 cm	Full thickness	34 days	10 days later	Left colectomy, transverse end colostomy
Anaspure [13]	63, M	AAA repair, axillo-bifemoral bypass	Rectum, 2 cm	Mucosa-submucosa	16 days	45 days after (dilatation)	Rectal stricture dilated endoscopically, favorable
Present study Case 1	47, M	LAR with TME,closure of diverting colostomy, radiotherapy?	Rectum, 7 cm	Mucosa-submucosa	1 month	3 weeks after (dilatation)	Stricture dilated endoscopically, favourable
Present study Case 2	49, M	Anterior rectal resection	Proximal left colon, 25 cm	Mucosa-submucosa	2 weeks	1 month later	Resection of the remaining proximal left colon, ileostomy (rectal stump laid open)
Present study Case 3	70, F	Right hemicolectomy	Ileocolic anastomosis	Mucosa-submucosa	3 weeks	5 months later	Resection of the anastomotic (ileocolic) segment, ileotransverse anastomosis
Present study Case 4	71, M	Sigmoid colon resection (IMA sacrified)	Descending colon, 40 cm	Full thickness	30 days	2 months later	Subtotal colectomy, ileorectal anastomosis

AAA=abdominal aortic aneurism, NR=not reported, LAR=low anterior resection, EMS=electrical muscle stimulation, TME=total mesorectal excision, M=male, F=female

possibly case 1 of this article are examples of such a situation. Three similar cases have been reported in the literature.[12–14] These occurrences were respectively attributed to chronic constipation and colonic stenosis (itself probably secondary to chronic ischemia),[12] to the use of an electrical muscle stimulation device in a patient with preexisting atherosclerosis and atrial fibrillation,[14] and to the repair of an abdominal aortic aneurysm.[13]

In the present study, ischemia leading to the passage of mucosal cast was secondary to a colorectal resection in all three cases (Table 1).

In case 1, the ischemia-producing effect of the total mesorectal excision, although negligible; the vascular injury to the left mesocolon; and probably the association of postoperative radiotherapy may all be incriminated in the ischemic process. In fact, the low colorectal anastomosis had already stenosed before the passage of the cast. The colostomy closure might have further aggravated the ischemia by increasing the oxygen demands while increasing also the luminal pressure of the previously defunctionalized colonic segment and so reducing the oxygen delivery to this vulnerable area. Nevertheless, the stricture could be easily dilated.

In case 2, although the left colic artery had been preserved, the remaining left colon could not be saved from an ischemia sufficient to cause mucosal sloughing and stricture formation. Moreover, low ileorectal anastomosis performed after the resection of the involved colonic segment developed a tight stricture again, although no evidence of a systemic disease, which might explain this recurrent stricture, could be detected.

Case 3 is quite unusual in that the ischemia had developed subsequent to a standard right colectomy without any comorbidity or any incident, such as a hypotensive episode, that could explain it. It is known that a considerable percent of acute bowel ischemia occurs in the absence of a defined vascular pathology.[1,2]

In the literature concerning transanal passage of a colonic cast, aortic surgery occupies by far the first range. Our series underlines the importance of this unusual presentation of gangrenous ischemia in colorectal, particularly in rectal, surgery. In fact, in three presented patients among four of this series, the bowel ischemia culminating in transanal colonic cast passage was secondary to IMA interruption.

Ischemia of the proximal descending colonic segment after high ligation of IMA yields a 25% incidence of devitalization of the terminal colon.[2] This incidence increases further when the hypogastric arteries are also impaired.[15,16] In fact, the existence of a watershed area in the splenic flexure (Griffith's point) is classical knowledge.[1,3] This problem may be prevented by conservation of the IMA, at least of its left colic branch in risky patients,[15,16] or by a more generous resection of the descending colon.[17] In Case 4, we think that if a more extended resection (as a left hemicolectomy) had been performed, the complication could have been omitted.

We would like to underline the importance of being aware of this form of presentation of bowel ischemia, not only following aneurysm surgery but also in the postoperative course of colorectal surgery. Diarrhea (not always bloody) besides other signs and symptoms should alert the surgeon to such an occurrence.

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Development of a Simple Model of Extrinsic Denervation of the Small Bowel in Mouse

Javairiah Fatima · Scott G. Houghton · Michael G. Sarr

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Abstract Small bowel transplantation (SBT) is associated with poorly understood enteric dysfunction. The study of SBT in mice is hindered by the technical difficulty of orthotopic SBT in the mouse. Our aim was to develop an easy preparation of extrinsic denervation of the entire jejunoileum in mice as a model of orthotopic SBT. All neurolymphatic tissues accompanying the superior mesenteric artery (SMA) and vein (SMV) were ligated just distal to the middle colic vessels. The SMA and SMV were then stripped of investing adventitia, and the mesentery to jejunum and colon were transected radially. Jejunum and colon were not transected and reanastomosed. To confirm extrinsic denervation 1, 3, and 6 months later, segments of small bowel were stained for protein gene product 9.5 (PGP9.5) and tyrosine hydroxylase (TH). Tyrosine hydroxylase immunoreactive intensity was then quantified using a semiquantitative analysis. Immunohistochemical fluorescence showed persistence of PGP9.5 immunoreactivity confirming enteric nerves in jejunoileum; however, there was no TH immunoreactivity in jejunoileum in denervated mice despite the expected preservation of TH immunoreactivity in the still-innervated duodenum at 1 month. At 3 months, sparse immunoreactivity for TH was present, and by 6 months, reinnervation of TH-containing nerves appeared similar to controls. Quantification of intensity at each time-point further confirmed this trend. This technique in the mouse accomplishes a complete extrinsic denervation of jejunoileum early postoperatively (1 and 3 months); reinnervation occurs by 6 months. This is an easily learned murine model of orthotopic SBT.

Keywords Small bowel transplantation · Mouse model · Extrinsic denervation · Microvascular surgery

Introduction

Small bowel transplantation for various problems of short gut syndrome or gut dysfunction continues to be a clinical dilemma because of frequent enteric dysfunction after transplantation.[1] Unlike the heart, lung, kidney, and liver, which function quite well despite the complete organ isolation and the obligate denervation necessitated by the transplantation procedure, small intestinal function is modulated extensively by extrinsic and enteric neural input.[2–5] The study of these aspects of gut dysfunction after transplantation in animal models will help us to understand the underlying mechanisms mediating the enteric dysfunction of transplantation.

The primary problems obligated by small bowel transplantation include immune phenomena, ischemia–reperfusion injury, disruption (albeit transient) of the lymphatic drainage, and intestinal denervation. Our aim was to develop and validate an experimental murine model to study the effects of the extrinsic intestinal denervation accompanying transplantation, while avoiding the confounding factors of immune effects or ischemia/reperfusion injury. Whereas others and we have developed and studied similar models in the dog[6] and rat,[7] we chose to develop this model in a mouse because its genome is well described. Therefore, apart from facilitating the understanding of physiologic

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J. Fatima · S. G. Houghton · M. G. Sarr (✉)
Department of Surgery,
Gastroenterology Research Unit (AL 2-435),
Mayo Clinic, 200 First Street SW,
Rochester, MN 55905, USA
e-mail: Sarr.Michael@mayo.edu

J. Fatima
e-mail: fatima.javairiah@mayo.edu

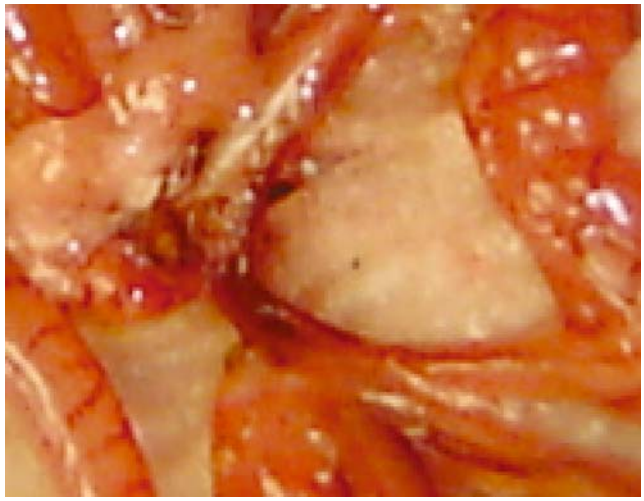

Figure 1 Superior mesenteric artery and vein after isolation from, and transaction of investing neurolymphatic tissues. Bowel continuity was not disrupted.

alterations in the small bowel attributed to extrinsic denervation, it will also allow us to create and evaluate genetically engineered models to study the molecular and genomic mechanisms mediating these effects.

Methods

Animal handling and care pre- and postoperatively was conducted in conformity with the NIH guidelines on the humane use and care of laboratory animals. Approval was sought from the Animal Care Committee of the Mayo Foundation.

Procedure

Eighteen adult c57bl6 mice weighing 20–25 g underwent extrinsic denervation (in vivo in situ neural isolation) of the small intestine from proximal jejunum to proximal colon. Isoflurane was used for induction, and pentobarbital was injected intraperitoneally (40 mg/kg) to maintain anesthesia. The bowel was exteriorized through a midline celiotomy. Using a Zeiss 10X/22B operating microscope, the proximal superior mesenteric artery (SMA) and superior mesenteric vein (SMV) were identified, and the encompassing neurolymphatic tissue was transected. Adventitia surrounding the SMA and SMV was then carefully teased away and transected until complete isolation of these vessels from the investing neurolymphatic tissues was achieved (Fig. 1). It is crucial to accomplish this maneuver without any blood loss; mice do not tolerate blood loss. The mesentery was then divided radially from the proximal SMA and SMV to the proximal jejunum and to the proximal colon, so that the only extrinsic connections to the jejunoileum were the SMA and SMV stripped of their investing fascia and the wall of the jejunum proximally and the ileum distally.

The bowel was placed back into the peritoneal cavity, which was then irrigated with 154 mM warm NaCl and closed with running 7-0 polyglactin in two layers. Mice were maintained in a warmed (85°F) recovery room for 24 h with free access to water and mouse chow and then returned to the animal housing facility.

Validation of Extrinsic Denervation

To confirm extrinsic denervation, immunohistofluorescence and colocalization of markers were performed using stains

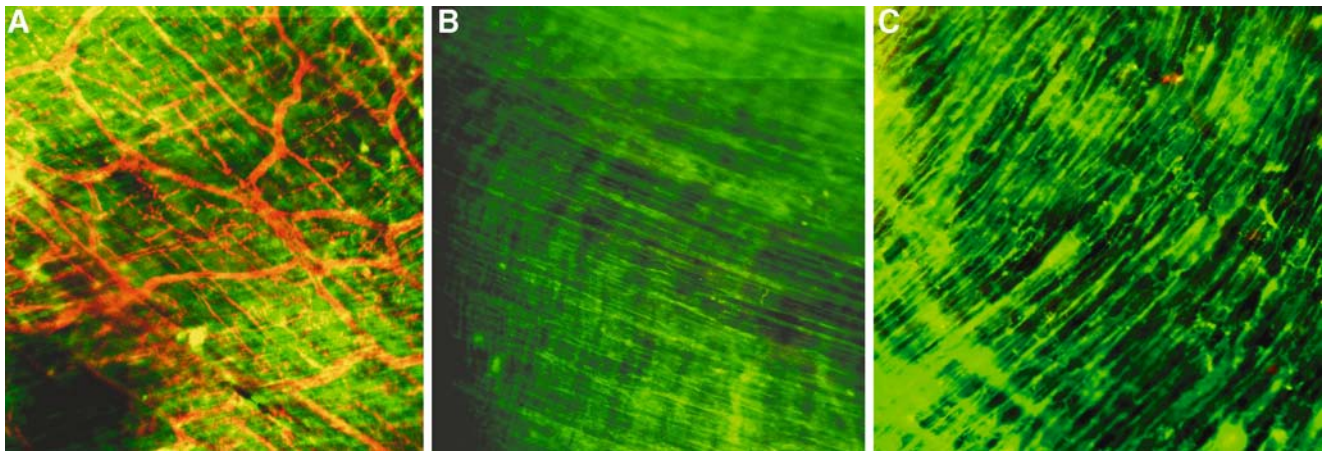

Figure 2 **a** Duodenal segment of mice 1 month after undergoing in situ neural isolation; shows colocalization of PGP9.5 with TH representing presence of sympathetic innervation. **b**, **c** Histologic sections of jejunal and ileal segments of the intestine 1 month after the procedure, respectively, showed complete absence of TH staining (sympathetic innervation) in the presence of staining with PGP9.5, affirming accomplishment of extrinsic denervation of the jejunoileum.

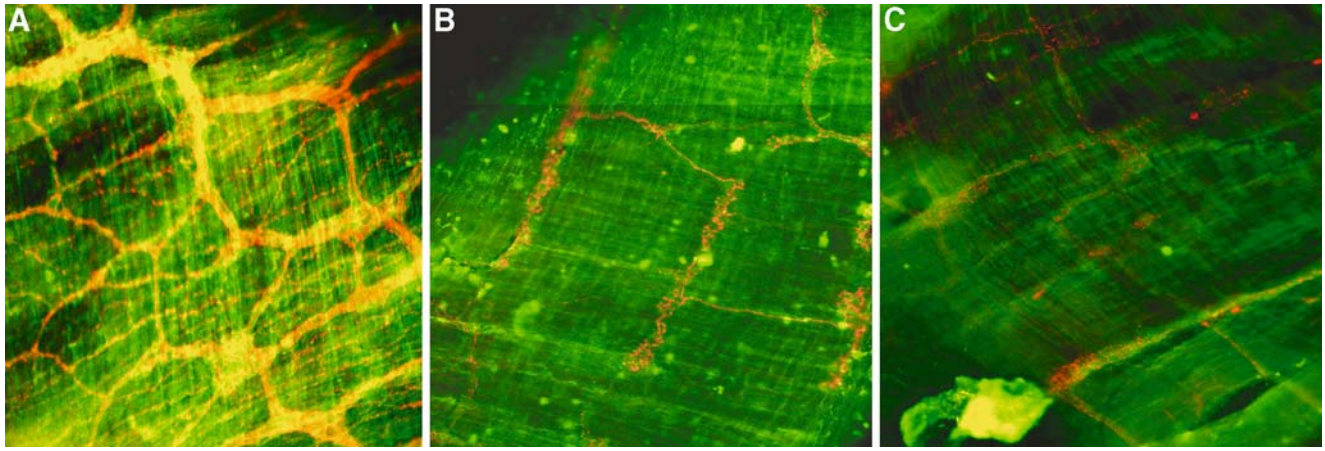

Figure 3 **a** Duodenal segment of mice 3 months after in situ neural isolation showing positive staining for both PGP9.5 as well as TH, indicating presence of sympathetic innerv ation. **b**, **c** Jejunal and ileal segments of the mouse small intestine 3 months after neural isolation of SMA and SMV, staining positively for PGP9.5 and weak staining for TH, representing initiation of regrowth of sympathetic innervation.

for protein gene product 9.5 (PGP9.5) and tyrosine hydroxylase (TH). PGP9.5 is a general neuronal stain that stains all types of neuronal cells, whereas the TH stain is specific for sympathetic neurons and has previously been used successfully to study denervation and reinnervation, because the only adrenergic innervation to the muscularis of the gut comes from the extrinsic (sympathetic) nerves.[8,9]

Samples from each segment of the intestine (duodenum, jejunum, and ileum) were harvested from all 18 mice that underwent this model of in situ neural isolation (n=6 at each time point: 1, 3, and 6 months) and from six normal, fully innervated mice. The intestinal segments were placed immediately into cold, freshly prepared, oxygenated Kreb's solution containing 3 μM nifedipine. Segments 1 cm^2 in area were pinned down on styguard; mucosa and submucosa were stripped away carefully until the smooth muscle layer of the muscularis containing the myenteric plexus remained as the most superficial layer. Tissue specimens were then placed in freshly prepared paraformaldehyde solution at 4°C for fixation overnight. Subsequently, samples were washed three times in phosphate-buffered saline (PBS) containing sodium azide (3 mM). Ten percent normal donkey serum (Chemicon International, Temecula, CA, USA) in PBS containing 0.3% Triton X-100 (Fischer Scientific, Fair Lawn, NJ, USA) was used for blocking. Tissues were then incubated in 5% normal donkey serum in PBS containing 0.3% Triton X-100 containing the primary antibody against TH (sheep polyclonal anti-TH, Chemicon International) at a 1:400 dilution and primary rabbit anti-PGP9.5 polyclonal unconjugated antibody (ABCAM, Cambridge, UK) at a dilution of 1:200. After 48 h, tissues were rinsed five times in PBS for 45 min and then placed in 2.5% normal donkey serum in PBS containing 0.3% Triton X-100 containing a 1:400 dilution of Alexa fluor 488 donkey antirabbit secondary antibody (Molecular Probes, Eugene, OR, USA) and a 1:200 dilution of CY3 conjugated anti-sheep

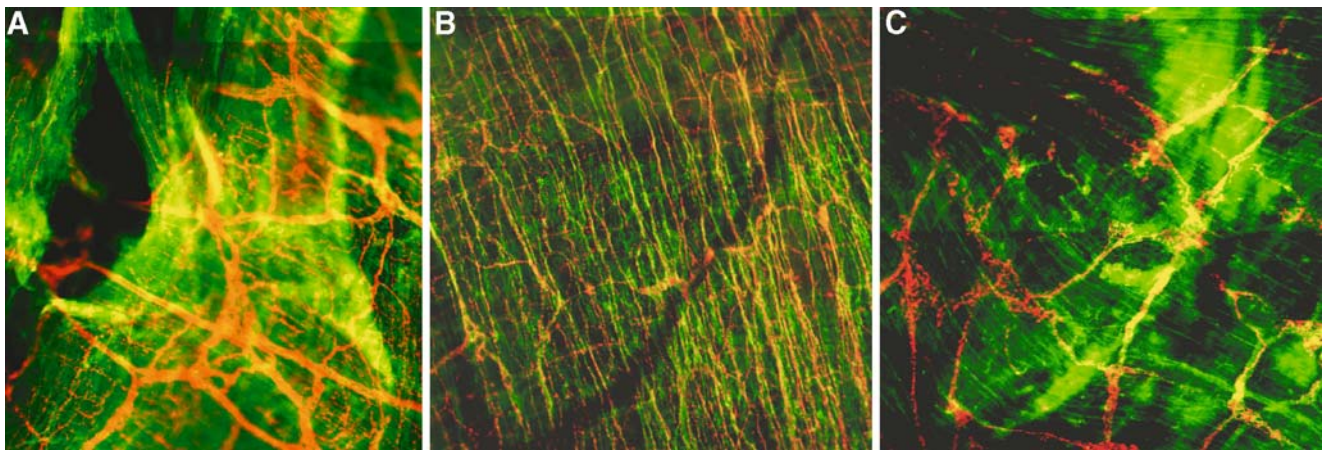

Figure 4 **a** Duodenal segment showing colocalization of PGP9.5 with TH 6 months after extrinsic denervation. **b**, **c** Histologic sections of jejunum and ileum in mice 6 months after extrinsic neural denervation, with an elaborate sympathetic nerve network after colocalization of PGP9.5 with TH, representing near-complete reinervation at 6 months.

secondary antibody (Chemicon International) for 24–36 h. Tissues were rinsed again in PBS five times for 45 min. Samples were stretched and mounted on a glass slide with a few drops of 10% glycerol in PBS and examined under a confocal microscope (Carl Zeiss Microimaging, Thornwood, NY, USA) at a magnification of ×10 to evaluate histofluorescence. Tissue specimens stained in the absence of primary antibody were used as negative controls.

Each image with immunohistofluorescence was then further analyzed to quantify the signal intensity of PGP9.5 and TH staining. The ratio of TH intensity to PGP9.5 was expressed as a percentage to represent the extent of innervation at each time point in the experiment (1, 3, and 6 months) in all three segments of the small intestine.

Results

Initially, this procedure has a steep learning curve if the surgeon does not have microvascular training. For the surgeon who has microvascular training, the isolation and skeletonization of the SMA and SMV are relatively straightforward. Some experience with handling of mouse tissue is needed, as experience with careful technique to keep the procedure free of blood loss is crucial. Once comfortable with the procedure, survival rates are 80–90%.

Surviving mice tolerated the operative procedure without any obvious complications such as diarrhea, malabsorption, or weight loss. In fully innervated control mice, colocalization of the TH stain (red) with the PGP9.5 stain (green) was represented by neurons appearing yellow (colocalization) in all the segments of the small bowel. Similarly, the duodenal segments of the mice undergoing our model of in situ neural isolation, when killed at 1, 3, and 6 months, also stained positively for PGP9.5 and TH with colocalization, indicating the presence of sympathetic innervation (Figs. 2a, 3a, and 4a), as would be expected, because the extrinsic denervation was selective for jejunoileum.

In contrast, in the jejunoileum at 1 month after extrinsic denervation, there was complete absence of TH immunoreactivity in the muscularis (indicative of absent sympathetic nerves), whereas persistence of PGP9.5 immunoreactivity (green stain) was evident in jejunum and ileum (Fig. 2b,c). At 3 months after extrinsic denervation, sparse immunoreactivity for TH was evident, as was a very faint colocalization with PGP9.5, suggestive of early reinnervation (Fig. 3b,c). Mice killed at 6 months had restoration of an elaborate neural network staining yellow, representing colocalization (and thus reinnervation), which appeared similar to the controls (Fig. 4b,c).

The signal intensity of each segment showed a corresponding amount of TH (extrinsic innervation) staining. Whereas the duodenum (control) in the denervated model showed 81, 71, and 90% TH staining at 1, 3, and 6 months, respectively, the denervated jejunum showed <5% TH staining intensity at 1 month, about 16% at 3 months, and about 75% at 6 months, demonstrating gradual reinnervation over time. Denervated ileum showed a similar pattern with the presence of <15% TH staining at 1 month after denervation, about 28% after 3 months, and reinnervation of extrinsic nerves to 89% after 6 months.

Discussion

Small bowel transplantation in mice is very demanding technically and has been performed by only a few select groups.[10–12] Our goal was to develop a model of intestinal (auto)transplantation in mice that was much less demanding technically and could be used to investigate neurally mediated mechanisms of genomic and molecular control of enteric function.

To avoid any ischemia/reperfusion injury, models of extrinsic denervation have been developed and validated previously in different animals to study the effects on intestinal physiology;[6,7] however, because the genome of most animals other than mice is not well-known, the study of molecular alterations occurring in intestinal cells after extrinsic denervation (transplantation) is not possible. In contrast, the mouse genome is well known, making it an ideal animal model for genetic and cellular level investigations via the use of genetically engineered strains of mice.

Obviously, validation of our denervation model in the mouse was necessary before adopting it for further investigations. All sympathetic innervation to the small intestine originates outside the bowel from neurons with cell bodies located in the sympathetic ganglia with their axons coursing along the mesenteric arteries supplying to the intestine.[13–15] Although there is some evidence of enteric adrenergic (dopaminergic) nerves in the submucosa, there are no adrenergic nerves in the muscularis after extrinsic denervation.[12] Thus, adrenergic immunoreactivity serves as a marker of extrinsic innervation of the intestinal wall. Hence, we adapted our previous models in the dog[6] and rat[7] to disrupt the neurolymphatic continuity along the SMA and vein to achieve complete extrinsic denervation of the mouse jejunoileum,[6,7] without the need for the demanding transection/reanastomosis of the blood supply, as well as the obligate ischemia/reperfusion phenomenon that accompanies all transplantation procedures. Immunohistofluorescent analysis for the nonspecific neuronal marker PGP9.5 confirmed the presence of intramural neurons, but the lack of immunofluorescence for TH, a marker of adrenergic nerves, confirmed extrinsic denervation at 1 month postop-

eratively. While preparing the specimen, care was taken to strip the mucosa and submucosa containing the submucosal plexus, and leaving behind only the muscular layers of the intestine containing the myenteric plexus as the specimen. A similar technique was utilized by Li et al. to demonstrate complete extrinsic denervation after disruption of neural continuity in the rat model.[16]

As with any model of denervation, reinnervation may occur. Previous investigations of extrinsic reinnervation in rat small bowel transplants and in our model of in situ extrinsic denervation in the rat have shown complete denervation of the jejunoileum early postoperatively.[14] By about 3 months postoperatively, however, sparse regrowth of sympathetic fibers was evident in the intestinal wall, and at 6–12 months, reinnervation of the small intestinal wall was well-established.[13] We further used semiquantitative analysis to confirm our observations. Our findings in the rat are very similar to these findings in the mouse.[7]

We did not also transect and reanastomose the proximal jejunum and distal ileum as in our previous canine and rat models.[6,7] Intestinal transection and reanastomosis in the mouse is also quite difficult technically. To be successful, many investigators have needed to keep the mice on a liquid diet postoperatively after intestinal anastomosis.[12] While we acknowledge that there are extrinsic nerves that travel within the wall of the small intestine for variable distances, our immunohistofluorescent staining suggests that the extent of such nerves in the mouse is minimal.[13]

Based on our findings, we conclude that our model of extrinsic denervation in mouse is a feasible and technically much easier, validated model of extrinsic denervation of the mouse small intestine and can be used to study the isolated effects of extrinsic denervation while avoiding the other potentially confounding factors that accompany transplantation (immune phenomena, ischemic/reperfusion, etc.). This model will allow investigation of the molecular mechanisms involved in the changes that occur in the intestinal cells posttransplantation. The reinnervation that occurs after 3 months can also be used as a model to study reinnervation.

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Surgical Therapy for Colorectal Metastases to the Liver

Timothy M. Pawlik · Michael A. Choti

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Abstract Colorectal cancer is the fourth most common type of cancer in the West and the second leading cause of cancer-related deaths in the United States. Approximately 35 to 55% of patients with colorectal cancer develop hepatic metastases during the course of their disease. Surgical resection of colorectal liver metastases represents the only chance at potential cure, and long-term survival can be achieved in 35 to 58% of patients after resection. The goal of hepatic resection should be to resect all metastases with negative histologic margins while preserving sufficient functional hepatic parenchyma. In patients with extensive metastatic disease who would otherwise be unresectable, ablative approaches can be used instead of or combined with hepatic resection. The use of portal vein embolization and preoperative chemotherapy may also expand the population of patients who are candidates for surgical treatment. Despite these advances, many patients still experience a recurrence after hepatic resection. More active systemic chemotherapy agents are now available and are being increasingly employed as adjuvant therapy either before or after surgery. Modern treatment of colorectal liver metastasis requires a multidisciplinary approach in an effort to increase the number of patients who may benefit from surgical treatment of colorectal cancer liver metastasis.

Keywords Colorectal metastasis · Liver · Surgery · Radiofrequency ablation · Chemotherapy

Introduction

Approximately 150,000 new cases of colorectal cancer occur each year in the United States, accounting for more than 55,000 cancer-related deaths.[1] Roughly one half of patients with colorectal cancer develop liver metastases during the course of their disease.[2] Of these, 15 to 25% present with synchronous liver metastases,[3–5] whereas an additional 20 to 25% develop metachronous hepatic tumors.[6–8] In 30% of patients with synchronous or metachronous liver metastases, the liver is the only site of metastatic disease.[9] Thus, 10,000 to 15,000 patients per year are candidates for local therapy for colorectal metastases.[8]

Surgical therapy for liver metastases remains the only therapy with potential for cure.[10] Patients with untreated metastatic colorectal cancer have a poor prognosis with a median survival of 6 to 12 months.[11–15] Although recent advances in systemic chemotherapy have led to improved tumor response rates, the median survival of unresected patients still ranges from 12 to 24 months and survival beyond 5 years is uncommon.[16–27] In contrast, long-term survival and potential cure after surgical resection for hepatic colorectal metastases have been demonstrated in numerous studies. Whereas this question has not been tested with randomized controlled trials, the preponderance of evidence supports a significant survival benefit to resection. In most series, the overall 5-year survival rates reported after hepatic resection with curative intent range from 35 to 58%.[10,28–36] These results are expected to

T. M. Pawlik · M. A. Choti
Division of Surgical Oncology, Department of Surgery,
The Johns Hopkins University School of Medicine,
Baltimore, MD, USA

M. A. Choti (✉)
Department of Surgery, Johns Hopkins Hospital,
600 North Wolfe Street Halsted 614,
Baltimore, MD 22187-6681, USA
e-mail: mchoti@jhmi.edu

improve even further with improved patient selection and multimodality approaches that include newer chemotherapy regimens.[37] As such, when indicated, resection of colorectal liver metastasis should be considered standard practice. In this report, we provide an overview of the issues relating to the therapeutic management of patients with colorectal liver metastasis.

Evaluation of the Patient with Hepatic Metastases

The diagnostic approach in patients with hepatic metastases is based on the available treatment options. In patients for whom further treatment is not being considered because of comorbid factors or patient choice, an exhaustive evaluation for the extent of disease may be unwarranted. In patients for whom systemic chemotherapy is being considered, an evaluation should facilitate monitoring of the response to treatment at all sites. A full evaluation is necessary to identify those patients who are candidates for local liver-directed therapy.

Selecting the Patient for Surgical Therapy

Over the past three decades, the perioperative mortality associated with hepatectomy has decreased from 20% in patients operated on before 1980 to close to 1% in patients undergoing liver resection in more recent years.[38–44] A significant aspect of the morbidity and mortality of liver surgery relates to patient selection. In deciding which patients will tolerate a major liver resection, a number of factors need to be considered, including patient comorbidities. The importance of patient age has long been debated, but a review of the literature shows that age alone is not an independent predictor of increased operative risk.[45,46] This fact is important, considering that a large percentage of patients referred for liver surgery, especially for malignancy, are elderly. In contrast, surrogate markers of general physiologic fitness such as the American Society of Anesthesiology (ASA) score and the preoperative Acute Physiology and Chronic Health Evaluation score do significantly influence the incidence of postoperative complications.[46,47] Patients with an ASA score >1 have been shown to have more than three times the mortality and twice the morbidity compared to those patients with an ASA of 1.[46] These patients with higher ASA scores undoubtedly reflect a group of patients with more underlying coronary artery disease, congestive heart failure, renal insufficiency, and other debilitating states that place this patient population at greater risk for postoperative complications. A major goal of the preoperative evaluation, therefore, is to identify patients who are at a high operative risk so that patients who represent a prohibitive risk can be excluded whereas those patients with manageable comorbidities can have these conditions addressed preoperatively in an attempt to reduce their risk.

Imaging

All patients being considered for resection of colorectal liver metastases should undergo preoperative computed tomography (CT) or magnetic resonance imaging (MRI) to evaluate the extent of intrahepatic disease and to exclude extrahepatic metastasis. Preoperative imaging should define the number of liver lesions, their distribution, and their proximity to major biliary and vascular structures. Computed tomography is also able to rule out pulmonary metastases with a high degree of sensitivity,[48] yet its cost effectiveness is less clear.[49,50] Although controversial, chest CT should be considered before resection of liver metastases, even in patients with a normal chest x-ray. A colonoscopy within the last 6 to 12 months is also mandatory to exclude anastomotic recurrence or a second colorectal primary tumor.

On contrast-enhanced CT, colorectal metastases appear as hypointense areas within the liver if the scan is obtained when the contrast is in the portal circulation. The sensitivity of detecting colorectal metastasis using earlier generation nonhelical contrast-enhanced CT was between 50 to 75%.[51–54] With the advent of contrast-enhanced helical CT, the sensitivity of CT has significantly improved. This is largely because helical CT permits rapid image acquisition, thereby allowing images to be obtained in the portal phase in a single run.[55] In one study, dual-phase helical CT was reported to have a sensitivity of 69 to 71% and a specificity of 86 to 91%, and was shown to be both an accurate and highly reproducible method for detecting and characterizing liver lesions.[56] With newer multiple detector helical CT, the sensitivity of identifying liver metastases now approaches 80 to 90%[55,57,58] as the thin collimation possible with multidetector row CT results in higher image resolution.

Spiral computed tomography during arterial portography (CTAP) was introduced in the late 1980s as a means to improve the sensitivity of detecting colorectal liver metastases. Initial studies suggested that CTAP was superior to the standard CT and MRI techniques that were available at that time, with CTAP sensitivity ranging from 75 to 90%.[51–53,58] More recent studies comparing CTAP with newer generation CT and MRI have failed to show an improved sensitivity with CTAP.[58,59] Computed tomography during arterial portography also has some notable disadvantages. It is an invasive procedure that necessitates angiography. Furthermore, the high sensitivity of CTAP comes at the expense of a high false positive rate and low specificity.[60–64]

Magnetic resonance imaging is another imaging modality frequently employed to characterize the extent of colorectal metastatic disease within the liver. Although the sensitivity of unenhanced MRI has been reported to be 64%,[51] contrast-enhanced MRI with agents such as gadolinium and ferumoxide has significantly improved the accuracy of detecting malignant liver tumors (80 to 90%).[65–70] Semelka et al.[63] reported that gadolinium-enhanced MRI was as sensitive as CTAP in detecting liver metastases, but had a greater specificity. Ferumoxide is an intravenous contrast agent that contains iron particles and is captured in the Kupffer cells of normal liver parenchyma and benign lesions but not metastases. The use of ferumoxide has been shown to improve the sensitivity of MRI to detect small metastatic lesions.[65,71,72] Specifically, T2-weighted ferumoxide-enhanced MRI has been reported to be superior to T2-weighted unenhanced imaging.[73,74] Whether ferumoxide is superior to gadolinium remains controversial.[75,76]

Perhaps the most promising imaging modality for the assessment of the extent of metastatic sites is whole body positron emission tomography (PET) (Fig. 1). Unlike CT and MR, which provide anatomic information, PET provides functional information related to metabolic activity. Although a number of radiopharmaceuticals have been labeled with positron emitters, 18F fluorodeoxyglucose (18F-FDG) is presently the most widely used PET radiopharmaceutical in oncology. When administered intravenously, 18F-FDG is taken up and accumulated in metabolically active cells. Malignant tissue with relatively increased uptake can be seen as areas of increased signal relative to the surrounding less metabolically active normal tissue.[77–79] More recently, PET has been combined with CT (PET/CT) in the same gantry, providing the advantage of anatomic localization of the PET scan abnormalities.[80–84]

Yeung et al.[85] has reported that combined PET/CT results in increased reader confidence and 53% fewer equivocal readings on the part of the radiologist, as well as an improved positive predictive value compared with PET alone. Currently, there is a general consensus that PET/CT is the most accurate method for staging advanced colorectal cancer, particularly when metastasectomy is being considered and when the patient has possible locoregional recurrent disease.[86–88] In a comparison of contrast-enhanced CT vs combined PET/CT, Selzner et al.[83] reported that CT and PET/CT provided similar information regarding hepatic metastases of colorectal cancer, with each modality having greater than 90% sensitivity for the detection of intrahepatic metastases. Combined PET/CT, however, was superior to CT alone for the detection of recurrent intrahepatic tumors after hepatectomy, extrahepatic metastases, and local recurrence at the site of the initial colorectal surgery.[83] Similarly, Joyce et al.[89] reported that PET/CT confirmed lesions

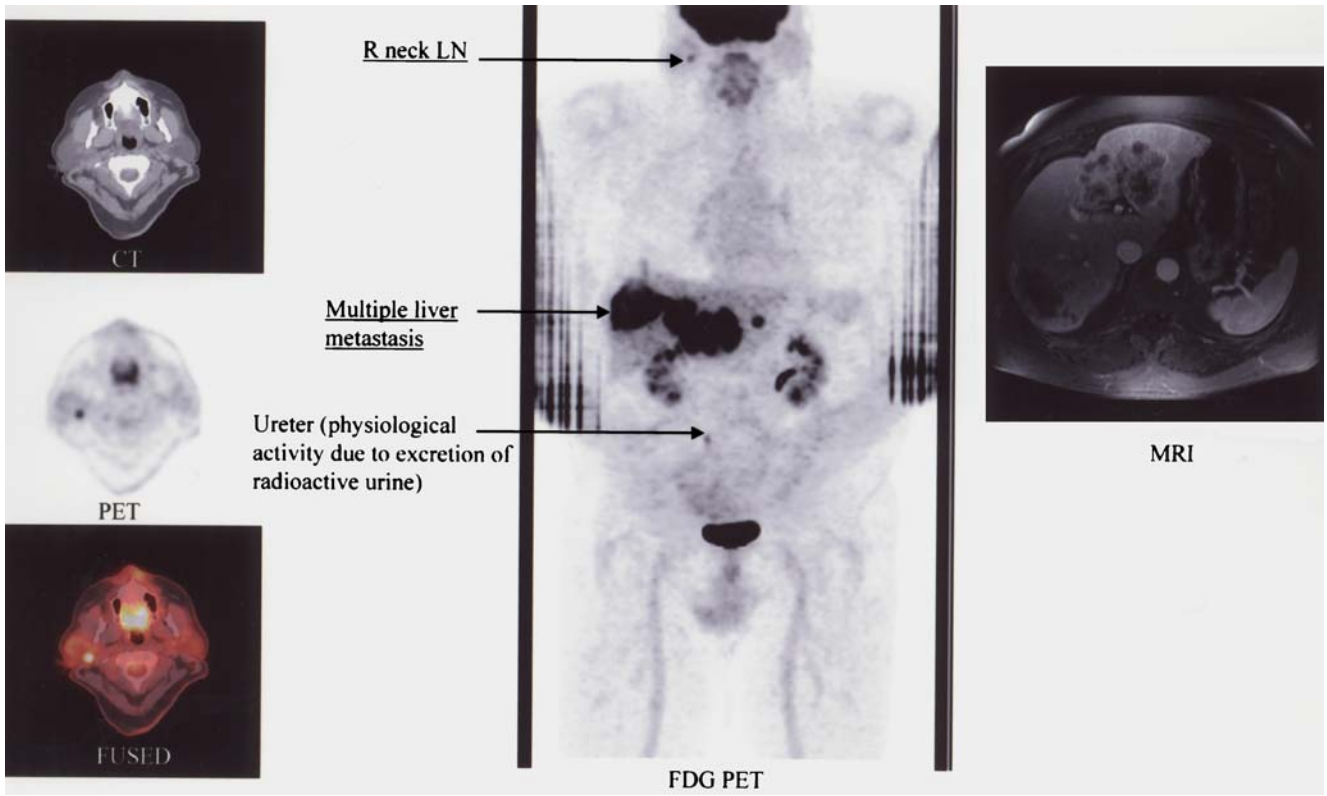

Figure 1 Positron emission tomography scan of patient with colorectal metastases. The FDG-avid lesions identified in the right and left side of the liver on PET scan correlated with the masses identified on the MRI. The PET/CT identified an additional distant metastasis in the right neck.

identified by conventional imaging techniques in 90% of patients but also identified additional findings in 32% of patients. In this study, PET resulted in a change in clinical management in 24% of cases.[89]

When possible, a PET/CT should be obtained before treatment with chemotherapy when staging patients with metastatic colorectal cancer. Treatment with chemotherapy can make a previously fluorodeoxyglucose (FDG)-avid lesion less metabolically active and therefore less prone to be detected on PET/CT. Therefore, obtaining a PET/CT before chemotherapy treatment increases the chances that all sites of metabolically active disease will be identified. In addition, obtaining a "baseline" PET/CT before treatment can be helpful to determine if chemotherapy successfully reduces PET-FDG uptake. This approach has previously been shown to accurately assess pathologic response to preoperative chemotherapy.[90] In fact, Guillem et al.[91] reported that metabolic tumor response to preoperative chemotherapy, defined by a decrease in FDG uptake, correlated with long-term outcome in patients with locally advanced rectal cancer. As such, the use of PET/CT may have both diagnostic and prognostic implications.

Decisions Regarding Resectability

In the past, resection of hepatic colorectal metastases was not attempted in patients who had more than three or four metastases, hilar adenopathy, metastases within 1 cm of major vessels such as the vena cava or main hepatic veins, or extrahepatic disease. More recent studies demonstrate, however, that patients with these clinicopathologic factors can achieve long-term survival after hepatic resection and therefore should not be excluded from surgical consideration. Specifically, the number of metastases is no longer considered a contraindication to surgery.[8,92,93] Similarly, contiguous extension to adjacent anatomical structures and local or regional recurrence at the site of the primary colorectal cancer are not contraindications to resection.[35] An increasing number of studies also indicate that although survival may be reduced in patients with extrahepatic colorectal metastases,[9,94,95] complete resection of limited extrahepatic disease can result in long-term survival.[96,97]

Taken together, these data have precipitated a shift in the definition of resectability from criteria based on the characteristics of the metastatic disease (tumor number, size, etc.) to new criteria based on whether a macroscopic and microscopic complete (R0) resection of the liver lesion as well as complete resection of any extrahepatic disease can be performed (Table 1). Currently, hepatic colorectal metastases should be defined as resectable when it is anticipated that the disease can be completely resected, two adjacent liver segments can be spared, adequate vascular inflow and outflow and biliary drainage can be preserved, and the volume of the liver remaining after resection (i.e., the "future liver remnant") will be adequate (at least 20% of the total estimated liver volume).[98,99] This definition of resectability represents a paradigm shift. Instead of resectability being defined by what is removed, decisions regarding resectability should now focus on what will remain after resection. Concern for the future liver remnant is particularly pertinent during preoperative evaluation of patients for an extended hepatectomy (resection of ≥5 liver segments), as some patients may be excluded from the benefit of a potentially curative resection because the anticipated liver remnant may be too small.

Table 1 Criteria Defining Resectability for Surgical Resection of Colorectal Liver Metastasis

Criteria
1. Macroscopic and microscopic (R0) treatment of the disease is feasible with either resection alone or resection combined with RFA.
2. Two adjacent liver segments can be spared.
3. Vascular inflow, outflow, and biliary drainage can be preserved.
4. Sufficient remnant liver volume (>20% of the total estimated liver volume).

Extended hepatectomy may allow for a curative resection in some patients with multiple or large tumors. In fact, in a significant proportion of patients, extended hepatectomy may be the only means available to achieve complete resection and provide a chance for cure. Recent studies have shown that extended hepatectomy can be performed with a near zero operative mortality rate and is associated with long-term survival.[98]

In general, 20% of the total liver volume appears to be the minimum safe volume that can be left after extended resection in patients with normal underlying liver. Computed tomography or MRI can now provide an accurate, reproducible method for preoperatively measuring the volume of the future liver remnant.[99] To avoid operating on patients with low volume future liver remnants, any patient who fails to show compensatory hypertrophy as a result of tumor growth and who has a future liver remnant of less than 20% should be considered for portal vein embolization (PVE) to induce hypertrophy of the contralateral liver lobe.[100] This method has been shown to increase both the size of the future liver remnant and the percentage of indocyanine green excretion and bile volume flow in the remnant liver.[101] Yet, no evidence, to date, clearly demonstrates improved outcomes after PVE compared to no PVE. It is likely that the selective use of PVE may enable the performance of an extended hepatectomy in a subset of patients who otherwise would not have been candidates for safe resection. In general, PVE needs to be performed only in patients being considered for an extended right hepatic resection; PVE is rarely necessary before extended left

hepatectomy because the right posterior sector typically constitutes about 30% of the total liver volume.[102,103]

Results of Liver Resection for Hepatic Colorectal Metastases

Overall, the perioperative mortality of liver resection for colorectal metastases is approximately 1% in most current reported series.[30,98,104] In experienced hands, even major hepatic resections (hemihepatectomy or extended hepatectomy), which are performed in about half of the cases, result in perioperative mortalities of less than 5%.[30,98] The potential for adverse outcome and the complexity of these operations justifies the recommendation that major liver resection be performed at centers and by surgeons with more than occasional experience with such procedures. One report analyzing the short-term outcome for liver resections in the state of Maryland indicated a clear relationship between hospital procedure volume and perioperative mortality.[105] Although operative mortality should be uncommon, the perioperative morbidity rate remains in the 20% range. The major morbidity associated specifically with liver resection includes hemorrhage, perihepatic abscess, bile leak and/or fistula, pleural effusion, and hepatic failure. Morbidity associated with hepatic resection has decreased in the past decade. This decreasing morbidity is multifactorial and undoubtedly related to better patient selection, improved anesthetic monitoring, greater understanding of hepatic anatomy, advances in surgical technique, and improved perioperative critical care. For example, devices such as vascular staplers and new instruments to transect the liver parenchyma have resulted in decreased blood loss and shorter operative times.[106]

With regard to survival, large series from the 1960s through the mid-1990s reported 5-year survival rates in the range of 33 to 36% for patients with colorectal liver metastases resected with curative intent.[7,10] However, more recent data from single institution[28,30] and multicenter[107] studies have shown an improved 5-year survival rate of 58% after complete resection of colorectal liver metastases (Table 2). This improvement in overall survival likely reflects improvement in patient selection, surgical technique, and more effective adjuvant therapy.

Table 2 Reported 5-year Survival After Resection of Colorectal Liver Metastasis with Curative Intent

Investigator	Year of Publication	Years Included in Study	5-year Survival (%)
Scheele et al.[7]	1995	1960–1992	39
Fong et al.[10]	1999	1985–1998	37
Choti et al.[30]	2002	1993–1999	58
Abdalla et al.[28]	2004	1992–2002	58
Fernandez et al.[255]	2004	1992–2002	58
Pawlik et al.[107]	2005	1990–2004	58

Major Indicators of Prognosis

Several clinicopathologic factors predictive of patient survival after hepatic resection have been identified. These include stage, grade, and nodal status of the primary colorectal tumor;[9,108–112] disease-free interval from diagnosis of primary tumor to diagnosis of liver metastases;[10,108,109,111] number and distribution of liver metastases;[34,108–112] level of preoperative carcinoembryonic antigen (CEA);[10,30,113] and presence of extrahepatic disease.[9,94,95] Fong et al.[10] have developed a clinical prognostic score based on a number of these clinicopathologic factors. The criteria found to be associated with poorer outcome included (1) positive nodal status of the primary tumor, (2) multiple tumors, (3) disease-free interval of less than 12 months between primary tumor and diagnosis of liver metastasis, (4) CEA level greater than 200 ng/ml, and (5) tumor size greater than 5 cm.

Whereas preoperative factors may be generally instructive, these factors should not be used to exclude patients from surgical consideration. Patients with one or multiple negative prognostic factors can still derive a significant survival advantage from hepatic resection of their colorectal metastases.[114] In fact, some investigators have suggested that molecular tumor biomarkers may be more useful than many traditional clinicopathologic factors at predicting survival after resection of hepatic colorectal metastases.[114–119] Specifically, proliferation markers such as Ki-67 labeling index[116,119,120] (also see p. 53 in De Jong et al.[121]), and human telomerase reverse transcriptase expression,[114,119] tritiated thymidine uptake,[122] and thymidylate synthase expression[118] have been shown to be more powerful predictors of recurrence and survival. These data serve to emphasize that traditional preoperative clinicopathologic factors are inadequate when attempting to define the true underlying biology of colorectal metastases. Therefore, all patients with metastatic disease that is deemed to be resectable—regardless of the associated clinicopathologic factors—should be considered as potential surgical candidates.

Operative Considerations

Intraoperative Assessment

Laparoscopic evaluation of the liver and abdominal cavity just before laparotomy has been advocated by some to reduce the number of patients unnecessarily undergoing full surgical exploration.[123–125] With recent refinements in lap-

aroscopic ultrasonographic devices, intraoperative hepatic ultrasonography can now complement visual laparoscopic assessment.[126,127] In one report, the use of diagnostic laparoscopy including laparoscopic intraoperative ultrasonography (IOUS) was shown to avoid unnecessary laparotomy in up to 25% of patients who were initially believed to have resectable disease.[128,129] However, with increasingly sensitive preoperative imaging techniques, including PET/CT, and the increasing use of ablation and resection of extrahepatic sites, fewer patients are being subjected to nontherapeutic laparotomy even without prior laparoscopy.

Intraoperative ultrasonography has been demonstrated to be an important tool for accurately staging liver tumors and frequently impacts intraoperative decision making.[130–134] The superiority of IOUS over preoperative radiologic images, as well as intraoperative inspection and palpation by the liver surgeon, has been demonstrated in numerous studies.[132,135–137] Specifically, compared with preoperative radiologic findings, IOUS has been reported to identify at least one additional malignant lesion in 10 to 12% of cases.[137] As such, IOUS may have an oncologic benefit by more accurately identifying all the malignant hepatic disease, thereby allowing for complete resection of all existing macroscopic disease.[128] Findings discovered by IOUS have been reported to change or guide surgical treatment in up to 67% of procedures.[132,133,135,137]

With IOUS, the liver is surveyed using a midfrequency (5.0 and 7.5 MHz) transducer probe. The 7.5-MHz probe can penetrate approximately 6 to 8 cm, which is usually sufficient during IOUS examination. Although the lower frequency transducers can provide deeper penetration, image resolution is significantly sacrificed.[138] It is imperative that IOUS is performed in a systematic manner to avoid missing small occult lesions. In general, IOUS using high-resolution ultrasound can detect 3- to 4-mm tumors when hyper- or hypoechoic relative to the liver parenchyma. Isoechoic tumors may be harder to detect. When identified, lesions should be imaged in both the transverse and sagittal planes to define the complete size and relation of the lesion to surrounding structures such as the portal or hepatic veins and biliary structures.

Nonanatomical vs Anatomical Resection and Extent of Surgical Margin

The basic tenets for the resection of colorectal liver metastasis include a clear pathologic surgical margin and the preservation of sufficient functional hepatic parenchyma. Whether to perform a nonanatomical resection or an anatomical resection, however, has been controversial.[139,140] Although the performance of an anatomical resection has been reported to improve tumor clearance and outcome in patients with hepatocellular carcinoma, similar findings have not been demonstrated in patients with colorectal liver metastasis.

Patients who undergo a nonanatomical resection have been reported to have a higher incidence of positive surgical margins and inferior survival compared with patients undergoing an anatomical resection.[141] Other studies, however, report similar rates of positive surgical margins and 5-year survival for patients treated with nonanatomical resection vs anatomical resection.[142,143] Kokudo et al.[142,143] reported comparable rates of a "minimum" surgical resection (i.e., margin of less than 2 mm) in patients undergoing nonanatomical vs anatomical resections. Therefore, the type of resection chosen for a particular patient should be individualized based on the number, size, location, and distribution of metastases. Whereas a small, superficial metastasis may be best treated with a nonanatomical resection, a large metastasis deeply located within the liver may be best treated with an anatomical, segment-oriented resection. Rather than dogmatically adhering to an anatomical vs nonanatomical approach, the surgeon's goal should be to resect all metastases with negative histologic margins while preserving sufficient functional hepatic parenchyma.

The importance of surgical margins status has been shown to be important in long-term outcomes after resection of colorectal liver metastases. Multiple studies have shown that a negative resection margin decreases local recurrence rates[107] and improves survival.[30,107] Pawlik et al.[107] reported that a positive resection margin was associated with a significantly higher risk of surgical margin recurrence and decreased overall survival. Similarly, Choti et al.[30] noted that patients who had a positive microscopic resection margin had a median survival of only 24 months compared with 46 months for patients with a negative surgical margin.

Whereas achieving a negative margin is important, the extent of margin clearance is controversial. Several earlier series concerning liver resection for colorectal liver metastasis have reported that one should attain at least a 1-cm margin[95,144] and if not possible, this should be a relative contraindication to surgery.[145] Cady et al.[144] reported that a surgical margin of less than 1 cm was associated with a significantly shorter disease-free survival. As a result, many centers adopted the "1-cm rule" as a minimal margin to obtain at the time of hepatic resection.[146,147] Other investigators[8,107] have reported more recently, however, that the actual width of the surgical margin has no effect on survival as long as the margin is microscopically negative. Altendorf-Hofmann and Scheele[8] noted that whereas patients with a microscopically positive margin had a worse prognosis compared with patients who had a microscopically negative margin, survival was not associated with the width of the negative surgical margin. Similarly, Pawlik et al.[107] noted that the width of a negative

surgical margin did not affect survival, recurrence risk, or site of recurrence after hepatic resection of colorectal metastases.

Surgeons should continue to plan hepatic resection preserving a "safety zone" and avoid routine use of "minimum margin" surgery. However, based on the aggregate data, a surgical margin of at least 1 mm appears to not place the patient at any increased risk of margin-related recurrences or worse prognosis. Therefore, a predicted margin of less than 1 cm after resection of hepatic colorectal metastases should not be used as an exclusion criterion for resection.

Hilar Lymph Node Metastases

Metastases to the hilar lymph nodes are recognized as an important predictor of prognosis. Unlike pericolic nodal disease, these nodes are felt to be "metastases from metastases" and are associated with poor outcome. Because of this, some investigators have considered hilar nodal metastasis a contraindication to hepatic resection of colorectal liver disease.[54,116,148,149] Other studies, however, have reported long-term survival in some patients with hilar nodal metastases and have concluded that this patient population may still benefit from hepatic resection.[139,150–152] In one study by Yasui et al.,[152] the 5-year survival rate of patients with colorectal liver metastasis and positive hepatic lymph nodes was 41.7%. In another study, patients with colorectal liver metastases and hilar lymphadenopathy had a 3-year survival rate of 38%.[153] Based on these data, the presence of hepatic lymph node metastasis, while certainly associated with worse outcome than when not present, should not be used as an exclusion criteria for hepatic resection. Whereas some authors[154] recommend that lymphadenectomy always be performed at the time of hepatic resection, the routine addition of this procedure to hepatectomy for metastatic colorectal liver disease remains controversial.

Two-stage Hepatectomy

Some patients with multiple liver metastases are not candidates for a complete resection by a single hepatectomy, even after PVE or downsizing by chemotherapy. A sequential—or two-stage—hepatectomy may therefore be needed to achieve complete surgical extirpation of all known metastatic disease.[155–157] Adam et al.[157] has reported a 3-year survival rate of 35% in 13 patients undergoing two-stage hepatectomy. In one approach to patients with extensive bilobar disease in whom a two-stage approach is planned, the metastases in the left hemiliver (the future functional liver remnant) are resected by wedge resections in the first stage. A right PVE is then performed and the remnant liver is allowed to hypertrophy. In the absence of any significant tumor progression, a right or extended right hepatectomy to resect the right liver metastases is performed as the second stage. The advantage of performing a limited, nonanatomic wedge resection in the first stage is the preservation of a maximal amount of liver parenchyma that will hypertrophy after PVE to become the functional liver remnant. A two-stage hepatectomy should only be performed with curative intent and the application of this strategy must be carefully considered to avoid posthepatectomy liver failure because of an inadequate liver remnant.

Tumor Ablation

Methods for local ablation have been developed in recent years with a goal of increasing the number of patients eligible for liver-directed therapy. The early experiences with ablation of metastases were primarily with hepatic cryosurgery. This technique relies on the destruction of a defined area within the liver by freeze/thawing, using probes cooled by liquid nitrogen or argon gas to subzero temperatures. Radiofrequency ablation (RFA) is currently the most commonly applied ablation method. A variety of other interstitial local ablative approaches have also been applied to liver metastases, including focused high-intensity ultrasound ablation,[158,159] laser thermotherapy,[160,161] and yttrium-90 seed implants.[162] More recently, thermal ablation using microwave-emitting devices has been gaining popularity.

Radiofrequency ablation involves the localized application of conductive thermal energy to destroy tumor cells. Specifically, alternating electric current in the range of radiofrequency waves (460 kHz) is applied from a generator through a needle electrode placed directly into the tumor.[163] As the temperature within the tissue becomes elevated beyond 50–60°C, proteins are denatured, cells are destroyed, and a zone of necrosis surrounding the electrode develops.[164] A variety of probe designs are available, including multielectrode deployable devices, cooled parallel probes, and saline infusion probes. These designs allow the capability to increase the speed and size of the zones of ablation. Tumors 4 cm or less in greatest diameter can typically be ablated with a single placement of a multielectrode array, creating spherical burn of up to 5–6 cm in diameter.[165] Larger tumors require multiple deployments of the needle electrode. Treatment should be planned such that the zones of coagulative necrosis overlap to ensure complete destruction of the tumor.[165] Ideally, a thermal zone of ablation measuring 1 cm wide around the tumor should be created to ensure destruction of the tumor and a rim of nonmalignant tissue. Tumors abutting major vascular structures can be safely ablated as the blood within these vessels acts as a heat sink to protect the vascular endothe-

lium from thermal injury while allowing complete coagulative necrosis of the tissue surrounding the vessel wall.[165,166] Radiofrequency ablation should not, however, be applied to lesions near the hilum; although the blood vessels can tolerate the heat generated from RFA, the large bile ducts in this area can be damaged, resulting in biliary strictures.[165]

Radiofrequency ablation can be performed percutaneously, laparoscopically, or with open surgery.[165,167] Percutaneous RFA is the least invasive and can be performed as an outpatient procedure. The disadvantage of this approach is the relatively inferior ability to detect and target small lesions compared with open RFA guided by IOUS.[168] In addition, some locations within the liver are inaccessible or associated with thermal injury to adjacent organs when using the percutaneous approach.[167] Laparoscopic RFA represents a minimally invasive method that allows for better staging with IOUS and better visualization (and thereby avoidance) of adjacent visceral structures. Laparoscopic RFA, however, can be technically challenging depending on the anatomic location of the metastasis. Radiofrequency ablation using an open surgical approach is the most invasive but perhaps the most effective. Open RFA provides the best visualization and targeting capability, and provides the opportunity to fully mobilize the liver, move away adjacent structures, and utilize inflow vascular occlusion if necessary. It can be done with other procedures, including with liver resection. When done alone, open RFA is generally preferred for patients with multiple tumors, lesions in difficult locations to access with other approaches, or if the tumor abuts a major structure to be avoided.[169,170]

Tumor ablation should not be viewed as a replacement for resection, but more as a supplement or extension of localized therapy in unresectable patients.[28,165,171] In general, patients with resectable colorectal liver metastasis should undergo resection—not ablation. The main indication for ablation is in patients who do not meet the criteria for resectability, but are candidates for liver-directed therapy based upon the presence of liver-only disease. Combining hepatic resection with ablation expands the number of patients who may be candidates for liver-directed surgical therapy, particularly as larger lesions that are less effectively treated with ablation can be resected and small lesions can be ablated. Adding RFA to hepatic resection has been reported to be well tolerated with a perioperative morbidity and mortality comparable to those seen after resection alone.[171]

Most data concerning outcome after ablation treatment of colorectal liver tumors has come from studies involving patients with unresectable disease who have had RFA as primary therapy. Procedure-related complications were infrequent with a complication rate lower than 10%.[169] Complications from RFA included bleeding, fever, pain, biliary fistulas, and perihepatic abscess.[165,169] Recurrence or persistence of metastatic disease at the site of the RFA has been reported to be less than 10% after open RFA.[28,167,169,172,173] Clearly, local recurrence after RFA is highly dependent on tumor size and location within the liver. Most treatment site failures occur in large tumors (greater than 5.0 cm).[169,172,173] Intrahepatic tumor recurrence has been reported to be uncommon after RFA of tumors less than 3 cm (3.6%) compared with lesions greater than 5 cm (50%).[165,174]

Survival after ablation is difficult to interpret, as many patients who undergo this therapy are characterized by a number of poor prognostic factors (i.e., "unresectable" disease, multiple tumors, bilobar location, etc.), thereby making comparisons to patients who have undergone complete surgical resection difficult. In general, studies with isolated RFA show a median survival of about 30 to 35 months and a 3-year survival rate of 20 to 36%.[171,175–177] In a recent study comparing recurrence and outcome after hepatic resection, RFA, and combined resection and RFA for colorectal liver metastases, the authors[28] reported a significantly worse disease-free and overall survival for patients treated with RFA. Although patient selection for RFA may have biased the results of the analysis, the study nonetheless serves to highlight the need for a randomized trial comparing resection vs ablation before this therapy can be accepted as a standard for patients with potentially resectable colorectal liver metastases.

A new and emerging thermal ablation technique involves the use of microwave ablation.[178–183] Microwave ablation offers several advantages over RFA, including the speed of delivery and perhaps the ability to generate a wider zone of active heating.[184] The capacity of microwave to generate larger zones of heat are probably related to the fact that, unlike RFA, microwave energy does not appear to be limited by charring and tissue desiccation.[179] To date the experience with microwave ablation has been limited. In a small study, Seki et al.[182] reported that in 15 patients treated with microwave ablation no recurrences occurred in the treated area, although the follow-up period was short. Additional studies are necessary before microwave ablation can be widely adopted.

Chemotherapy

Conventional first-line chemotherapeutic regimens for metastatic colorectal disease contain fluorouracil (5-FU) in addition to leucovorin, which potentiates 5-FU inhibition of DNA and RNA synthesis in malignant cells (Table 3).[20] Using a bolus administration regimen, patients treated with 5-FU and leucovorin have reported response rates ranging from

Table 3 Results of Selected Chemotherapy Trials for Metastatic Colorectal Cancer

Investigator(s)	Year	Chemotherapy Regimen	Response (%)	Median Time to Progression (months)	Overall Survival (months)
Saltz et al.[17]	2000	Bolus 5-FU/Leucovorin	21.0	4.3	12.6
Saltz et al.[17]	2000	IFL	31.0–39.0	7.0	14.8–15.0
Goldberg et al.[20]	2004				
Douillard et al.[19]	2000	FOLFIRI	34.8–54.2	6.7–8.5	17.4–20.1
Koehne et al.[256]	2003				
de Gramont et al.[21]	2000	FOLFOX	45.0–50.7	8.7–9.0	16.2–19.5
Goldberg et al.[20]	2004				
Hurwitz et al.[25]	2004	IFL + Bevacizumab	44.8	10.6	20.3
Cunningham et al.[200][a]	2004	FOLFIRI + cetuximab	22.9	4.1	8.6

[a] Second line treatment in irinotecan-refractory metastatic colorectal cancer.

20 to 30% and a median survival of 11.5 months.[16,17,185–187] Although delivery of 5-FU by continuous infusion has improved the response rate and toxicity profile, patients treated with continuous infusion 5-FU alone did not enjoy a statistically significant difference in median survival.[18,188]

Over the past decade, additional chemotherapeutic and biologic agents have been found that have significantly increased activity against colorectal cancer. Irinotecan is a topoisomerase I inhibitor that was initially introduced as monotherapy for patients with metastatic colorectal cancer refractory to 5-FU.[22,23,189] Saltz et al.[17] subsequently showed that combining irinotecan with bolus 5-FU/leucovorin (i.e., IFL regimen) resulted in a higher response rate (39%), longer progression-free survival time, and longer overall survival time (14.8 months) compared with bolus 5-FU/leucovorin alone. At around the same time, Douillard et al.[19] showed that irinotecan in combination with continuous infusion of 5-FU/leucovorin (i.e., FOLFIRI regimen) produced better response rates and longer progression-free and overall survival compared to 5-FU/leucovorin alone.

Oxaliplatin, a cisplatin derivative with a similar mechanism of action to other platinum compounds, is another agent that has demonstrated significant activity in treating colorectal metastasis.[21,190] Recently, the combination of oxaliplatin and infusional 5-FU/leucovorin (i.e., FOLFOX regimen) was found to be less toxic and more efficacious than the bolus irinotecan/5-FU/leucovorin regimen.[20] Whether FOLFOX or FOLFIRI is better as first-line chemotherapy remains controversial as they have comparable response rates.[191] What may be more compelling is that when these regimens are used sequentially when progression or toxicity occurs, regardless of the order, survival is prolonged.

In addition to novel cytotoxic agents, new molecular targeted therapeutic approaches have been developed. Bevacizumab, a monoclonal antibody to vascular endothelial growth factor A that inhibits angiogenesis, has been studied in phases II and III trials in patients with metastatic colorectal cancer.[25,192] The addition of bevacizumab to cytotoxic chemotherapy has been shown to improve response rates and progression-free survival.[25,192] In one study, adding bevacizumab to bolus IFL increased the response rate from 35 to 45% and the median overall survival from 15.6 to 20.3 months.[25] Currently, bevacizumab is being combined with infusional chemotherapy as first-line therapy in many patients with metastatic colorectal cancer.

It is important to note that treatment with bevacizumab has important implications for patients being considered for surgery. Specifically, in patients undergoing hepatic resection of colorectal metastases, the use of bevacizumab-containing regimens may result in increased risk of bleeding, wound healing complications, or even impaired liver regeneration.[193] The mean serum half-life of bevacizumab is approximately 20 days, but the range varies between 11 and 50 days.[194] In general, a 6- to 8-week waiting period after the last dose of bevacizumab is recommended before performing an elective hepatic resection and a similar delay is recommended to initiate this antibody therapy after liver resection.[193]

The epidermal growth factor receptor (EGFR) signaling pathway has also been targeted by new biologic agents aimed at blocking the downstream signaling cascade that leads to tumor cell proliferation, migration, and inhibition of apoptosis.[195] Cetuximab, an anti-EGFR monoclonal antibody, has been shown to have activity as a single agent, and when used in combination with cytotoxic chemotherapy, results in synergistic antitumor response.[196–199] Saltz et al.[26] reported a phase II study in which the response rate of cetuximab plus irinotecan in patients with irinotecan-refractory colorectal metastasis was 22.5%. In another study by Cunningham et al.,[200] response rates of 10.8% for cetuximab alone and 22.9% for cetuximab plus irinotecan were reported. Ongoing trials are addressing the role of cetuximab as first-line therapy and in combination with

bevacizumab. In addition, other biologic agents targeting the EGFR are currently under investigation.

Downsizing of Unresectable Metastases

The improved efficacy of chemotherapy agents has not only allowed increased patient survival in the noncurative setting, but has also allowed a subset of previously unresectable patients to undergo liver surgery after tumor downsizing.[201–203] By reconsidering the initial unresectability of patients who strongly respond to chemotherapy, several investigators have shown that survival can be achieved by liver resection in a significant proportion of patients who otherwise would have had a poor outcome.[201–203] Specifically, about 15 to 20% of patients with initially unresectable disease (defined as either the inability to resect all tumor while leaving 20% of the total liver volume or by concomitant presence of extrahepatic metastases) have significant tumor downsizing to the point that the metastatic disease can ultimately be considered resectable (Fig. 2).[201,203] Adam et al.[202] reported that rescue surgery for unresectable colorectal liver metastases downsized by chemotherapy resulted in a 5-year survival rate of 33%. Other investigators[204–206] have substantiated these findings and resection of initially nonresectable liver metastases after systemic chemotherapy has become increasingly adopted worldwide.

Adjuvant and Neoadjuvant Chemotherapy for Resectable Disease

The role of adjuvant chemotherapy after potentially curative resection of liver metastases is uncertain. Given that the majority of patients with hepatic metastasis experience recurrence after resection, undetectable disease probably often exists at the time of surgery. Systemic treatment of this subclinical disease with adjuvant chemotherapy is therefore rational. No prospective study has examined the use of adjuvant systemic chemotherapy after complete resection of liver tumors. Yet, based largely on randomized data on the benefit of adjuvant systemic chemotherapy for stage III disease and the high response rates in stage IV patients with measurable disease, the use of a combination regimens of chemotherapy is reasonable, which is often recommended as stage IV adjuvant therapy after liver resection, at least when the patient is chemonaïve.

There is also increasing interest in using chemotherapy preoperatively for patients who present with *resectable* hepatic colorectal liver metastases. The rationale for this policy has been supported by the better prognosis obtained with neoadjuvant chemotherapy and surgery, compared with immediate surgery in patients with multinodular colorectal liver metastases.[207,208] Preoperative systemic chemotherapy may also be associated with a lower (3%) rate of positive surgical margins compared with immediate resection (9 to 19%).[209,210]. A trial of chemotherapy also provides time to identify those patients who will develop progressive disease and therefore may not benefit from liver resection.[207] Allen et al.[211] reported that patients whose disease did not progress while they were receiving neoadjuvant chemotherapy experienced improved survival after liver resection compared with patients who did not receive chemotherapy. In a study by Adam et al.,[207] patients who had tumor progression while receiving neoadjuvant chemotherapy only had an 8% overall 5-year survival rate vs 37% for patients who had an objective tumor response to preoperative chemotherapy. These data call into question the utility of hepatic resection in patients who fail to respond to neoadjuvant therapy and may have important implications for the therapeutic strategy adopted by medical oncologists and surgeons.[207]

Although the use of newer chemotherapeutic agents may improve the resectability of some colorectal metastases, the effect that these agents have on the underlying liver parenchyma remains ill-defined. Some studies have associated the use of oxaliplatin with an increased incidence of hepatic sinusoidal obstruction,[212] whereas others have suggested that irinotecan may be associated with steatosis.[213] Fernandez et al.[213] reported that preoperative administration of oxaliplatin or irinotecan was associated with an increased risk of steatohepatitis, especially in the obese. This study, however, included only 37 patients and needs to be confirmed in a large cohort of patients.

The decision to give chemotherapy before or after surgery should be individualized and based on the specific clinical situation (Table 4). In patients who are chemonaïve with multiple metastases and stage IV disease at presentation, neoadjuvant chemotherapy may be appropriate. Neoadjuvant chemotherapy also may be warranted in a patient who is marginally resectable, in which case a response would improve the outcome of the resection. In contrast, if a patient has comorbidities or there is concern about chemotherapy causing hepatoxicity in a patient who will require an extended resection, initial surgery would be more appropriate. The other factor to consider is whether a patient has been exposed to chemotherapy or not, either in the metastatic setting or in the adjuvant setting. If a patient has received adjuvant chemotherapy for his or her primary disease and the disease recurred early, then one may be less inclined to use neoadjuvant chemotherapy.

Regional Therapy

Regional chemotherapy has been proposed as treatment for unresectable colorectal disease or as adjuvant therapy after hepatic resection.[214–218] Although several randomized con-

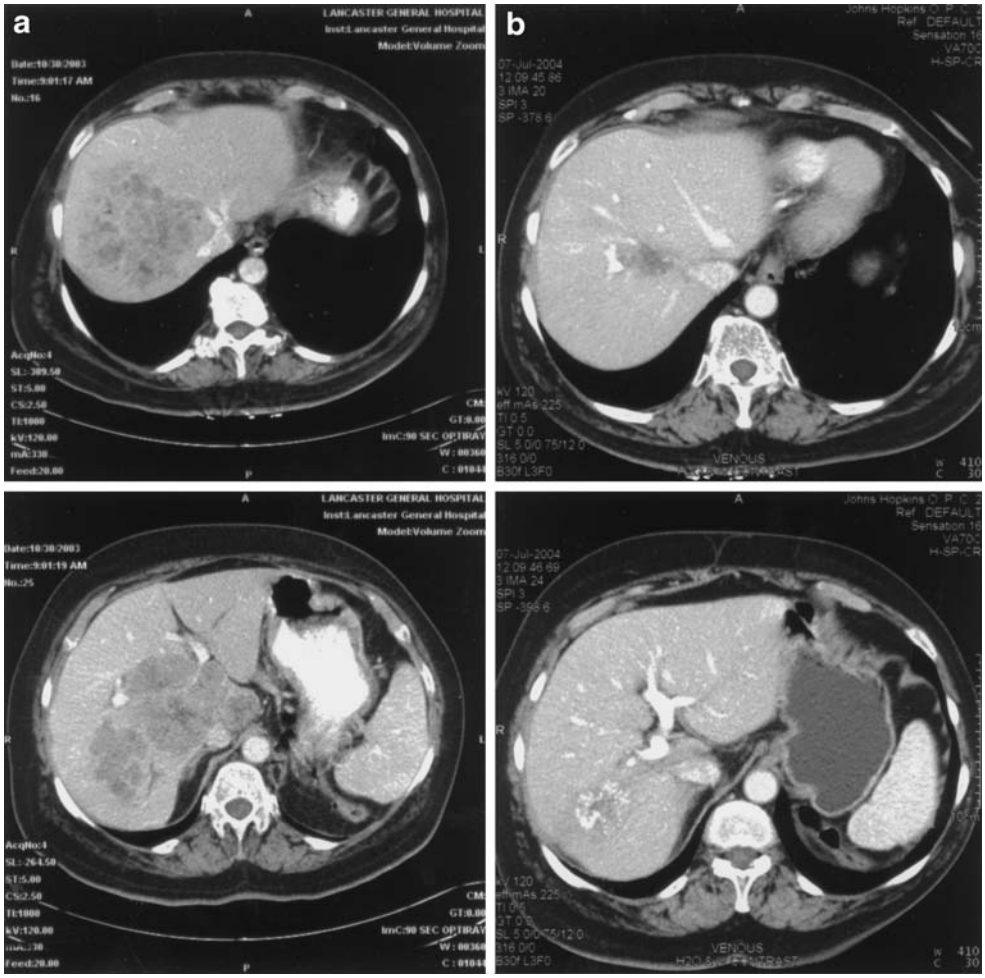

Figure 2 **A** A CT scan showing unresectable large hepatic metastases with tumor abutting the right and middle hepatic veins, and involving the portal vein bifurcation. **B** A CT scan of the same patient after treatment with preoperative systemic chemotherapy. Note the dramatic reduction in the size of the tumor mass and the shrinkage of tumor away from the portal vein bifurcation, thereby making the tumor amenable to resection.

trol trials have demonstrated that hepatic arterial infusion (HAI) chemotherapy with fluorodeoxyuridine (FUDR) produced better response rates in patients with unresectable colorectal liver metastases than systemic chemotherapy, an improvement in survival was not detected.[214–216,218] Hepatic arterial infusion as adjuvant therapy after hepatic resection has also not been shown to confer an improvement in long-term survival—although the study was not powered to evaluate survival as a primary endpoint.[217]

Most studies have shown that the majority of patients with recurrence after hepatectomy for liver metastases develop extrahepatic disease (either distant only disease or distant disease plus intrahepatic recurrence).[107,219–222] Hepatic arterial infusion may be appropriate in patients with liver only unresectable/unablatable disease, or perhaps in those patients who have failed systemic chemotherapy and have liver only disease. The use of HAI in the adjuvant setting remains controversial and is currently the subject of an ongoing clinical trial (NSABP-C-09). This study compares capecitabine and oxaliplatin either alone or in combination

Table 4 Preoperative vs Postoperative Chemotherapy for Resectable Colorectal Liver Metastases

Clinical Situations
Advantages:
1. Allows time for other metastatic sites to declare themselves and become clinically evident.
2. Allows for in vivo gauge of chemoresponsiveness, facilitating postoperative chemotherapy planning.
3. Response may allow for easier resection and increased rate of negative surgical margins.
4. Response may be a prognostic factor.
Disadvantages:
1. Tumors may progress to unresectable status.
2. Perioperative morbidity may be increased because of hepatotoxicity of chemotherapy.
3. Possible loss to surgical follow-up.
4. Patient anxiety and desire to have the tumor resected as soon as possible.

with HAI with FUDR in patients who have had resection or ablation of hepatic metastases.

Another possible local therapeutic option for unresectable or unablatable liver metastases is intraarterial embolization. Whereas more commonly used with hypervascular tumors such as hepatocellular carcinoma, transarterial chemoembolization (TACE) involves the selective administration of chemotherapy combined with the embolization of the vascular supply of the tumor. Transarterial chemoembolization treatment is intended to deliver a locoregional dose of chemotherapy while also inducing selective ischemia to the liver tumor.[223,224] Multiple studies[225–229] report that response to TACE is measured in months and all patients have eventual progression of disease with little improvement in survival. Transarterial chemoembolization, therefore, has a limited clinical role in the treatment of patients with colorectal liver metastases. More recently, intraarterial therapy with yttrium-90 microspheres has gained some popularity.[230–233] This approach may achieve greater response rates than TACE in hypovascular tumors such as colorectal metastases. Currently, radioembolization is being used in selected cases in the palliative setting. Its role in the management of hepatic colorectal cancer awaits further trials to demonstrate a benefit.

Special Considerations

Management of Synchronous Presentation of Primary Colorectal Cancer and Hepatic Metastases

The optimal timing for surgical resection of synchronous colorectal liver metastasis and the primary tumor has not been well defined. Traditionally, most investigators have recommended a staged approach with initial resection of the primary colorectal tumor followed by hepatic resection 8 to 12 weeks later.[33,35,109,234,235] Proponents of this approach cite an increased morbidity associated with the combined procedure. More recently, with advances in surgical and perioperative care, several studies have reported that simultaneous resection of colon and liver tumors results in morbidity, length of hospital stay, and perioperative mortality comparable to staged resection.[236–239] In addition, because a second laparotomy can be avoided, simultaneous resection of the primary colon tumor and hepatic metastasis may be preferred as it permits earlier completion of surgical therapy, thereby allowing more prompt initiation of adjuvant therapy.[236] The final decision whether to perform a simultaneous or staged resection, however, should be individualized. Simultaneous resection

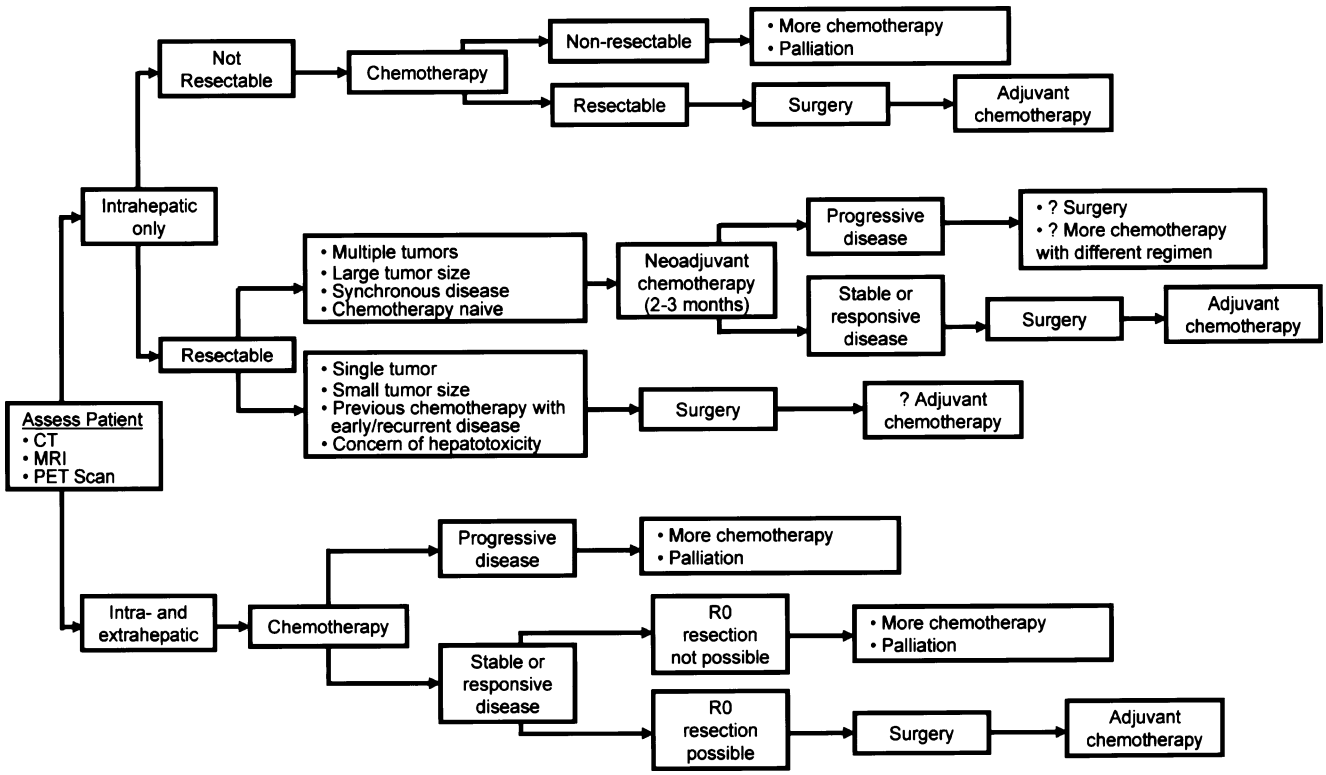

Figure 3 Proposed treatment algorithm for patients with colorectal metastases. Among other factors, therapy should be dictated by site of disease (intra- vs extrahepatic) and response to chemotherapy. Because more efficacious chemotherapy agents have allowed a subset of previously unresectable patients to undergo liver surgery after tumor downsizing, patients should be reassessed on a routine basis.

may be more appropriate in patients who require either a straightforward colon resection (i.e., right hemicolectomy) and a major liver resection or in those patients who require a more complex colorectal resection (i.e., abdominoperineal resection) and a limited liver resection (i.e., wedge or left lateral sectionectomy). Patients who require both a complex colorectal and hepatic resection are probably best managed with a staged approach. Ultimately, the decision whether to perform a staged or simultaneous resection needs to be based on the experience of the surgeon, the estimated time necessary to perform both operations, and the flow of the case intraoperatively.

Repeat Hepatectomy

After hepatic resection of colorectal liver metastasis, 50 to 60% of patients will have a recurrence.[220,221,240–243] In a subset of patients, the disease will recur solely as isolated intrahepatic disease[220,240–242] and repeat hepatic resection may therefore be indicated. Although repeat liver resection may be technically more demanding and difficult, most series report comparable perioperative morbidity and mortality compared with the first hepatectomy.[155,243,244] Five-year overall survival rates of 16 to 41% have been reported from several institutions.[242,243,245] Repeat hepatectomies can provide long-term survival and should be offered to patients based on the same criteria as those used for initial hepatectomy.[243]

Extrahepatic Metastatic Disease

Extrahepatic disease has traditionally been considered a contraindication to resection of hepatic colorectal cancer metastasis.[9,31,32,104] However, improvements in morbidity and mortality rates after hepatectomy, as well as the advent of more effective systemic chemotherapy agents, have prompted several investigators[246–249] to attempt surgery for some of these patients. Elias et al.[249] reported that the 5-year survival rate after hepatectomy for colorectal liver metastasis and simultaneous resection of extrahepatic disease with curative intent was 29%. The distribution of extrahepatic disease included hilar lymph nodes, peritoneal carcinomatosis, retroperitoneal nodes, and lung metastasis. No statistically significant difference in outcome was found relating to the site of extrahepatic disease; however, this may have been because of insufficient statistical power. Patients with peritoneal carcinomatosis or extrahepatic disease at multiple sites did tend to have a worse survival than patients with single-site extrahepatic disease.[249] In contrast, patients with only pulmonary metastasis as a site of extrahepatic disease have been shown to have a particularly good outcome after complete metastasectomy of both liver and lung disease.[250–253] Five-year survival rates ranging from 22 to 48% have been reported after surgery for colorectal pulmonary metastasis.[250–254] Consequently, extrahepatic disease in colorectal cancer patients with liver metastasis should no longer be considered a contraindication to hepatectomy. Patients with simultaneous hepatic and extrahepatic colorectal metastasis do, however, need to be well selected for surgery. Position emission tomography and computed tomography can often be useful in evaluating the full extent of metastatic disease.[89] In those patients with extrahepatic metastases, preoperative chemotherapy should be strongly considered, especially in patients with evidence of multivisceral metastatic disease.

Conclusion

Liver resection currently represents the only potentially curative therapeutic option for hepatic colorectal metastasis with 5-year survival rates of 35 to 58%.[10,28–36] By understanding the diagnostic and therapeutic options, patients with colorectal metastases can be treated with a rational approach (Fig. 3). Recent improvement in whole body and hepatic imaging has allowed for more accurate selection of patients with colorectal liver metastasis. Preoperative imaging is now able to detect minimal burdens of metastatic disease that in the past would have been difficult to detect. Traditional clinicopathologic factors, although helpful in stratifying patients with regard to prognosis, should not be used to exclude otherwise resectable patients from surgery. The use of modern surgical techniques is reducing perioperative morbidity and mortality, whereas PVE and preoperative systemic chemotherapy can expand the population of patients who are candidates for surgical treatment. In addition, more active systemic chemotherapy agents are being increasingly employed as adjuvant therapy to improve survival in resected patients.

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Usefulness of Computed Tomography as a Preoperative Diagnostic Modality in a Case with Acute Jejunogastric Intussusception

Yasushi Hashimoto · Shuji Akagi · Yoshihiro Sakashita · Michio Takamura · Hiroshi Iwako · Yusuke Watadani · Norifumi Shigemoto · Taijiro Sueda

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Keywords Jejunogastric intussusception · Gastric surgery · Intestinal obstruction · Complications

Abbreviations

JGI	jejunogastric intussusception
CT	computed tomography
GI	gastrointestinal
US	ultrasonography

Case Report

A 72-year-old man was admitted to our hospital with an acute abdomen 1 h after the abrupt onset of hematemesis and upper abdominal pain. His medical history included a distal gastrectomy for gastric cancer 15 years previously and a subsequent gastrojejunostomy with Braun's anastomosis because of an anastomotic stricture. Physical examination at the time of admission revealed diffuse abdominal tenderness with muscular guarding and a palpable firm mass in the left upper quadrant.

A plain X-ray of the abdomen showed dilatation of the small intestine, but this finding was not specific enough to lead to a diagnosis. A subsequent abdominal computed tomography (CT) showed intestinal loops intussuscepted into the patient's severely dilated gastric remnant through the gastrojejunostomy (Fig. 1a). Contiguous CT sections identified a normal afferent loop and intussuscepted efferent loops extending into the lower abdomen (Fig. 1b, c). Together, these findings suggested that the intussusception into the stomach involved the efferent loop, indicating the presence of a type II jejunogastric intussusception (JGI).[1,2] The patient was immediately taken into surgery.

Surgery revealed a severely dilated stomach stump and an 80-cm-long efferent intestinal loop that had intussuscepted in a retrograde direction at the gastrojejunostomy into the remnant gastric lumen, passing over the Braun's anastomosis, which is in agreement with the preoperative diagnosis made by CT (Fig 2). After unsuccessful efforts to reduce this invagination by Hutchinson's procedure,partial resection of a 100-cm-long small intestine, with end-to-end anastomosis and efferent loop fixation, was subsequently performed. The resected specimen was found to be gangrenous without perforation for a distance of 10 cm below the Braun's anastomosis. No abnormalities such as a tumor, ulcer, diverticulum, or stenosis were identified that could have acted as a leading point for the intussusception. The JGI in this case was thus considered to be a late complication of gastrojejunostomy with Braun's anastomosis.

Y. Hashimoto · Y. Sakashita · M. Takamura · H. Iwako · Y. Watadani · N. Shigemoto
Department of Surgery, Oda Municipal Hospital,
1428-3 Yoshinaga, Oda-cho Oda,
Shimane 694-0063, Japan

S. Akagi
Department of Internal Medicine, Oda Municipal Hospital,
1428-3 Yoshinaga, Oda-cho Oda,
Shimane 694-0063, Japan

Y. Hashimoto (✉) · T. Sueda
Department of Surgery, Division of Clinical Medical Science,
Programs for Applied Biomedicine,
Graduate School of Biomedical Sciences,
Hiroshima University,
Kasumi 1-2-3, Minami-ku,
Hiroshima 734-8551, Japan
e-mail: hashimoto@hiroshima-u.ac.jp

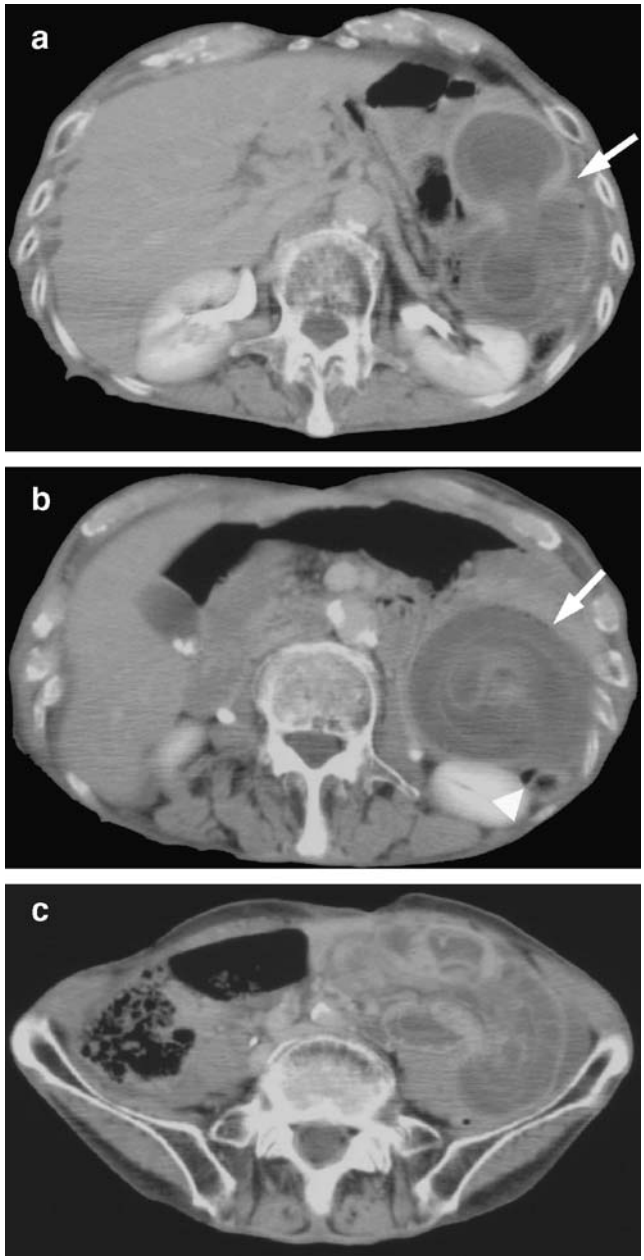

Figure 1 Abdominal CT findings. **a** The intestinal loops are intussuscepted into the severely dilated gastric remnant through the gastrojejunostomy in the upper abdomen (arrow). **b** Layer structure in the intestinal loop, highlighting intussuscepted efferent loops (arrow) and a normal afferent loop (arrowhead). **c** This CT section, contiguous with subpanels **a** and **b**, shows the presence of intussuscepted efferent loops in the lower abdomen.

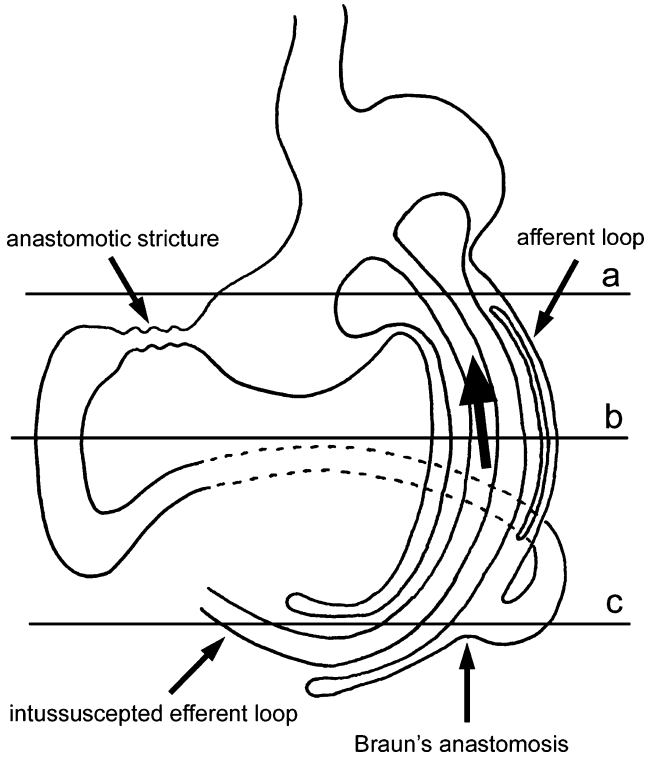

Figure 2 Schematic diagram of the intussusception. An approximately 80-cm segment of efferent jejunal loop intussuscepted retrogradely into the remnant gastric lumen through the jejunogastric anastomosis, passing over the Braun's anastomosis. The arrow shows the route of the invagination. Lines a, b, and c correspond to the position of CT sections in Fig. 1a–c, respectively.

Discussion

Jejunogastric intussusception is a rare complication of gastric surgery and has been associated with various methods of gastroenteric anastomosis, including the Roux-en-Y anastomosis.[3] Jejunogastric intussusception has been classified into three types according to the invaginated loop[1,2]: afferent loop invagination (type I), efferent loop invagination (type II), and the intussusception of both loops (type III). A type II intussusception is observed in most cases and corresponds to the form of intussusception identified precisely by CT in our case. Clinically, JGI also presents in two forms, acute and chronic.[2] The acute form presents with the peritoneal stimulating sign, and in most cases, requires immediate surgical exploration as was seen in the present case. Delaying surgery for more than 48 h raises the mortality rate.[1] Therefore, immediate reduction of the intussuscepted segment is the most effective treatment because of the significant risk of severe complications. In our case, the presence of a nonviable segment of intussuscepted intestine required surgical resection; early diagnosis enabled this procedure to be performed in a timely fashion.

Studies have indicated that gastrography, gastroendoscopy, or ultrasonography (US)[2,4] can establish the diagnosis of JGI preoperatively by identifying dilatated loops of small intestine within the stomach. Gastroendoscopy and gastrography occasionally can be diagnostic[4] but are contraindicated in the management of acute JGI when the peritoneal-stimulating sign is present. On the other hand, abdominal US, a widely used and less invasive method for diagnosing abdominal disease,

could not detect the dilation and obstruction of the intestine in the present case because of profuse intestinal gas. Indeed, it is unlikely that US can play a major role in the diagnosis of JGI because massive intestinal gas is frequently seen in such cases.

Because of its recent advent as an imaging technique, abdominal CT has been able to visualize intraabdominal pathological findings in greater detail than was previously possible.[5] We believe that abdominal CT is the most useful procedure for identifying JGI when profuse abdominal gas prevents US from visualizing invagination. Prompt abdominal CT in advance of exploratory laparotomy should be considered in all postgastrectomy patients with severe abdominal pain of unclear etiology. In conclusion, this case demonstrates the need for clinicians to consider JGI as a possible cause of abdominal pain in patients with a history of gastric surgery. Performing abdominal CT studies in such patients may enable early diagnosis and prompt surgical intervention.

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Hernia Sac Laparoscopy under Spinal Anesthesia for Evaluation of Reduced Incarcerated Inguinal Hernia

Kazuaki Takabe

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Abstract Hernia sac laparoscopy (laparoscopy through an inguinal hernia sac) is a useful method to evaluate the viability of the self-reduced bowel of incarcerated inguinal hernia that is suspected for strangulation, and avoid unnecessary exploratory laparotomy. On the other hand, peritoneal insufflation for laparoscopy is best avoided in patients with severe chronic obstructive pulmonary disease or poor cardiac output. Here, we describe a 78-year-old male with chronic obstructive pulmonary disease and congestive heart failure, whose incarcerated inguinal hernia self-reduced when he was given spinal anesthesia. Bowel viability was in question, so hernia sac laparoscopy without gas was performed, which allowed us adequate evaluation of the reduced bowel by positioning alone, avoiding both exploratory laparotomy and peritoneal insufflation. In our case, hernia sac laparoscopy under spinal anesthesia without pneumoperitoneum was sufficient to obtain necessary information with minimal surgical stress.

Keywords Hernia sac · Inguinal hernia · Incarceration · Spinal anesthesia · Laparoscopy

A 78-year-old man with known right inguinal hernia had 6 hours of severe constant pain and a right groin mass that extended to his scrotum, accompanied by nausea. He had a background of chronic obstructive pulmonary disease (COPD), coronary artery disease, myocardial infarction, congestive heart failure, type II diabetes mellitus, and hypertension. His chest x-ray showed centrilobular emphysema with honeycombing, calcified apical granulomas, and both hilar and mediastinal lymphadenopathy attributed to idiopathic pulmonary fibrosis.

Upon presentation, he had a tender, hard, nonreducible right groin mass measuring 7×5 cm. His scrotum was erythematous and tender. His abdomen was diffusely tender, but not rigid. He was afebrile and had a normal white blood cell count. The hernia could not be reduced using analgesia, sedation, or Trendelenberg positioning; therefore, he was taken to the operation room for open exploration and hernia repair.

Spinal anesthesia using 15 mg of Bupivacaine at the level of L3-4 was chosen, as the patient had COPD and cardiac risks. When the patient was positioned supine after induction of spinal anesthesia, his hernia spontaneously reduced by itself. Upon exploration, we noted a thick, empty hernia sac. Dark, turbid discharge was noted to be emanating from the peritoneal cavity, suggesting that strangulated bowel may have been reduced into the abdominal cavity.

We inserted a 5-mm trocar through the hernia sac, after placing an air-tight purse-string suture on the hernia sac and putting the patient in Trendelenburg position. A subserosal hematoma was seen in a portion of small bowel immediately below the hernia sac, which was presumably the piece of bowel that had been incarcerated (Fig. 1). All visible bowel looked viable and displayed peristalsis. The purse-string suture was closed, the sac was amputated at the internal ring, and we completed a routine herniorraphy with mesh plug and patch. There has been no complication or recurrence over a follow-up period of 2 years.

Discussion

Standard management of incarcerated inguinal hernia is to manually reduce the hernia, and to explore the abdomen when

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K. Takabe (✉)
Department of Surgery, University of California San Diego School of Medicine,
402 Dickinson Street, Code 8402,
San Diego, CA 92103, USA
e-mail: ktakabe@comcast.net

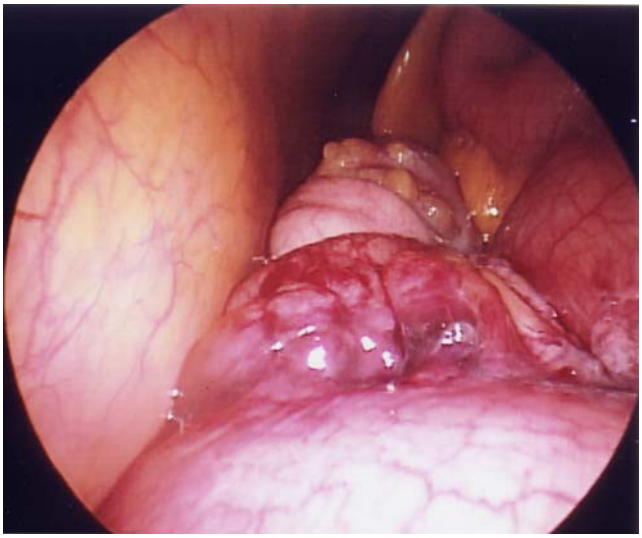

Figure 1 Hernia sac laparoscopy view of the bowel segment, which self-reduced from incarcerated inguinal hernia. The limited view does not allow evaluation of the whole abdomen. However, it is sufficient to adequately evaluate the reduced portion of the bowel.

bowel ischemia is suspected. The potential for reducing gangrenous intestine occurs in up to 1% of cases, but diagnosing intestinal ischemia is notoriously difficult,[1] with only a long period of incarceration correlating with non-viability of the incarcerated viscus.[2] Therefore, it is essential to evaluate the viability of the incarcerated hernia content. When self-reduction of the hernia content occurs before this evaluation, abdominal exploration may be unavoidable.

Hernia sac laparoscopy, the introduction of the laparoscope through an open inguinal hernia sac, is utilized in pediatric surgery to assess the contralateral inguinal ring for a patent processus vaginalis.[3] It has also been shown that hernia sac laparoscopy is a useful method to evaluate the viability of a reduced segment of the bowel after incarcerated inguinal hernia to avoid laparotomy.[4] When pneumoperitoneum is obtainable, hernia sac laparoscopy allows for a good view of the entire abdomen. Some have advocated retrieving the hernia contents through the hernia sac by obtaining another port after evaluation by hernia sac laparoscopy.[5] In a prospective laparoscopic series, only five out of 24 cases needed bowel resection, implying that bowel remains viable in most incarcerated inguinal hernias.[6] This is in agreement with another study in which incarcerated inguinal hernias were intentionally reduced in the operating room, and only one in three patients displayed hemorrhagic fluid in the hernia sac, implying the need for further exploration.[7] Taken together, these results suggest that bowel ischemia does not occur frequently, and exploratory laparotomy may be replaced by hernia sac laparoscopy. Our case had a good indication for hernia sac laparoscopy, as both the clinical course and the dark hemorrhagic fluid in the hernia sac suggested strangulation.

The adverse effects of carbon dioxide pneumoperitoneum for laparoscopy on the cardiopulmonary system have been well described.[8] Peritoneal insufflation causes an increase in intraabdominal volume and pressure, which proportionally decreases pulmonary compliance and vital capacity. This may result in hypercapnea and/or hypoxia. Absorption from the peritoneum increases delivery of carbon dioxide to the lung as much as 50%, which may lead to acidosis in patients with sepsis, COPD, or poor cardiac output. Increased intraabdominal pressure increases preload, with resultant increase in cardiac work. It may also decrease cardiac output in hypovolemic patients. Hypercapnea can cause myocardial depression. Our patient was at high risk for these complications because of COPD and congestive heart failure caused by myocardial infarction.

Our major concern was the quality of visualization, as it has been reported that visualization is impaired by free peritoneal fluid and edematous intestines.[9] We found that this was not the case, and we were able to obtain sufficient exposure by Trendelenberg positioning alone without pneumoperitoneum. This was mainly because the bowel in question was located immediately below the hernia ring, which should usually be the case.

Hernia sac laparoscopy can be very useful in this limited setting, preventing unnecessary laparotomy after spontaneous reduction of incarcerated hernia upon induction of anesthesia, including spinal anesthesia.

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