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Hepaticojejunostomy—Analysis of Risk Factors for Postoperative Bile Leaks and Surgical Complications

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Abstract Anastomoses between the jejunum and the bile duct are an important component of many surgical procedures; however, risk factors for clinically relevant bile leaks have not yet been adequately defined. The objective of this study was to describe the incidence of bile leaks after hepaticojejunostomy and to define predictive factors associated with this risk and with surgical morbidity. Between October 2001 and April 2004, hepaticojejunostomies were performed in 519 patients in a standardized way. Patient- and treatment-related data were documented prospectively. A bile leak was defined as bilirubin concentration in the drains exceeding serum bilirubin with a consecutive change of clinical management or occurrence of a bilioma necessitating drainage. Surgical morbidity occurred in 15% of patients, the incidence of a bile leak was 5.6%. Multivariate analysis confirmed preoperative radiochemotherapy, preoperative low cholinesterase levels, biliary complications after liver transplantation necessitating a hepaticojejunostomy, and simultaneous liver resection as risk factors for bile leakages, whereas biliary complications after liver transplantation necessitating hepaticojejunostomy, simultaneous liver resection, and diabetes mellitus were significantly associated with postoperative surgical morbidity. Our results demonstrate that hepaticojejunostomy is a safe procedure if performed in a standardized fashion. The above found factors may help to better predict the risk for complications after hepaticojejunostomy.

Keywords Hepaticojejunostomy · Bile leak · Risk factors · Surgical morbidity

Introduction

Anastomoses between the biliary and the gastrointestinal system are commonly performed in abdominal surgery. The first surgical anastomosis between the biliary and the gastrointestinal system was created using the gall bladder and the colon.[1] The first cholecystojejunostomy was then performed in 1887 in a patient with a metastasized periampullary carcinoma.[2] In 1891, Sprengel described the first anastomosis between the common bile duct and the duodenum.[3] A hepaticojejunostomy was first reported by Dahl 1909, modifications of this procedure were then later published by many different authors.[4,5] Since those days, hepaticojejunostomy has remained an important component of many surgical procedures, including pancreaticoduodenectomy for benign and malignant neoplasms, liver transplantation, resection of bile duct tumors, palliative surgical approaches for unresectable obstructive tumors, repair after bile duct injuries, and surgical procedures for chronic pancreatitis and choledocholithiasis. Biliary leaks after hepaticojejunostomy represent a major complication carrying a high risk for prolonged hospital stay, biliary peritonitis, and the need for placing interventional drainages or even relaparotomy.[6,7] Despite the frequent use of

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this type of anastomosis, a precise analysis of risk factors associated with postoperative bile leaks is currently lacking in the literature. The aims of this study were, therefore, to describe the incidence of bile leaks after hepaticojejunostomy and to define predictive factors associated with this risk.

Patients and Methods

The study included all 519 patients who underwent hepaticojejunostomy in a standardized way (interrupted sutures using PDS 5–0, no transanastomotic stent) between October 1, 2001, and May 30, 2004, at the Department of Surgery, University of Heidelberg, Germany. The morbidity, mortality, and bile leak rate of a subgroup of patients has been reported in an earlier manuscript.[8] Patient demographics such as age and gender, ASA score, type of operation, duration of operation, surgeon experience, blood loss, transfusions, body mass index, preoperative laboratory values [bilirubin, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, γ-glutamyl transpeptidase, cholinesterase], hospital course, and postoperative complications were analyzed. The patients' characteristics are listed in Table 1. The normal values for the used laboratory parameters are as follows: bilirubin, <1.0 mg/dl; ALT and AST, <50 U/l; alkaline phosphatase, 40–130 U/l; γ-glutamyl transpeptidase, <60 U/l; and cholinesterase, 5.32–12.9 kU/l.

A bile leak was defined as bilirubin concentration in the drains exceeding serum bilirubin with a consecutive change of clinical management or occurrence of a bilioma necessitating drainage. Postoperative morbidity was divided into surgical and medical morbidity. Surgical morbidity included the following complications: postoperative anastomotic leak (any anastomotic leak), stenosis of the hepaticojejunostomy, wound infection, intra-abdominal abscess, peritonitis, postoperative hemorrhage, intraperitoneal hematoma, lymphatic leaks, bilioma, abdominal wall dehiscence, and visceral perforation. Medical morbidity included all other complications such as postoperative respiratory, cardiac, renal, and gastrointestinal complications.

Statistical Analysis

Statistical computations were performed with JMP (SAS institute, Cary, NC, USA) and SPSS (SPSS, Chicago, IL, USA). Continuous variables were expressed as medians and were compared using the Wilcoxon test, whereas categorical variables were compared using Fisher's exact test or chi-square test. Multivariate logistic regression was performed by including factors with a p value ≤ 0.05 in the univariate analysis. Statistical significance was defined as $p \leq 0.05$.

Table 1 Patient Characteristics of 519 Patients Undergoing Hepaticojejunostomy

Variable	*n*	%
Gender		
Male	318	61
Female	201	39
ASA status		
I	12	2
II	264	51
III	237	47
IV	6	1
Diagnosis		
Redo after liver transplantation[a]	6	1
Benign pancreatic tumor	28	5
Cholangitis/choledocholithiasis	33	6
Liver cirrhosis	24	5
Chronic pancreatitis	68	13
Bile duct tumors/gallbladder cancer	34	7
Pancreatic cancer	317	61
Other	9	2
Surgical procedure (in addition to hepaticojejunostomy)		
Liver resection	10	2
Hepaticojejunostomy only	162	31
Liver transplantation	26	5
Total pancreatectomy	21	4
Pancreaticoduodenectomy	300	58
Perioperative transfusion		
Yes	143	28
No	519	72
Morbidity		
Yes	178	34
No	341	66
Surgical morbidity		
Yes	76	15
No	443	85
Medical morbidity		
Yes	123	24
No	396	76
Postoperative bile leak		
Yes	29	5.6
No	490	94.4
Mortality		
Yes	7	1.3
No	512	98.7

[a] Biliary complications after liver transplantation necessitating a hepaticojejunostomy

Results

Patient Characteristics and Hospital Course

The median age of the 519 patients included in the study was 61 years (range: 52–69 years). The median operative time was 5.7 h (range 4.25–7 h), the median blood loss was 600 cm^3 (range, 300–1,000 cm^3) and the median hospital

Table 2 Univariate Analysis of Factors Associated With Postoperative Bile Leaks

Variable	Bile Leak	No Bile Leak	*p* Value
Gender			
Male	21 (7%)	297 (93%)	ns (0.4)
Female	8 (4%)	193 (96%)	
Age[a] (years)	64 (54–70)	61 (52–69)	ns (0.7)
Diagnosis[b]			
Redo after liver transplant[c]	3 (50%)	3 (50%)	0.003
Benign pancreatic tumor	0 (0%)	28 (100%)	ns (0.4)
Cholangitis	2 (6.0%)	31 (94%)	ns (0.7)
Liver cirrhosis	2 (7.7%)	22 (92.3%)	ns (0.6)
Chronic pancreatitis	3 (4.4%)	65 (95.6%)	ns (1.0)
Bile duct tumor	4 (11.8%)	30 (88.2%)	ns (0.1)
Pancreatic cancer	13 (4.1%)	304 (95.6%)	ns (0.08)
Others	2 (22%)	7 (78%)	ns (0.09)
Procedure[b]			
Liver resection	5 (50%)	5 (50%)	<0.0001
Liver transplantation	2 (7.7%)	24 (92.3%)	ns (0.7)
Total pancreatectomy	0 (0%)	21 (100%)	ns (0.6)
Pancreaticoduodenectomy	11 (3.7%)	289 (96.3%)	0.03
Hepaticojejunostomy	11 (6.8%)	151 (93.2%)	ns (0.4)
Vascular resection			
Yes	0 (0%)	41 (100%)	ns (0.2)
No	29 (6.1%)	449 (93.9%)	
Intraoperative radiation			
Yes	2 (15.4%)	11 (84.6%)	ns (0.2)
No	27 (5.3%)	479 (94.7%)	
Preoperative radiochemotherapy			
Yes	6 (12%)	44 (88%)	0.05
No	23 (4.9%)	446 (95.1%)	
Diabetes mellitus			
Yes	3 (7.1%)	39 (92.9%)	ns (0.7)
No	26 (5.5%)	451 (94.5%)	
ASA status			
I/II	16 (6%)	260 (94%)	ns (0.9)
III/IV	13 (5%)	230 (95%)	
Surgeon's experience			
>30 hepaticojejunostomies	12 (4%)	299 (96%)	0.05
≤30 hepaticojejunostomies	17 (8%)	188 (92%)	
Perioperative transfusion			
Yes	12 (8.4%)	131 (91.6%)	ns (0.1)
No	17 (4.5%)	359 (94.5%)	
Preoperative bilirubin[a] (mg/dl)	1.3 (0.7–3.4)	1 (0.6–3.3)	ns (0.3)
Preoperative ChE[a] (kU/l)	4.4 (3.4–6.8)	6.2 (4.3–8.6)	0.003
Preoperative AP[a] (U/l)	198 (91–458)	163 (94–365)	ns (0.5)
Preoperative AST[a] (U/l)	30 (17–71)	28 (15–73)	ns (0.7)
Preoperative ALT[a] (U/l)	48 (22–167)	46 (20–120)	ns (0.8)
Preoperative GT[a] (U/l)	190 (34–330)	94 (27–281)	ns (0.4)
Body mass index[a]	23.9 (22–26)	24 (22–26)	ns (0.9)
Operation time[a] (hours)	6 (3.8–7.3)	5.8 (4.3–6.75)	ns (0.7)
Blood loss (cm^3)	800 (225–1,100)	600 (300–1,000)	ns (0.6)

ns=not significant, AP=alkaline phosphatase, GT=glutamyl transpeptidase, ChE=cholinesterase
[a] Median and interquartile range
[b] Each factor was individually tested against all the others
[c] Biliary complications after liver transplantation necessitating a hepaticojejunostomy

stay was 12 days (range 9–15 days). A total of 24 different surgeons performed the hepaticojejunostomies in this series; 21 of them were attending surgeons, 12 surgeons had experience of more than 10 bilioenteric anastomoses and three surgeons of more than 30 in the study period. A leak of the bilioenteric anastomosis occurred in 5.6% (29 patients). Table 1 summarizes patient characteristics, the underlying diagnoses, and the procedures performed and gives an overview of the morbidity and mortality.

Univariate Analysis of Factors Associated With Bile Leaks

Univariate analysis showed that certain types of operation were significantly associated or not associated with a postoperative bile leak. Patients undergoing liver resection in combination with a hepaticojejunostomy developed bile leaks in 50% (five of 10 patients, $p<0.0001$), whereas patients undergoing pancreaticoduodenectomy developed this complication in only 3.7% (11 of 289 patients, $p<0.03$). Other surgical procedures were not significantly associated with a postoperative bile leak. Patients with biliary complications after liver transplantation necessitating a subsequent hepaticojejunostomy developed postoperative bile leaks in 50% of cases ($p=0.0028$). Patients who had received preoperative radiochemotherapy also had a significantly higher risk for suffering a bile leak, with six of 50 patients (12%, $p=0.05$) developing this complication. Patients who developed a bile leak had significantly lower preoperative cholinesterase levels compared to patients without this complication [median 4.4 kU/l (interquartile range 3.4–6.8) vs 6.2 kU/l (interquartile range 4.3–8.6); $p=0.003$], with preoperative cholinesterase measurements being performed in 485 patients. The surgeon's experience was a significant factor on univariate analysis: Patients treated by surgeons with a personal experience of more than 30 hepaticojejunostomies developed significantly fewer bile leakages compared to patients operated on by less experienced surgeons (4 vs 8%; $p=0.05$) (Table 2).

Table 3 Multivariate Analysis of Factors Associated With Postoperative Bile Leaks

Variable	Odds Ratio	95% CI	*p* Value
Surgeon's experience (>30)	0.5	0.2–1.2	ns (0.1)
Preoperative RxCx	3.83	1.4–10.9	0.01
Preoperative ChE-level	0.87	0.75–1.0	0.05
Redo after LTPL[a]	12.8	2.2–77	0.005
Liver resection	19.2	4.6–83	<0.0001
Pancreaticoduodenectomy	0.68	0.27–1.7	ns (0.4)

CI=confidence interval, ns=not significant, RxCx=radiochemotherapy, ChE=cholinesterase, LTPL=liver transplantation

[a] Biliary complications after liver transplantation necessitating a hepaticojejunostomy

Multivariate Analysis of Factors Associated With Bile Leaks

The results of the multivariate analysis are depicted in Table 3. Liver resection, biliary complications after liver transplantation necessitating a hepaticojejunostomy, preoperative radiochemotherapy, and preoperative cholinesterase level were significantly associated with postoperative bile leaks in this analysis.

Univariate Analysis of Factors Associated With Surgical Morbidity

The univariate analysis of factors associated with postoperative surgical morbidity is shown in Table 4. Patients undergoing liver resection in combination with a hepaticojejunostomy developed surgical complications significantly more often compared to patients undergoing other surgical procedures. When analyzing the effect of the underlying diagnosis, pancreatic cancer (11%, 38 of 317 patients, $p=0.04$) and biliary complications after liver transplantation necessitating a hepaticojejunostomy (four of six patients, $p=0.001$) showed a significant association with surgical morbidity. Other factors significantly associated with postoperative surgical complications were diabetes mellitus, ASA status III/IV, and individual experience of the operating surgeon (more than 30 hepaticojejunostomies). Low preoperative levels of cholinesterase were also associated with surgical morbidity (5.3 vs 6.3 kU/l; $p=0.005$).

Multivariate Analysis of Factors Associated With Surgical Morbidity

Multivariate analysis revealed that simultaneous liver resection ($p=0.001$), biliary complications after liver transplantation necessitating a hepaticojejunostomy ($p=0.04$), diabetes mellitus ($p=0.03$), and surgeon's personal experience of over 30 hepaticojejunostomies ($p=0.05$) were independently and significantly associated with surgical morbidity (Table 5).

Discussion

This study aimed to identify risk factors for prediction of postoperative bile leaks and surgical morbidity in patients undergoing hepaticojejunostomy for different diagnoses. The incidence of clinically relevant bile leaks after hepaticojejunostomy varies considerably depending on the type of procedure. In patients undergoing pancreaticoduodenectomy or pancreatectomy, bile leak rates of 0 to 5% have been described.[9–13] Patients undergoing hepaticojejunostomy for repair of bile duct injury – a group of patients

Table 4 Univariate Analysis of Factors Associated With Surgical Morbidity

Variable	Morbidity	No Morbidity	*p* Value
Gender			
Male	50 (16%)	268 (84%)	ns (0.4)
Female	26 (13%)	175 (87%)	
Age (years)[a]	61 (51–68)	61 (52–69)	ns (0.9)
Diagnosis[b]			
Redo after liver transplant[c]	4.(67%)	2 (33%)	0.005
Benign pancreatic tumor	3 (11%)	25 (89%)	ns (0.8)
Cholangitis	7 (21%)	26 (79%)	ns (0.3)
Liver cirrhosis	5 (21%)	19 (79%)	ns (0.4)
Chronic pancreatitis	9 (13%)	59 (87%)	ns (0.9)
Bile duct tumors	8 (24%)	26 (76%)	ns (0.1)
Pancreatic cancer	38 (11%)	279 (88%)	0.04
Other	2 (22%)	7 (78%)	ns (0.6)
Procedure[b]			
Liver resection	6 (60%)	4 (40%)	0.001
Liver transplantation	6 (23%)	20 (77%)	ns (0.2)
Total pancreatectomy	2 (10%)	19 (90%)	ns (0.8)
Pancreaticoduodenectomy	37 (12%)	263 (87%)	ns (0.1)
Hepaticojejunostomy	25 (15%)	137 (85%)	ns (0.8)
Vascular resection			
Yes	6 (15%)	35 (85%)	ns (1.0)
No	70 (15%)	408 (85%)	
Intraoperative radiation			
Yes	3 (23%)	10 (77%)	ns (0.4)
No	73 (14%)	433 (86%)	
Preoperative radiochemotherapy			
Yes	9 (18%)	41 (82%)	ns (0.5)
No	67 (14%)	402 (86%)	
Diabetes mellitus			
Yes	11 (26%)	31 (74%)	0.04
No	65 (14%)	412 (86%)	
ASA status			
I/II	31 (11%)	245 (89%)	0.02
III/IV	45 (19%)	198 (81%)	
Surgeon's experience			
>30 hepaticojejunostomies	36 (12%)	275 (88%)	0.02
≤30 hepaticojejunostomies	39 (19%)	166 (81%)	
Perioperative transfusion			
Yes	25 (17%)	118 (83%)	ns (0.3)
No	51 (14%)	325 (86%)	
Preoperative bilirubin[a] (mg/dl)	1.3 (0.7–3.8)	1.0 (0.5–3.3)	ns (0.2)
Preoperative ChE[a] (kU/l)	5.3 (3.7–7.6)	6.3 (4.4–8.8)	0.005
Preoperative AP[a] (U/l)	177 (81–432)	163 (95–365)	ns (0.9)
Preoperative AST[a] (U/l)	30 (17–72)	28 (14–73)	ns (0.5)
Preoperative ALT[a] (U/l)	41 (21–108)	46 (20–121)	ns (1.0)
Preoperative GT[a] (U/l)	127 (28–287)	94 (27–287)	ns (0.6)
Body mass index[a]	24.4 (22–27)	23.8 (21–26)	ns (0.3)
Operation time (hours)	6 (4.3–7)	5.8 (4.3–6.8)	ns (0.4)
Blood loss (cm^3)	700 (400–1,200)	600 (300–1,000)	ns (0.4)

ns=not significant, AP= alkaline phosphatase, GT= glutamyl transpeptidase, ChE= cholinesterase

[a] Median and interquartile range

[b] Each factor was individually tested against all the others

[c] Biliary complications after liver transplantation necessitating a hepaticojejunostomy

that was very rare in our study – leak in about the same magnitude.[14,15] In the context of liver transplantation, biliary leaks occur in 1–25% of patients; this incidence seems to be unrelated to the type of biliary reconstruction (hepaticojejunostomy or choledocho-choledochostomy).[16,17] Biliary resection for hilar cholangiocarcinoma, which is often combined with liver resection, carries a higher complication rate; in one report the incidence of intra-abdominal abscesses was 30%, the incidence of a sterile bile collection/leak 11%.[18]

Table 5 Multivariate Analysis of Factors Associated With Surgical Morbidity

Term	Odds Ratio	95% CI	*p* Value
Redo after LTPL[a]	6.5	1.2–50.5	0.04
Pancreatic cancer	0.7	0.4–1.3	ns (0.3)
Liver resection	9.3	2.4–39	0.001
Diabetes mellitus	2.4	1.04–5.2	0.03
ASA I/II	1.4	0.8–2.5	ns (0.2)
Surgeon's experience (>30)	0.6	0.3–0.99	0.05
Preoperative ChE level	0.12	0.01–1.3	ns (0.1)

CI=confidence interval, LTPL=liver transplantation, ns=not significant, ChE=cholinesterase

[a] Biliary complications after liver transplantation necessitating a hepaticojejunostomy

An exact definition of predictive factors associated with bile leaks might help to better manage these patients; however, a detailed analysis is currently not available in the literature. Therefore, the main objective of our study was to describe the incidence of postoperative bile leaks after hepaticojejunostomy and to define predictive factors associated with this risk.

One might debate the bile leak definition we used, as this definition is critical for a study describing the incidence of bile leaks and its risk factors. We chose a definition that we view as clinically relevant and pragmatic. Patients receiving a hepaticojejunostomy are routinely drained in our institution; however, the drains are routinely removed on the first or second postoperative day. Therefore, routine measurements of the bilirubin concentration in the drained fluid are not performed in our institution. Only patients in whom the clinical management had to be altered, such as a delayed removal of the surgically placed drains, administration of antibiotics, or surgical or interventional drainage of a bilioma, were viewed as having a bile leak in this study. In our hands and with the chosen definition, biliary leakage occurred in 5.6% of patients receiving a hepaticojejunostomy. As expected, the incidence of a bile leak showed a vide variation when comparing different underlying diagnoses and surgical procedures. As hepaticojejunostomy is performed in a standardized fashion in our institution, the incidence of bile leaks seems to be largely associated with factors that cannot be influenced by the surgeon. Risk factors for postoperative bile leaks might interact with each other; therefore, it is essential to perform a multivariate analysis of potential risk factors. Multivariate analysis identified simultaneous liver resection, biliary complications after liver transplantation necessitating a hepaticojejunostomy, preoperative radiochemotherapy, and low preoperative cholinesterase levels as independently associated with a postoperative bile leak. It should be noted that some of the procedures associated with bile leaks (liver resection, redo after liver transplant) were only performed in low numbers; although we obtained statistically significant results, this might influence the validity of our findings.

In patients undergoing liver resection, the jejunum is often not anastomosed to the common hepatic duct but rather to the right or left hepatic duct or even smaller bile ducts. This could explain the higher incidence of bile leaks in this group of patients. This is supported by results from a recent study from the Netherlands.[6] Some authors, however, have reported large series with bile leaks rates of only around 10–25%.[18–22] Only few patients underwent liver resection in addition to hepaticojejunostomy in our series, which might also explain the high bile-leak rate in this subgroup of patients. However, liver resection per se carries a risk of a bile leak from the transsected liver surface.[23] As the exact location of the bile leak was not defined in all our patients, we cannot rule out that in some of these patients the liver parenchyma and not the bilioenteric anastomosis leaked. In addition, patients undergoing liver resection generally have a temporarily reduced liver function. In these patients, healing of the bilioenteric anastomosis might be impaired. This hypothesis is supported by our observation of an association of low cholinesterase levels with a higher leakage rate. A low cholinesterase level indicates poor liver protein synthesis, which again might influence anastomotic healing.[24,25]

The higher incidence of bile leaks of the patients who developed biliary complications after liver transplantation with the need of relaparotomy and creation of a hepaticojejunostomy is easily explainable, given the difficulty of these procedures and the underlying diagnoses of these patients.

Interestingly, preoperative radiochemotherapy was associated with an increased incidence of bile leaks. Recently, several studies demonstrated that preoperative radiotherapy is associated with postoperative wound complications in patients with rectal cancer and soft tissue sarcomas.[26,27] Radio- and/or chemotherapy obviously has an impact on anastomotic and wound healing, an effect also demonstrated in our study.

A recent study found preoperative endoscopic biliary drainage and a high body mass index to be associated with bile leaks.[6] We were not able to confirm body mass index as a risk factor in that respect. We elected not to include preoperative biliary stenting in our analysis, as the vast majority of patients with pancreatic cancer and bile duct tumors presented with a biliary endoprothesis in a place which would bias the result of the statistical analysis.

We also analyzed factors associated with surgical morbidity. Multivariate analysis defined biliary complications after liver transplantation necessitating a hepaticojejunostomy, simultaneous liver resection, diabetes mellitus,

and surgical experience as risk factors. Of these factors, surgical experience warrants further discussion. The so-called volume–outcome relationship in regard to experience of the surgeon and hospital on postoperative outcome has been well described in the literature.[28–31] Interestingly, we did not find an effect of the surgeon's experience on the bile leak rate in our study, which might be the consequence of the standardized fashion that this anastomosis is performed at our institution. Regarding surgical morbidity, however, surgeon's experience became a prognostic factor. Therefore, individual experience remains an important factor for perioperative outcome, even in a high-volume institution.

In summary, in the presented series of 519 patients undergoing hepaticojejunostomy, bile leaks occurred in 5.6% of patients. The low leak rate demonstrates that hepaticojejunostomy per se adds minimally to perioperative morbidity and mortality if performed in a standardized fashion. We were able to identify factors associated with surgical morbidity and postoperative bile leaks. The findings of this study might allow a better risk stratification of patients undergoing hepaticojejunostomy.

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Mechanical Bowel Preparation for Elective Colorectal Surgery with Primary Intraperitoneal Anastomosis by a Single Surgeon: Interim Analysis of a Prospective Single-Blinded Randomized Trial

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Abstract We report an interim analysis of a prospective single-blinded randomized trial designed to investigate whether preoperative mechanical bowel preparation influences the rate of surgical-site infection and anastomotic failure after elective colorectal surgery with primary intraperitoneal anastomosis performed by a single surgeon. Patients scheduled to undergo an elective colorectal procedure with a primary intraperitoneal anastomosis were randomized to receive either oral polyethylene glycol lavage solution and enemas (group A) or no preparation (group B). Surgical-site infection and anastomotic failure were investigated. Of 97 patients included, 48 were assigned to group A and 49 to group B. Twelve (12.4%) developed wound infections, six in each group (12.5 vs. 12.2%; NS). Intra-abdominal sepsis was only seen in group A (n=3, 6.3%). Anastomotic failure occurred in four patients in group A (8.3%) vs. two patients in group B (4.1%) (NS). The overall complication rate in group A was 27.1%, vs. 16.3% in group B. The number needed to harm was 9.3. Our interim analysis of a prospective single-blinded randomized trial suggests that a surgeon may have the same or even worse outcomes when mechanical bowel preparation is routinely used for colorectal surgery with primary intraperitoneal anastomosis.

Keywords Mechanical bowel preparation · Colorectal surgery · Surgical-site infection · Anastomotic failure · Randomized clinical trial

Introduction

Mechanical bowel preparation (MBP) became a standard practice as a measure to decrease the high morbidity associated with colorectal surgery in the past. Infection and anastomotic failure were the two most feared complications following resection of the large bowel, and cleansing was thought to be essential to prevent them.[1] However, the basis for this indication was completely intuitive: the benefits of routine preoperative MBP had never been clinically proven and some experimental support was obtained retrospectively.[2] Over the last decade, several groups have shown no superiority of different cleansing protocols over no preparation for the prevention of either postoperative wound infection or anastomotic leakage.[3–9] Moreover, several meta-analyses reported that standard MBP was associated with an increased risk of anastomotic dehiscence.[10,11] However, this latter finding was difficult to interpret.[12]

In spite of the published data, MBP with cathartic solutions and enemas is an established practice for general and colorectal surgeons, as shown by surveys and guidelines.[13–16] There is no clear explanation for the incongruence between the standard of care and the science supporting it, but methodological flaws and heterogeneity in study design may have biased the outcomes and prevented

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surgeons from translating "scientific evidence" into their individual clinical practice.

At our department, MBP with either polyethylene glycol (PEG) or sodium phosphate and enemas has been unimpeachably used for decades, but several years ago, we questioned how omitting MBP would affect the practice of an individual surgeon. Thus, a prospective single-blinded randomized trial was designed to investigate whether omission of preoperative MBP increases the rate of surgical-site infection (SSI) and anastomotic failure after elective colorectal surgery performed by the same surgeon. Here, we report an interim analysis.

Patients and Methods

Starting October 2001, all patients scheduled to undergo an elective colorectal procedure with a primary intraperitoneal anastomosis but without intraoperative colonoscopy and to be operated on by the same surgeon were included in the study if they (1) had not had an endoscopic exploration in the prior week, (2) were 18 years of age or older, and (3) had given informed consent. Exclusion criteria are presented in Fig. 1. Patients enrolled in the study were subsequently admitted and randomized (computer-generated numbers) to receive either 3 l of PEG lavage solution orally plus conventional enemas over 24 h (group A) or to have no MBP (group B) prior to surgery. No intravenous fluids were administered as a part of the preoperative protocol. Patient's compliance with the cleansing protocol was supervised and assessed by a registered nurse. In both arms, dietary restrictions were limited to 12 h prior to surgery. Antibiotic prophylaxis consisted of intravenous administration of gentamicin and metronidazole (80 and 500 mg, respectively) 30 min before surgery and every 8 h postoperatively (three doses). Antithrombotic prophylaxis was based on preoperative and postoperative administration of subcutaneous low-molecular-weight heparin (enoxaparin 20 or 40 mg depending on individual risk factors). The peritoneal cavity was always approached in a standard fashion: midline skin incision with a scalpel, the subcutaneous fat and the fascia were dissected, and hemostasis was achieved with monopolar electrocautery. The wound edge was always isolated and protected with a circular plastic drape (3M, Madrid, Spain). Anastomoses were hand-sewn or stapled according to the preference of the surgeon (hand-sewn anastomoses were favored except when they were judged to be more difficult to perform). No additional irrigation with antibiotic or antibacterial solutions was used during the operation. Intra-abdominal drains were never used after the primary procedure and skin incisions were always closed with staples.

The primary end point was SSI and the secondary end point was anastomotic leakage. Patients were followed for SSI (wound infection + intra-abdominal sepsis) and anastomotic failure within 30 days after surgery by a trained surgeon who was not involved in the study. Surgical-site infection was diagnosed and classified following the definitions made in the 1999 CDC guidelines[17] as superificial incisional SSI, deep incisional SSI, and organ/space SSI. Anastomotic failure was diagnosed if there was a fecal fistula, an anastomotic dehiscence was identified at reoper-

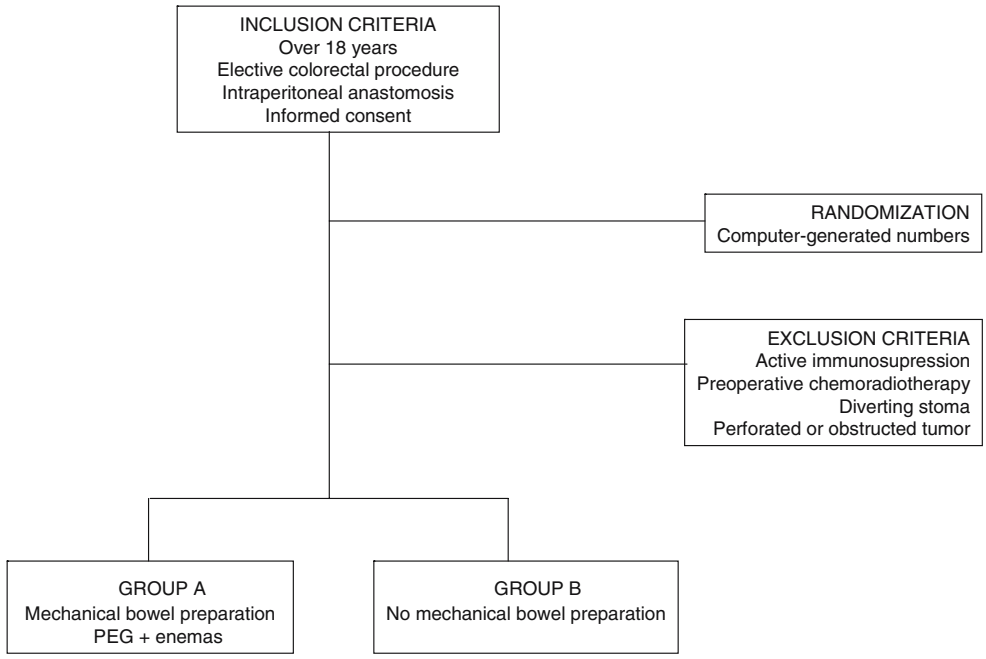

Figure 1 Study design. Patients on active immunosupression or preoperative chemoradiotherapy were excluded from the study before randomization.

Table 1 Preoperative Demographics, Indication for Surgery, and Procedure-related Data

	Overall (n=97)	Group A (n=48)	Group B (n=49)	p
Age (years)	67±14	66±12	68±14	NS
Sex (F/M)	48/49	26/22	23/26	NS
IBD	6/97	3 (6%)	3 (6%)	NS
CRC	76/97	36 (75%)	40 (82%)	NS
Other	15/97	9 (19%)	6 (12%)	NS
AB prophylaxis	100%	100%	100%	NS
Enoxaparin	100%	100%	100%	NS
Anastomosis				
SB–LB	39/97	15 (31%)	24(49%)	NS
LB–LB	58/97	33 (69%)	25 (51%)	NS
Hand-sewn	56/97	23 (48%)	33 (67%)	NS
Stapled	41/97	25 (52%)	16 (33%)	NS

IBD=inflammatory bowel disease, CRC=colorectal cancer, AB=antibiotics, SB=small bowel, LB=large bowel

ation or during postmortem, and/or clinical suspicion was confirmed by a radiological test (CT scan).

Historical data from our department had shown that our expected wound infection rate after elective colorectal operations with routine MBP was about 10% for clean–contaminated procedures and about 30% for dirty surgery (unpublished data). Most randomized trials and meta-analyses of MBP usually show lower infection rates for elective procedures. However, a recent report found a higher incidence, up to almost 30% of patients undergoing open elective surgery, and half of them being diagnosed after discharge.[18] Because prevention of postoperative infections is the rationale for the use of MBP,[17] we considered that it would be clinically relevant for an individual surgeon if, by omitting MBP, his/her SSI rate (incisional + organ/space) would reach those figures. In other words, omission of MBP would convert clean–contaminated elective colorectal surgery into a dirty procedure. As a result, a sample size of 62 patients for each group was calculated to detect an increase in the SSI rate from 10 to 30% with an α error of 0.05 and a power of 80% for a two-tailed comparison. Data were entered into a computerized database and analyzed with the SPSS software package. The number needed to treat or number needed to harm (NNH) was calculated as the inverse of the increase in relative risk. Student's t, Pearson's Chi square and Fisher exact tests were used for statistitcal analysis as indicated. Statistical significance was defined as $p<0.05$. The trial was approved by the ethics committee of Hospital Clinico San Carlos.

Results

Up to July 2005, 110 patients who met the inclusion criteria had been enrolled in the study. Two patients (2%) were preoperatively excluded because they were on active immunosuppressive therapy for severe connective tissue disorders. Subsequently, 108 patients were randomized, but 11 of them (10%) were excluded from this interim analysis because they met at least one of the exclusion criteria (diverting stoma in nine cases, contained perforation in one patient, and unresectable tumor in one patient). Of the remaining 97 patients, 48 were randomly assigned to group A and 49 to group B. No significant differences in demographics were found between groups as presented in Table 1. Antibiotic and antithrombotic prophylaxis were systematically used in both groups. No discontinuation of the cleansing protocol was needed in group A. With regard to fecal load, 52 out of the 97 patients included in the analysis were found to have solid stool in the anastomotic stumps. Solid feces were easily removed before fashioning the anastomosis (three out of 48 patients in group A and all patients in group B). No remarkable contamination of the surgical field was reported.

With regard to the primary end point (Table 2), SSI occurred in 15 of the 97 patients included in the study, with superficial incisional SSI being the most frequent presentation (group A=12.5% and group B=12.2%; NS). There were three organ/space SSIs, but all of them occurred in group A (6.3%). Thus, although SSI was more frequent in patients receiving MBP, there was no statistical difference

Table 2 Primary End Point: SSI

	Overall (n=97)	Group A (n=48)	Group B (n=49)	p
Incisional SSI	12 (12.5%)	6 (12.5%)	6 (12.2%)	NS
Organ/space SSI	3 (3.1%)	3 (6.2%)	0	NS
Total SSI	15 (15.5%)	9 (18.7%)	6 (12.2%)	NS

Table 3 Secondary End Point: Anastomotic Dehiscence Rate by Group and Intestinal Segment Involved in the Anastomosis

Anastomosis	Overall	Group A	Group B	*p*
SB–LB	1/39 (2.6%)	1/15(6.6%)	0/24 (0)	NS
LB–LB	5/58 (8.6%)	3/33 (9.1%)	2/25 (8.0%)	NS
Total	6/97 (6.2%)	4/48 (8.3%)	2/49 (4.1%)	0.05[a]

SB=small bowel, LB=large bowel
[a] Fisher's exact test

between groups. Besides, the technique used to construct the anastomosis was not found to be associated to a statistically significant increase in the SSI rate, with eight infections in 41 patients (19.5%) for stapled anastomosis vs. seven infections in 56 patients (12.5%) for hand-sewn anastomosis.

As shown in Table 3, the overall rate of anastomotic failure was 6.3% (n=6). Not surprisingly, it was higher in those cases in which two segments of the large bowel were involved in the anastomosis, irrespective of whether patients received MBP or not. With regard to the anastomotic technique, no statistically significant difference in the dehiscence rate between stapled and hand-sewn anastomoses was found, with two failures in 41 patients (4.9%) vs. four failures in 56 patients (7.1%), respectively. However, the most interesting finding was that patients receiving MBP had almost twice the frequency of anastomotic dehiscence compared to patients without colonic cleansing: four patients in group A (8.3%) vs. two patients in group B (4.1%). This difference achieved a p value of 0.05 using Fisher's exact test.

When the frequency of SSI and anastomotic failure, which are the reasons for the preventive use of MBP, were added, the complication rate in group A was 27.1% vs. 16.3% in group B. This difference did not achieve statistical significance, but the calculated NNH was 9.3. Therefore, ten patients needed to receive preoperative MBP for one extra patient to develop one of these complications, compared to no preparation.

Mortality occurred in five patients (5%), with a similar rate in both groups (6.2% in group A vs. 4% in group B; NS). All deaths occurred in patients older than 80 years or with advanced disease (Table 4), but mortality was related to a previous anastomotic dehiscence in only two cases (one patient with a right hemicolectomy and another who died of an acute myocardial infarction after recovering from a prior dehiscence in the left colon).

Discussion

In this interim analysis of a single-blinded prospective randomized trial, we have found that a surgeon who omitted preoperative MBP for elective colorectal procedures with a primary intraperitoneal anastomosis did not have a greater SSI rate when compared to the standard practice. In addition, his anastomotic failure rate was not increased, either. In fact, the risk of developing a leak doubled for patients who received preoperative MBP with PEG and conventional enemas. Although the sample size was small, this trend towards a higher rate of anastomotic dehiscence in patients submitted to colonic cleansing almost reached statistical significance (p=0.05).

With the present study, we have tried to avoid the methodological flaws attributed to previous large trials addressing the same issue[19] and to overcome one of their major drawbacks, that is, the "surgeon factor." It is clear that differences in the surgical technique and in technical expertise among surgeons, and even hospitals, may influence their postoperative results and, consequently, surgeon volume has emerged as an important predictor of in-hospital outcome for colorectal resections.[20] However, to achieve a large sample size, most randomized trials[3–9] studying the impact of MBP on postoperative outcomes did not control for that variable (different surgeons with different trainings and different numbers of cases, multiple hospitals, etc). Therefore, it could be argued that technical issues might have biased the results. This may be one plausible explanation for the lack of acceptance of "scientific evidence" by the majority of surgeons practicing colorectal surgery.[19] Therefore, we decided to conduct a trial with patients operated on by the same surgeon, who was blinded to the preoperative MBP protocol used in every case. Substratification by segments involved in the anastomosis or anastomotic technique was not carried out

Table 4 Description of Patients Who Died Within 30 days After the First Procedure

Patient	Age	Gender	MBP	Indication	Anastomosis/type	Cause of Death
1	80	M	Yes	CRC	SB–LB/Hand-sewn	Leak/MOF
2	84	F	Yes	CRC	LB–LB/Stapled	Pneumonia
3	80	F	Yes	AD	SB–LB/Hand-sewn	CHF
4	68	M	No	MTX	LB–LB/Stapled	Leak/AMI
5	82	M	No	CRC	LB–LB/Stapled	Pneumonia

CRC=colorectal cancer, SB=small bowel, LB=large bowel, AD=angiodysplasia, MTX=metastatic disease, MOF=multiple organ failure, CHF=congestive heart failure, AMI=acute myocardial infarction

because these factors have not been shown to affect our primary end point.[21] We only included patients with intraperitoneal anastomosis to reduce selection bias. Obviously, these restrictions have prolonged the duration of the study and limited its sample size but have given us a tighter control over the experimental conditions (surgical technique and postoperative management).

Interestingly, even after controlling for the "surgeon factor," our results are qualitatively similar to those reported in other randomized trials and meta-analyses.[3–12] We have also found that the incisional SSI rates are equivalent for patients with and without preoperative colonic cleansing. Quantitatively, our results are comparable, although slightly poorer, to those from other groups.[3–9] Most recently published randomized trials have reported SSI rates under 10%, whereas our figures are 18.8 and 12.2% in patients with and without MBP, respectively. This poorer result may be explained by variations in the population recruited, by variations in surgeon or hospital expertise, and/or by bias in the definition and detection of postoperative infections. For example, Smith et al.[18] showed, in a retrospective study, that the postoperative infection rate after colorectal surgery by a single surgeon was higher than expected and, interestingly, half of the cases had been diagnosed following discharge.

Anyway, our observed equivalence in SSI rates between groups suggests that no significant reduction in wound and abdominal cavity contamination during elective colorectal surgery is achieved by adding MBP, irrespective of whether there is a reduction in "bowel contents and live microorganisms" in the colon[17] or not. Although not specifically investigated, we agree with a recent study by Mahajna et al.,[22] who showed that inadequate (or even adequate) MBP leads to a higher incidence of intraoperative spillage of liquid feces and subsequent postoperative infections.

With regard to anastomotic failure, we have found an increased risk of dehiscence in the MBP group that almost reached statistical significance, even with a small sample size, but with no difference in postoperative mortality. This observation is in agreement with results reported in meta-analyses.[12] Many factors could explain why a "clean colon" is more likely to heal poorly than a full large bowel. First, MBP may induce local changes, like those reported by Buscher et al.,[23] that would interfere with healing. Second, fluid and electrolyte disturbances are often seen after the use of MBP,[24] which may put sick and older patients at a higher risk.

Finally, interpretation of results from randomized trials investigating MBP is difficult because statistical and clinical significance do not always run parallel. The number needed to treat is a good measure to interpret the clinical significance of the comparison between two mutually exclusive treatments and it may help us answer the following question: Is the difference in postoperative infection and anastomotic dehiscence rates between MBP and no cleansing large enough to be considered clinically relevant? A NNH of 10 for MBP, as seen in our study, means that, for every ten patients submitted to preoperative colonic cleansing, one more complication will develop compared to patients not receiving it. This suggests that it may be sound clinical judgement to omit preoperative mechanical colonic cleansing in patients undergoing elective colorectal procedures with intraperitoneal anastomosis, except in specific scenarios (e.g., intraoperative colonoscopy).

In conclusion, our interim analysis of a prospective single-blinded randomized trial of MBP for large bowel surgery, although underpowered, suggests that an individual surgeon may have the same or even worse outcomes in terms of SSI and anastomotic failure rates if he/she routinely uses preoperative MBP with poliethylenglycol and conventional enemas. We should wait for the final analysis before drawing a definitive conclusion.

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A New Drug Delivery System Targeting Ileal Epithelial Cells Induced Electrogenic Sodium Absorption: Possible Promotion of Intestinal Adaptation

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Abstract We previously demonstrated the induction of the epithelial sodium channel, prostasin, and 11β-hydroxysteroid dehydrogenase type 2 and activation of sodium transport mediated by those molecules in the remnant ileum after total proctocolectomy. The aims of the present study were to develop a new drug delivery system that targets ileal epithelial cells and to enhance local mineralocorticoid action without systemic effects. Orally administered D-aldosterone-containing D,L-lactide/glycolide acid copolymer microspheres are absorbed in the rat terminal ileum and released aldosterone. Blood and terminal ileal tissues were collected 2 weeks after the administration of the microspheres, and the aldosterone concentrations, mRNA, and protein expressions of the above molecules and sodium transport were evaluated. Significantly high levels of tissue aldosterone in the absence of elevated plasma levels were detected in the microspheres-treated rats. Epithelial mRNA and protein expression of the above molecules increased significantly in the microspheres-treated animals. Electrogenic sodium transport in the ileum was enhanced in the microspheres-treated rats. Aldosterone-containing microspheres successfully induced the expression of the above molecules and activated sodium transport in the ileal mucosa, both of which are essential for intestinal adaptation. Pre- and/or postoperative treatment with this drug may compensate for the excessive loss of sodium and water following proctocolectomy.

Keywords Total proctocolectomy · Intestinal adaptation · Aldosterone · Ulcerative colitis

Introduction

Total proctocolectomy (TPC), followed by ileoanal anastomosis, is an established surgical treatment for ulcerative colitis and familial adenomatous polyposis. The kidney is the major organ to keep fluid and electrolyte balances in the body and adapt well by molecular induction of aldosterone-associated molecules for sodium transport.[1] It is well known that these patients suffer from persistent postoperative diarrhea and frequent bowel movement due to the absence of the colon. Dehydration and electrolyte imbalance also occur in patients with acute or infectious enteritis even after a long postoperative interval. Chronic watery diarrhea substantially decreases quality of life.[2] Therefore, the promotion of intestinal adaptation in the remnant small bowel is critical to diminishing diarrhea and improving the quality of postoperative life.

Homeostasis of water, as well as electrolyte balance, is regulated in the gastrointestinal tract primarily by the renin–angiotensin–aldosterone system. In both animals[3,4] and

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humans,[5] increased plasma aldosterone is one consequence of TPC. We assessed the mechanisms responsible for adaptive or compensatory changes in response to watery diarrhea following TPC using a rat TPC model. Interestingly, plasma aldosterone, but not corticosterone, increased concomitantly with the induction of regulating fluid balance and sodium transport in the ileum, such as α-, β-, and γ-subunits of the epithelial sodium channel (ENaC), 11β-hydroexysteroid dehydrogenase type 2 (11β-HSD2), and prostasin.[3,4,6] Those molecular inductions linked to functional improvements of sodium absorption. Both basal and aldosterone stimulated electrogenic sodium absorption, which is amiloride sensitive, were enhanced in the remnant ileal mucosa.[3,4]

The binding of aldosterone to the mineralocorticoid receptor (MR) results in an increase in the number and activity of ENaC at the apical cell surface, although the responsible intracellular mechanisms are not fully understood. Coexpression of 11β-HSD2 with MR is required for the regulation of sodium absorption by aldosterone in colonic epithelial cells. The specificity of aldosterone binding to MR is maintained by 11β-HSD2, which converts glucocorticoids to their respective receptor inactive metabolites.[7,8] Others and we reported that the expression of 11β-HSD2 and the β- and γ-subunits of ENaC are down regulated in the inflamed colonic epithelia associated with ulcerative colitis. On the one hand, decreased expression of these molecules in epithelial cells may contribute to severe diarrhea.[9–12] On the other hand, enhanced expression of these molecules may increase sodium and water absorption from the lumen and, thereby, diminish diarrhea. We previously reported that continuous aldosterone infusion in rats fully induced the α-, β-, and γ-subunits of ENaC, 11β-HSD2, and prostasin in the distal small intestine,[6] where MR is constitutively expressed.[13] If aldosterone alone has a primary role in the induction of these molecules,[14] the selective enhancement of the aldosterone concentration in the ileal tissues may be a novel therapeutic approach to induce the subunits of ENaC, 11β-HSD2, and/or prostasin to alleviate persistent diarrhea following TPC.

A major impediment to such therapy is targeting the ileal mucosa to enhance the tissue aldosterone concentration. In the present study, we employed a novel drug delivery system using D,L-lactide/glycolide copolymer (PLGA) microspheres. Biodegradable microspheres in various sizes have been used (a) to induce an effective immune response,[14] (b) to deliver medication of mucosal inflammation,[15–18] and (c) in cancer therapy.[19,20] Orally administered microspheres are thought to be absorbed at the gut-associated lymphoid tissues, the largest of which are the Peyer's patches.[21,22] Phagocytic microfold cells (M cells) and tissue macrophages take up the microspheres, which subsequently release adsorbed molecules, e.g., phospholipase A2,[14] dexamethasone,[15–18] and 5-fluorouracil.[19,20] The aims of the present study are (a) to develop aldosterone-incorporated microspheres, (b) to demonstrate the induction of molecules that regulate sodium transport in the ileum in the absence of systemic effects using aldosterone-containing microspheres, and (c) assess the therapeutic value of D-aldosterone-containing microspheres to promote postoperative intestinal adaptation.

Materials and Methods

Animals

Male Sprague–Dawley rats (200–250 g; Japan SLC, Shizuoka, Japan) were housed in the animal facility at Tohoku University Institute for Experimental Animals (Sendai, Japan). The animals were maintained on a 12-h light/dark cycle, fed a standard rat chow (Nippon Nosan, Yokohama, Japan) and provided tap water ad libitum.

Preparation of D-Aldosterone-Containing PLGA Microspheres

PLGA microspheres were synthesized by simple polycondensation of DL-lactic acid at 180°C under reduced in the absence of catalyst. D-aldosterone- (Sigma Chemical, St. Louis, MO, USA) incorporated microspheres were prepared by the solvent-evaporation method using a double emulsion, as previously described.[21] In brief, 0.4 mg of D-aldosterone (W1) was poured into 1 ml of methylene choride containing 200 mg of PLGA microspheres (O), and emulsified by sonication to form a W1/O emulsion. The emulsion was added to 2 ml of a 1-wt.% polyvinyl alcohol (weight-averaged M_r=5,400; degree of saponification, 79.85 mol%) aqueous solution (W2) saturated with methylene choride at room temperature and agitated by a vortex mixer to form a double emulsion. The W1–O–W2 double emulsion was stirred by an impeller (200 rpm) at room temperature until the methylene choride was completely evaporated. The microspheres were collected by centrifugation (5,000 rpm, 5 min, 4°C), washed three times with cold distilled water and lyophilized. The D-aldosterone-containing microspheres were further fractionated by counterflow centrifugal elutriation. Each milligram of microspheres contained approximately about 2×10^{-3} mg of D-aldosterone.[15–17,23] The D-aldosterone-containing microsphres were sized by microscpopy using a reference scale (Fig. 1). We adjusted the diameter of the D-aldosterone-containing microspheres with approximately 10 μm to fix microspheres in the ileal mucosae for long periods.

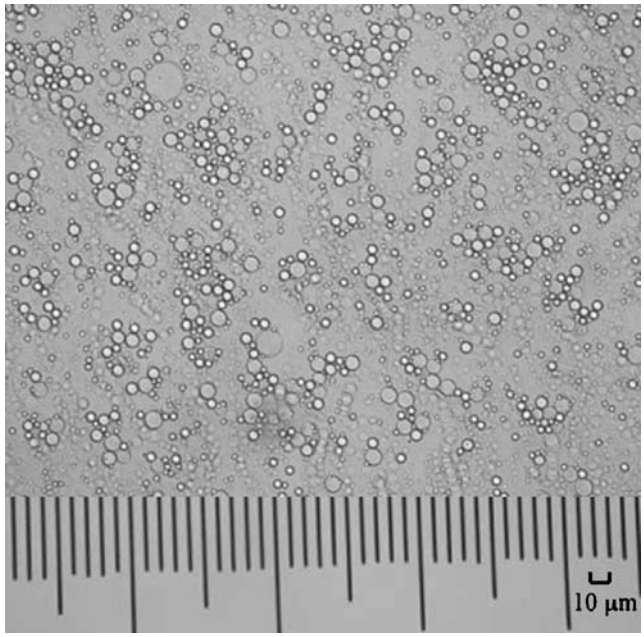

Figure 1 Photograph of PLGA microspheres. *Bar* indicates 10 μm.

In Vitro Release of Aldosterone from PLGA Microspheres

D-aldosterone-containing PLGA microsphares were suspended in normal saline (1 mg/ml) and incubated at 37°C in a shaking bath. Small amounts of incubation media were collected after 0, 3, 6, 12, 24, 36, 48, and 72 h, and each concentration of aldosterone was measured using an Aldosterone EIA Kit (Cayman Chemical, Ann Arbor, MI, USA).

Protocols for Oral Administration of PLGA Microspheres

Rats were divided into four groups (groups A–D); group A (n=8), no treatment; group B (n=5), PLGA microspheres (0.1 mg/g body weight/day) alone; group C (n=5), free D-aldosterone (5×10^{-4} mg g^{-1} day^{-1}) alone; and group D (n=12), D-aldosterone-containing PLGA microspheres (0.1 mg g^{-1} day^{-1}, which was equivalent to 5×10^{-4} mg g^{-1} day^{-1} of D-aldosterone). All substances were administered orally. Rats were anesthesized with ether and killed after 2 weeks of microsphere or D-aldosterone treatment. Blood was collected immediately and the terminal ileum (10 cm in length) was removed. This protocol was approved by the Tohoku University Animal Care Committee.

Measurement of Aldosterone Concentration in Plasma

Whole blood was collected from the abdominal aorta between 12:00 AM and 2:00 PM (to avoid diurnal variation) in tubes containing EDTA and aprotinin, and plasma was separated from whole blood by centrifugation at 1,500 rpm for 5 min. Plasma aldosterone concentrations were measured using an Aldosterone EIA Kit.

Measurement of Aldosterone Concentration in Ileal Tissues

Steroids were extracted from the ileal tissues according to the method of Shih and Tseng[24] with minor modifications. One-centimeter segments of the terminal ileum were weighed, placed in absolute ethanol (30 ml/g tissue), homogenized, and extracted in 10 vol of methanol and chloroform (2:1, v/v). The chloroform fraction was collected after centrifugation and air-dried. The residue was dissolved in 70 ml methanol, mixed with 30 ml $CaCl_2$ (1 M), and kept at −80°C for 4 h. The methanol fraction was collected and evaporated, and the residue was resuspended in 10 ml dichloromethane. Two milliliters each of water, 0.1 N NaOH, and 0.1 N acetic acid were added and the solution was mixed in a funnel. Dichloromethane was evaporated and the remaining residue was dissolved in methanol and used to measure the aldosterone concentration.

Isolation of Intestinal Epithelial Cells and Extraction of Total RNA

Epithelial cells were isolated from 10-cm segments of the terminal ileum as previously described.[3] The segments were inverted and incubated in Hank's balanced salt solution (HBSS) containing dithiotheritol (1.5 mg/ml) to remove the mucous. The mucosal segments were successively incubated (3×) in HBSS containing EDTA (1 mM) for 45 min. The resultant supernatants were centrifuged at 1,500 rpm for 5 min and the pellets were resuspended in RPMI 1640 (Gibco BRL, Gaithersburg, MD, USA). The purity and viability of the epithelial cells were assessed using trypan blue exclusion; values were consistently greater than 90% with minimal contamination by mononuclear cells. The cells were pelleted and lysed with guanidium thiocyanate solution, and the RNA was isolated using a cesium chloride gradient. The quantity and quality of the RNA were determined by A_{260}nm and by staining with ethidium bromide after gel electrophoresis, respectively.

Quantitative Reverse Transcription-Polymerase Chain Reaction

The amounts of α-, β-, γ-subunits for ENaC, prostasin, 11β-HSD 2, α1-, β1-subunitis of Na^+/K^+-ATPase, and sodium glucose cotransporter-1 (SGLT-1) mRNAs were measured by quantitative reverse transcription-polymerase chain reaction (RT-PCR). Complementary DNAs (cDNAs) were generated as previously described.[6] Two microliters of diluted RT mixture was used for mRNA quantification in duplicate using QuantiTect SYBR Green PCR Kit (Quiagen K.K., Tokyo, Japan) and ABI GeneAmp 5700 (Applied Biosystems Japan, Tokyo, Japan) according to the manufacturers' protocols.[6] The primer sequence for quantitative RT-

PCR was determined with Primer Express software (PE Applied Biosystems, Foster City, CA, USA) (Table 1). The dissociation curves of the amplified products displayed a single peak, demonstrating that only specific products were synthesized. Amplified products were preliminarily subcloned into a pCRII TOPO cloning vector using a TA cloning kit (Invitrogen, Tokyo, Japan). The sequence of inserted cDNA was confirmed using an AutoCycle Sequencing Kit (Pharmacia Biotech, Tokyo, Japan) and ALF Express DNA Sequencer (Pharmacia Biotech). The relative quantification of target and β-actin mRNAs was calculated using the comparative threshold cycle number for each sample fitted to a four-point standard curve. The standard curve was constructed using a serial dilution of total RNA extracted from the kidneys or ileum of control rats. The expression levels were normalized to β-actin mRNA. The amplification profile consisted of initial incubation at 50°C for 2 min, initial denaturation at 95°C for 10 min, followed by the specified 40 cycles of 94°C for 30 s, 50°C (for α-, β-, γ-subunits of ENaC, prostasin, 11β-HSD2, α1- and β1-subunits of Na^+/K^+-ATPase, and SGLT-1), 55°C (for β-actin) for 1 min and 72°C for 1 min.

Table 1 Primer Sequence Used for Quantitative RT-PCR

	Sequence
ENaC-α	
Up	5′-CGAAGCCTTGTAGTGTGATCA-3′
Down	5′-TCTGCAAGGACAGCATCTCG-3′
ENaC-β	
Up	5′-CCTCCCAACTATGACTCCCTGA-3′
Down	5′-TGGCCTCTTTGGACAAGGGC-3′
ENaC-γ	
Up	5′-ACGCTAACCCTGACTTAGCCTG-3′
Down	5′-CTTGTCCCAATGTCAATGGTTG-3′
Prostasin	
Up	5′-ACCTTCTCCCGCTACATCAGAC-3′
Down	5′-TCCCTTAACATAGCCAGCGC-3′
11β-HSD2	
Up	5′-GCTCATCACCGGTTGTGACATGGTT-3′
Down	5′-TCCTGGTTGTGTCATGAACAGGGC-3′
SGLT-1	
Up	5′-CATCCTCTTCGCTATCAGCGTC-3′
Down	5′-GATGCCGTTGATGTTCACCA-3′
Na^+/K^+-ATPaseαl	
Up	5′-TGGATCAATGATGTGGAGGACA-3′
Down	5′-CTGCACTACCACGATACTGACAAA-3′
Na^+/K^+-ATPaseβl	
Up	5′-CGTGCAGTTCACCAACCTCA-3′
Down	5′-AAGCGTCCCTGAAAACGGT-3′
β-actin	
Up	5′ACCACCACAGCTGAGAGGGA-3′
Down	5′CCGATAGTGATGACCTGACCG-3′

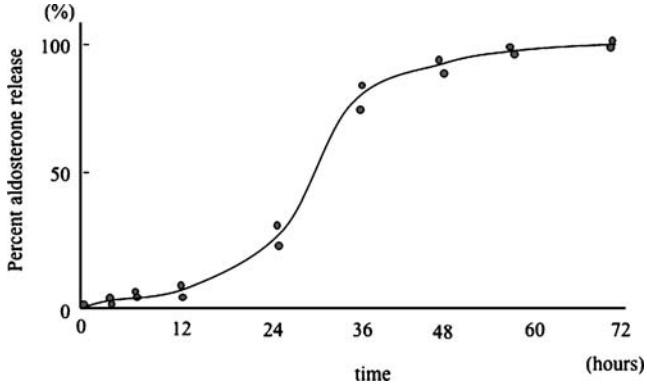

Figure 2 In vitro release of aldosterone from PLGA microspheres. A release curve was constructed from two separate experiments. D-aldosterone-containing microspheres were incubated in saline with constant shaking. Each points the percent concentration of aldosterone at each interval. Maximal concentration was 97% and aldosterone release plateaued 48 h after the start of incubation.

Immunohistochemistry for α-Subunit of ENaC

We used segments of terminal ileum (5 mm) from groups A, B, C, and D and the distal colon from group A. Tissues were fixed in formalin, embedded in paraffin, sectioned at 3-μm thickness, and stained with hematoxylin and eosin to assess the effects of aldosterone-conjugated PLGA microspheres on mucosal morphology and the relative abundance of Na^+ channels in the enterocyte apical membrane. The ENaC α-subunit alone can form a fully functional amiloride-sensitive sodium channel, whereas the coexistence of β- and γ-subunits with α-subunits leads to greater expression of amiloride-sensitive sodium conductance.[25,26] The sections were immuno-stained as previously described with minor modifications[27] using a polyclonal antibody directed against the α-subunit (1:50) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Rabbit immunoglobulins conjugated to a peroxidase-labeled amino acid polymer [HISTOFINE Simple Stain PO (MULTI), Nichirei, Tokyo, Japan] was used as the secondary antibody. The antigen–antibody complex was visualized using 3, 3′-diaminobenzidine (DAB) solution (1 mM DAB, 50 mM Tris–HCl buffer, pH 7.6, and 0.006% H_2O_2) and counterstained with hematoxylin. The specificity of the immunohistochemical staining was confirmed by replacing the primary antibody with phosphate-buffered saline.

Measurement of Amiloride-Sensitive Short Circuit Current

Amiloride-sensitive short circuit current (Isc) was measured in vitro using an Ussing chamber. Terminal ileal segments (2 cm in length) were collected 2 to 4 cm from the ileocecal junction in group A, B, C, and D rats. Colonic mucosae of the distal colon in group A were also used as positive reaction control tissues. We previously demonstrated that a terminal segment, rather than proximal intestine, is essential

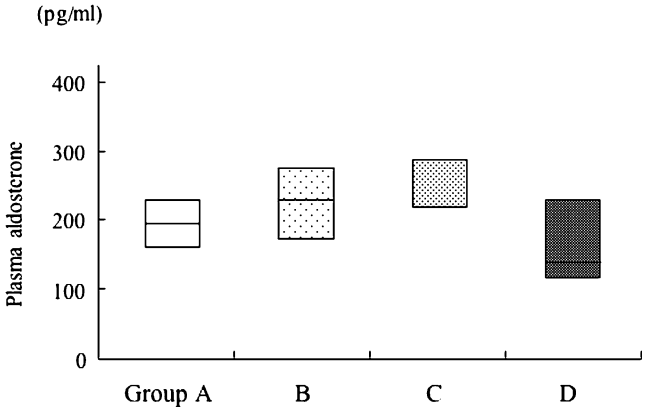

Figure 3 Plasma concentration of aldosterone. Blood was collected after 2 weeks of microsphere or D-aldosterone treatment. *Group A*, no treatment; *B*, PLGA microspheres alone; *C*, free D-aldosterone alone; *D*, D-aldosterone-containing PLGA microspheres. No significant differences were seen between the groups (n=3).

to measure altered sodium absorption after TPC[4]. The isolated segments were opened and rinsed, and the submucosal tissue and muscle layer was removed. The tissues were mounted vertically between acrylic resin chambers (surface area of 0.1 cm^2; Physiologic Instruments, San Diego, CA, USA). Each chamber contained 8 ml of bathing solution and the temperature was maintained at 37°C. The mucosal solution contained 119 mM NaCl, 21 mM $NaHCO_3$, 2.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 8.5 mM mannose. The serosal solution was similar, except mannose was replaced with 2.5 mM glutamine, 5 mM glucose, 0.5 mM β-hydroxybutyrate (sodium salt), and 3×10^{-4} mM tetrodotoxin. The solutions were aerated with 95% O_2 and 5% CO_2 (pH 7.4). The tissues were continuously short-circuited, with a compensation for fluid resistance between the potential-sensing bridges, by using a voltage-clamping amplifier (CEZ9100; Nihon Kohden, Tokyo, Japan). The transepithelial potential was measured using 1-M-KCl electrodes with the transepithelial current being applied across the tissue through a pair of Ag/AgCl electrodes kept in contact with the mucosal and serosal bathing solution by using 1-M-NaCl agar bridges. Isc was considered positive when the current flowed from the mucosa to the serosa, and the transepithelial resistance was calculated from the change in current in response to voltage pulses according to Ohm's law. The viability of the mucosa was confirmed by the addition of glucose to the mucosal buffer at the conclusion of the experiments. Amiloride-sensitive Isc was measured by the addition of 0.1 mM amiloride (a blocker of ENaC) into the mucosal buffer and calculated by the decline in the Isc.

Statistics

Values are presented the median + percentile. Data were tested for significance by Mann–Whitney's U-test with $P<0.05$ being considered significant.

Results

Spontaneous Release of Aldosterone from D-Aldosterone-Containing Microspheres In Vitro

A release curve, constructed from two separate experiments, demonstrated that 48% of the aldosterone incorporated into

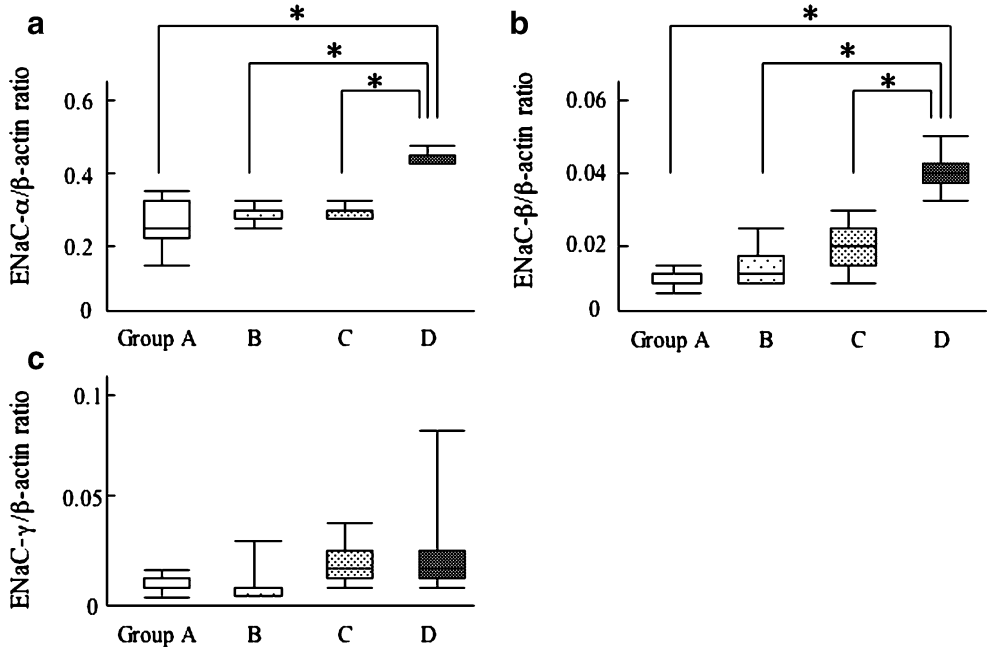

Figure 4 ENaC subunit mRNA expression in the terminal ileum. Epithelial expression of ENaC α- (**a**) and β-subunit (**b**) mRNAs were evaluated by quantitative RT-PCR. *Group A*, no medication; *B*, PLGA microspheres alone; *C*, free D-aldosterone alone; *D*, D-aldosterone-containing PLGA microspheres. The amount of each mRNA was measured in duplicate. Expression levels were normalized to β-actin mRNA (n=5, *asterisks* represent $P<0.05$).

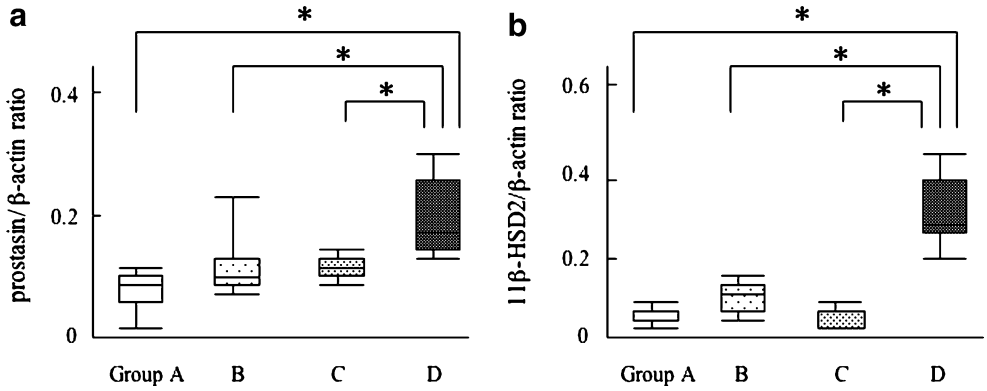

Figure 5 Expression of 11β-HSD2 and prostasin mRNAs. Epithelial expression of prostasin (**a**) and 11β-HSD2 (**b**) mRNAs were evaluated by quantitative RT-PCR. *Group A*, no medication; *B*, PLGA microspheres alone; *C*, free D-aldosterone alone; *D*, D-aldosterone-containing PLGA microspheres. The amount of each mRNA was measured in duplicate. Expression levels were normalized to β-actin mRNA (n=5, *asterisks* represent P<0.05).

the microspheres was released within 24 h. The maximal concentration achieved was 97% and the value plateaued at 48 h after the start of incubation (Fig. 2).

Tissue Concentrations of Aldosterone in the Terminal Ileum

The tissue concentrations of aldosterone in groups A, B, and C were below the sensitivity of the assay system, whereas the value in group D was approximately 200 pg/tissue g.

Plasma Concentrations of Aldosterone

The median aldosterone levels in the plasma were approximately 170–270 pg/ml in all of the groups, including after treatment with microspheres for 2 weeks (Fig. 3).

Expression of ENaC α-, β-, γ-Subunits, Prostasin, and, 11β-HSD2 mRNA

Significant increases in the expression of the α- and β-subunit mRNAs were detected only in group D (Fig. 4a, b). No significant differences were detected in the α and β subunit mRNAs in groups A, B and C. Similarly, there was no significant increase in γ-subunit mRNA expression in group D (Fig. 4c). The expression of both prostasin and 11β-HSD2 mRNAs was significantly elevated in group D, but not in the other groups (Fig. 5a, b).

Expression of SGLT-1, Na+/K+-ATPase α1- and β1-Subunit mRNAs

Quantitative RT-PCR demonstrated that no differences were found in the levels of SGLT-1 or in the Na^+/K^+-ATPase α1-

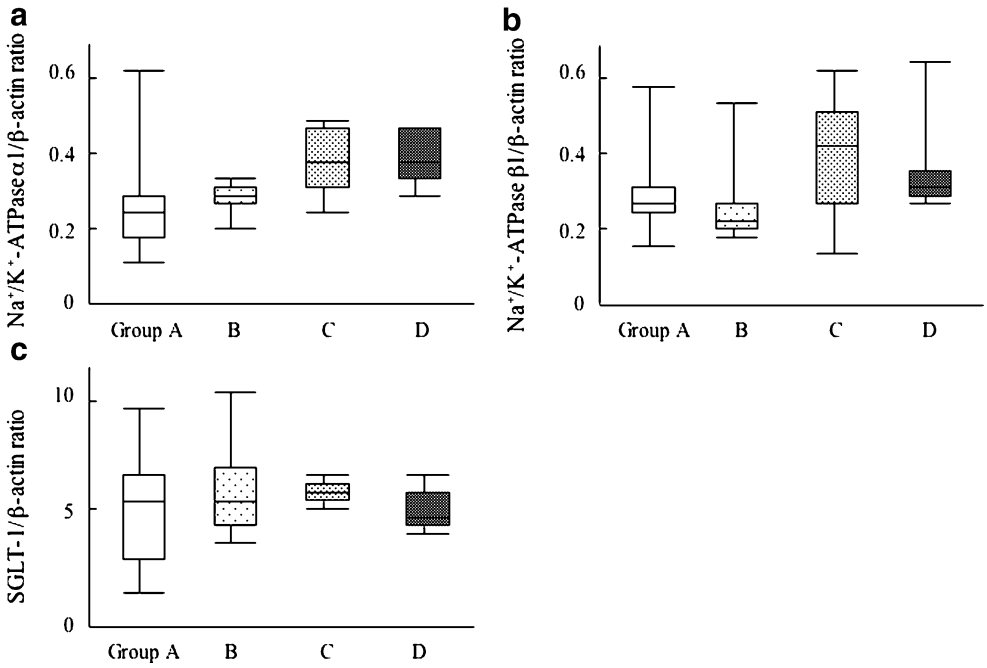

Figure 6 Messenger RNA expression of Na^+/K^+-ATPase subunit and SGLT-1 mRNAs. Epithelial expression of the α1- (**a**) and β1- (**b**) subunits of Na^+/K^+-ATPase, and SGLT-1 (**c**) mRNAs were evaluated by quantitative RT-PCR. *Group A*, no medication; *B*, PLGA microspheres alone; *C*, free D-aldosterone alone; *D*, D-aldosterone-containing PLGA microspheres. The amount of each mRNA was measured in duplicate. The expression levels were normalized to β-actin mRNA.

and β1-subunit mRNAs among all the experimental groups (Fig. 6a–c).

Immunohistochemistry for α-Subunit of ENaC in the Terminal Ileum

The distal colon in group A was used as the positive control. Immunoreactivity for the α-subunit of ENaC was detected only in the apical surface of the colonic epithelial cells as a very thin layer (Fig. 7a, b) and not in the crypt cells or immune and nonimmune cells in the lamina propria. No immunoreactivity was observed in the ileal samples from groups A, B, and C (Fig. 7c, d). However, we observed positive immunoreactivity for the α-subunit in the brush border of surface epithelia in group D (Fig. 7e, f). Phosphate-buffered saline replaced the primary antibody as a negative control and no immunoreactivity was observed (data not shown).

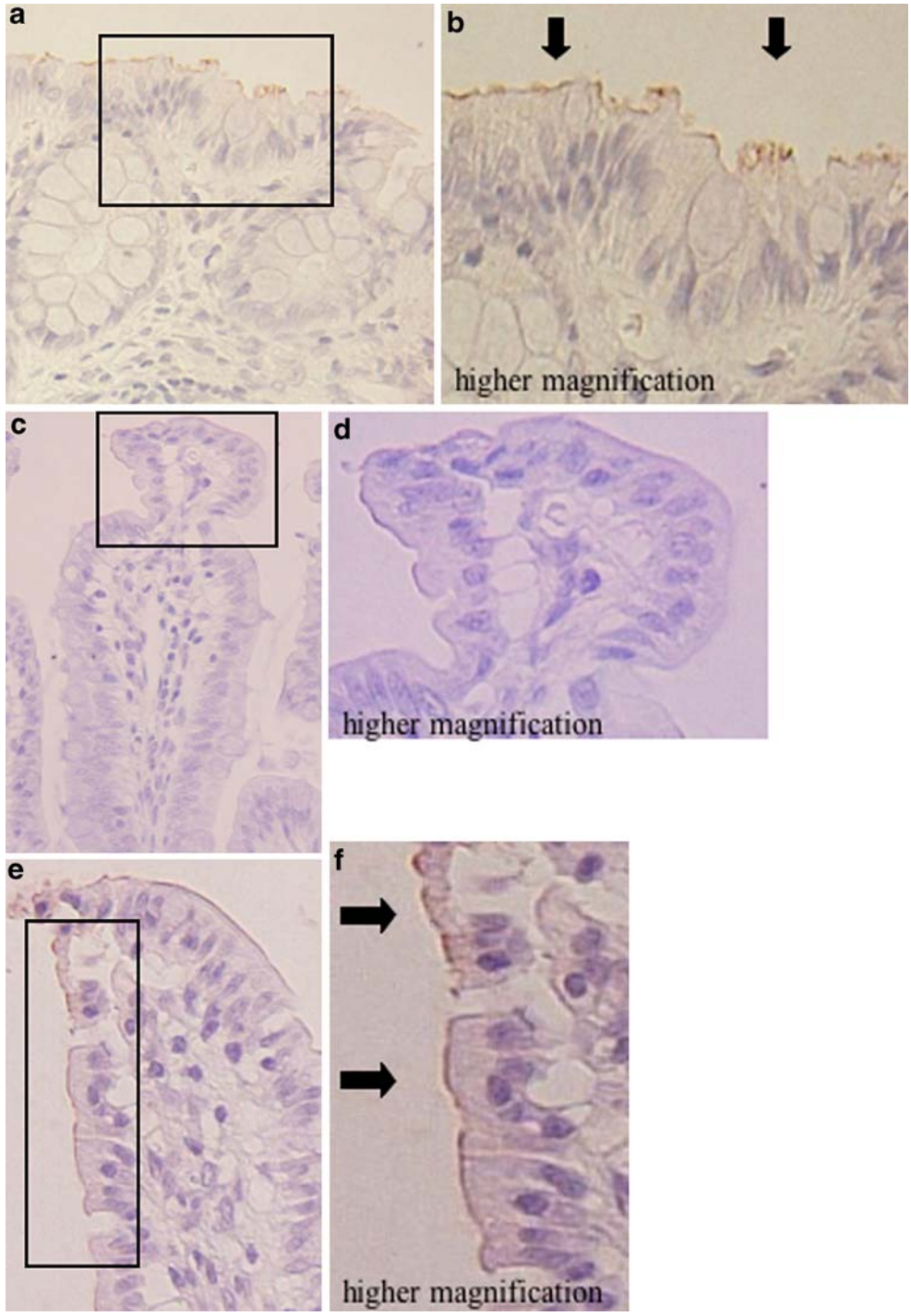

Figure 7 Immunostaining of the distal colon and terminal ileum with anti-α-ENaC. Tissues were obtained from groups A (no medication) and D (treated with D-aldosterone-containing PLGA microspheres). **a**, **b** Distal colon from group A. **c**, **d** Terminal ileum from group A. **e**, **f** Terminal ileum from group D. Note the positive immunoreactivity for the ENaC α-subunit in surface ileal epithelial cells of **e** and **f** but not in **c** and **d**.

Histological Examination of the Terminal Ileum

Systemic adrenal steroids have been reported to alter the mucosal architecture of the small intestine.[28] However, we did not detect any changes in the terminal ileum, e.g., villous height, crypt depth, infiltration of inflammatory cells into the mucosa, or the ratio of goblet cells to enterocytes in any of the groups (data not shown).

Measurement of Amiloride-Sensitive Sodium Transport in the Ileal Mucosae

The basal levels of Isc were approximately 50 μA/cm^2, and they were stable following the addition of 0.1 mM amiloride to the mucosal buffer in groups A, B, and C, suggesting that these tissues were devoid of amiloride-sensitive electrogenic sodium transport (Fig. 8a). However, the addition of amiloride to the tissues from group D animals resulted in a significant decrease in the Isc, suggesting the presence of electrogenic sodium transport (Fig. 8b).

Discussion

The overall objective of the present study is to establish optimal management for the persistent diarrhea, electrolyte imbalance, and dehydration that often develops postoperatively in patients undergoing TPC. Our data suggest that the use of D-aldosterone-containing PLGA microspheres may promote postoperative intestinal adaptation without significant systemic side effects. Because circulating aldosterone plays an important role in the pathogenesis of various vascular diseases, such as hypertension, endothelial dysfunction, and myocardial fibrosis,[28] we measured plasma aldosterone. The plasma aldosterone levels were similar in all of the experimental groups.

PLGA microspheres <5 μm in diameter are taken up into Peyer's patches in rats and transported via the mesenteric lymph nodes to the systemic lymphoid tissues, such as the spleen, whereas microspheres 5–10 μm in diameter do not enter the mesenteric lymph nodes but remain fixed in the Peyer's patches for longer periods.[21,22] Therefore, we developed microspheres with a diameter of approximately 10 μm to enhance the release of D-aldosterone in the ileal mucosae with minimal mineralocorticoid release into the systemic circulation. Our intent was to avoid these systemic effects by preventing microspheres from exiting the terminal ileum. There was no evidence of aldosterone-induced pathology, e.g., swelling of endothelial cells, on histological examination[29–31] in the ileal mucosa following 2 weeks of treatment with the microspheres. To date, microspheres containing various drugs have been used in numerous experimental models and have exhibited local effects in the absence of serious systemic side effects.[14–20]

In the present study, we measured mRNAs of α-, β-, and γ-subunits of ENaC, prostasin, 11β-HSD2, α1- and β1-subunits of Na^+/K^+-ATPase, and SGLT-1. The ENaC

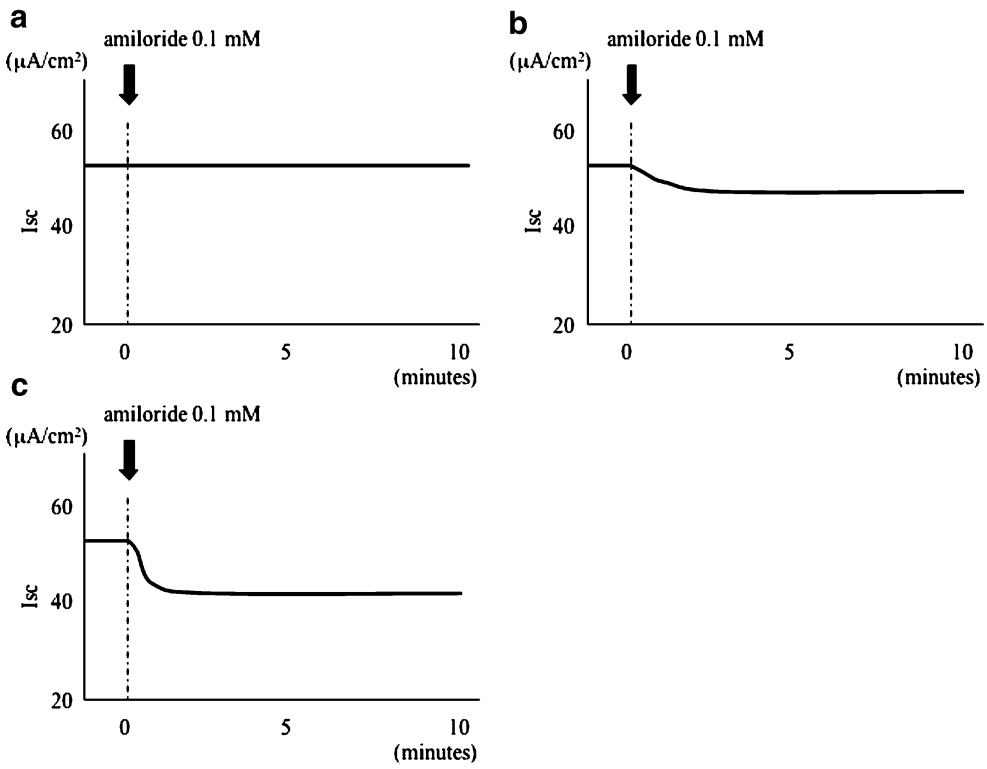

Figure 8 Isc in the terminal ileum. Typical tracings from group A (no treatment) (**a**) and group D (D-aldosterone-containing PLGA microspheres) (**b**) are shown. Trace from the distal colon in group A is also presented (**c**). The addition of 0.1 mM amiloride resulted in a significant decline in the Isc in group D. *Arrows* indicate when amiloride (0.1 mM) was added to the mucosal solution.

plays a major role in amiloride-sensitive sodium absorption from the apical side of epithelial cells. Prostasin, a membrane-bound serine protease, was initially found in mammalian urine[32] and its coexpression with ENaC increases sodium transport in renal and bronchial epithelial cells and Xenopus oocytes.[33,34] 11β-HSD2, an epithelial cell enzyme, is essential in conferring aldosterone its specificity for the nonselective MR by inactivating local glucocorticoids.[8] Thus, this enzyme stimulates aldosterone-mediated sodium absorption by a mechanism that involves the apical ENaC and the basolateral Na^+/K^+-ATPase. The Na^+/K^+-ATPase consists of α- and β-subunits, each of which has isoforms. Epithelial cells are thought to express α1- and β1-heterodimers.[35] This enzyme is present in the basolateral membrane and actively extrudes sodium from the cells into the interstitial milieu. SGLT-1 is expressed in the brush border membrane of small intestinal enterocytes and is responsible for active glucose absorption.[36,37] The transport of glucose is coupled to sodium transport down an electrochemical potential gradient into the cells. The administration of D-aldosterone-containing PLGA microspheres increased the expression of ENaC α- and β-subunits mRNA, as well as those of prostasin and 11β-HSD2, but not those of SGLT-1 or the α1- and β1-subunts of Na^+/K^+-ATPase. These data are similar to those obtained in the aldosterone-infused rats, although they lack the statistical significance observed in ENaC γ-subunit induction, suggesting that D-aldosterone-containing PLGA microspheres increased the level of aldosterone in the ileal mucosa. The increase in the amiloride-sensitive electrogenic Isc and the immunolocalization of the α-subunit of ENaC to the ileal surface in rats receiving aldosterone-containing microspheres supports our contention. However, we did not observe an induction of the α1- and β1-subunits of Na^+/K^+-ATPase, as was observed in aldosterone-infused and total colectomized rats.[6] One possible explanation is that Na^+/K^+-ATPase activity may be enhanced posttranscriptionally and/or this enzyme may originally have a wide range of capacity for sodium extrusion. Another possibility is the existence of undefined mechanisms for sodium extrusion in the remnant small intestine. Nevertheless, D-aldosterone-containing PLGA microspheres appear to enhance intestinal sodium absorption via the induction of aldosterone-associated molecules. Therefore, the pretreatment of patients scheduled for TPC with this therapy may promote intestinal adaptation and diminish postoperative diarrhea.

We successfully activated electrogenic sodium transport, which is essential for post-TPC adaptation of the ileum, by introducing a novel drug delivery system. Pre- and postoperative treatment with this drug may functionally compensate for the loss of sodium and water due to the absence of the entire colon. However, several questions must be resolved prior to any clinical trials with D-aldosterone-containing PLGA microspheres. For example, is this drug really free from adverse drug effects after long-term treatment? Furthermore, it must be demonstrated that D-aldosterone-containing PLGA microspheres decrease the loss of sodium and water from stools, even in the postoperative pouch.

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Adenocarcinoma of the Ampulla of Vater: T-Stage, Chromosome 17p Allelic Loss, and Extended Pancreaticoduodenectomy are Relevant Prognostic Factors

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Abstract
Objective To evaluate the prognostic significance of different clinico-pathological and molecular factors, and to compare survival after standard and extended pancreaticoduodenectomy (PD) in ampulla of Vater adenocarcinoma (AVAC).
Summary Background Data There are discordant data on factors affecting prognosis, and hence therapeutic choices, in AVAC.
Patients and Methods Clinical-pathological factors were evaluated in 59 patients, subjected to PD for AVAC; in 42 subjects information on chromosome 17p and 18q allelic losses (LOH) and microsatellite instability (MSI) was also available. The association between survival and type of PD was investigated in the 25 patients operated between 1990 and 2001 (16 standard and nine extended).
Results The overall 5- and 10-year tumor-related survival rates were 46% and 33%, respectively. Sixteen patients had T-stages 1–2, 14 T-stage 3, and 29 T-stage 4 cancers. Chromosome 17p and 18q LOH were detected in 23 (55%) and 15 cases (36%), respectively, and in 12 cases (29%) coexisted. Five cases were MSI-positive (12%). At univariate analysis, poor survival was

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associated with cancer ulceration (P=0.051), poor differentiation (P=0.008), T-stage 4 (P<0.001), nodal metastases (P=0.004), chromosome 17p (P<0.001) and 18q LOH (P=0.002), and absence of MSI (P=0.009). At multivariate analysis, only T-stage (P=0.002) and 17p LOH (P=0.001) were independent predictors of survival. All patients with MSI-positive cancers were long-survivors (>12 yrs), whereas only 30% of MSI-negative cancer patients survived at 5 years. Extended pancreaticoduodenectomy was associated with a 3-year disease-related survival higher than standard resection (83% vs 31%; P=0.018).

Conclusion MSI and chromosome 17p status allow to better define prognosis within ampullary cancers at the same stage. Surgery alone resulted curative in MSI-positive cancer patients, whereas it was inadequate in patients showing allelic losses, who might benefit from adjuvant therapy. In this observational study, extended PD was associated with increased survival compared to standard procedures.

Keywords Ampullary carcinoma · Pancreaticoduodenectomy · Extended pancreatic resection · Microsatellite instability · Chromosome 17p allelic losses

Introduction

Ampulla of Vater adenocarcinoma (AVAC) is the second most common periampullary carcinoma, representing about 10 to 30% of patients undergoing Whipple resection.[1,2] Surgery alone allows to cure about 50% of patients affected by AVAC. Prognostic factors, and hence therapeutic strategies, are still controversial. These include several parameters such as tumor size, differentiation grade, loco-regional invasion, and nodal involvement. Local spread of the tumor (T-stage) is the only established prognostic factor for AVAC; nevertheless, short- and long-term survivors coexist within the same T class after pancreaticoduodenectomy, as reported by Yamaguchi and Nishihara.[3]

In 1995, Klempnauer et al.[4] confirmed that prognosis of AVAC cannot be explained by classic risk factors and anticipated that "new techniques of molecular biology will lead to a better understanding of the differential biological behavior of these tumors".

As far as the type of resection is concerned, most authors identify pancreaticoduodenectomy as the treatment of choice, whereas a few others consider local resection (ampullectomy) as an adequate intervention. During the 1990s, extended pancreaticoduodenectomy for the treatment of pancreatic and periampullary carcinomas has spread in clinical practice,[5,6] although few reports describe the effectiveness of this technique in AVAC.[7,8] Moreover, the role of adjuvant chemoradiotherapy after AVAC resection is still controversial.[9,10]

The aims of the present study are: 1) to evaluate the prognostic significance of a combination of clinico-pathological and molecular factors in AVAC patients to plan adjuvant treatment when appropriate, and 2) to compare survival after standard and extended pancreaticoduodenectomy.

Patients and Methods

Patients Under Study

From January 1970 to August 2001, 82 patients underwent surgical resection for tumors of the ampulla of Vater at Clinica Chirurgica (1970 October 1992), renamed Chirurgia Generale C (November 1992 to August 2001) of the Department of Surgery of the University of Verona. From these, 18 cases were excluded for diagnosis different from AVAC, namely, villous adenoma (n=9), carcinoid tumor (n=2), small-cell neuroendocrine carcinoma (n=3)[11], colloid carcinoma (n=3), and signet-ring-cell carcinoma (n=1) (Table 1). After neglecting four additional patients died postoperatively and one patient deceased in hospital from surgical complications, 59 patients were considered for risk factor analysis.

All 64 patients with AVAC underwent pancreaticoduodenectomy (PD) and this intervention represented the 15.7% of all PD performed for whatever tumor, benign or malignant, in our Unit (n=408). Standard PD was the only intervention for AVAC until 1993, when the extended procedure was introduced. The surgical technique used for the extended PD (Fig. 1) has been previously described.[6,12,13] Briefly, the pancreas was divided at the left margin of the aorta, all the pre-aortic connective, lymphatic, and neural tissues were

Table 1 Tumors of Ampulla of Vater Treated at Clinica Chirurgica, Later Renamed as Chirurgia Generale C of the Department of Surgery, University of Verona

Histologic type	No. of cases	Surgery
Adenoma	9	8 ampullectomies, 1 PD
Carcinoid tumors	2	2 ampullectomies
Adenocarcinoma	64	64 PD (9 extended, 55 standard)
Colloid carcinoma	3	3 PD (3 standard)
Small-cell neuroendocrine carcinoma	3	3 PD (3 standard)
Signet-ring-cell carcinoma	1	1 PD (1 extended)

PD = Pancreaticoduodenectomy

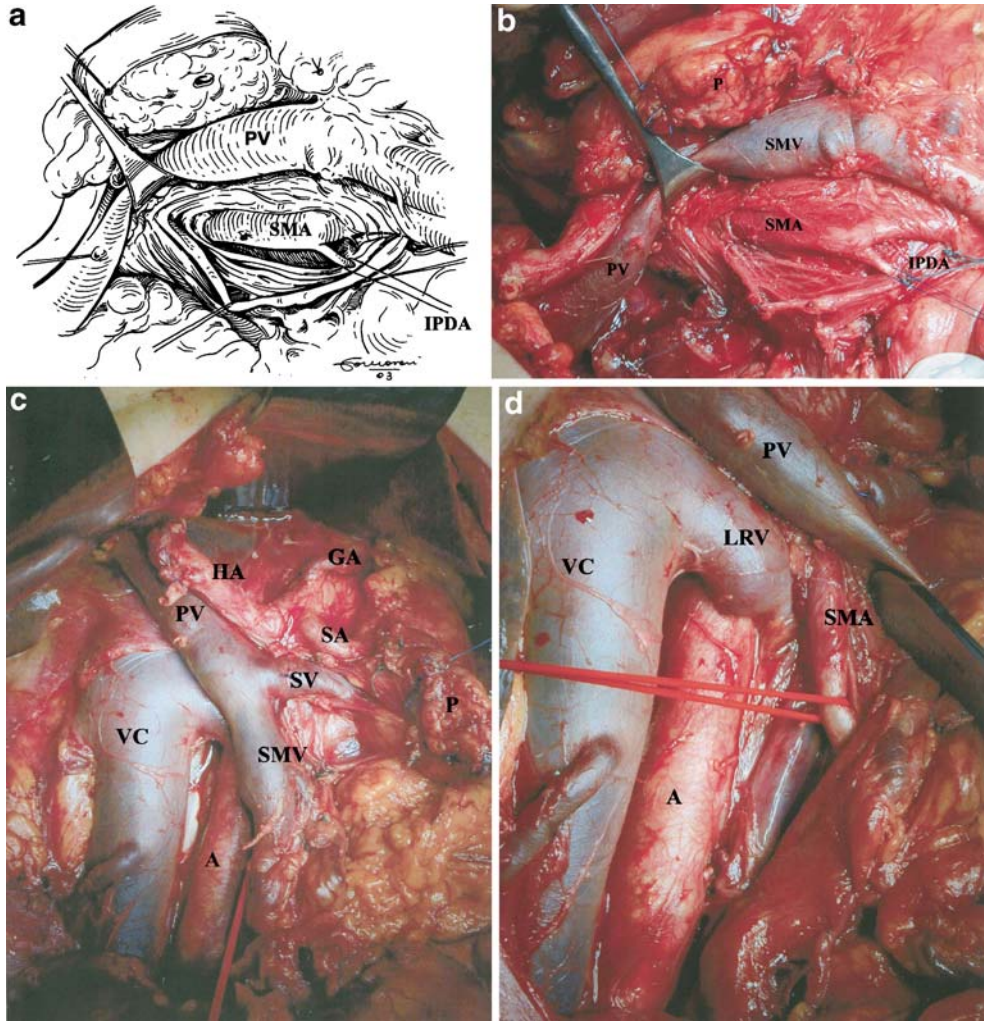

Fig. 1 a. Schematic drawing, illustrating dissection of Superior Mesenteric Artery (SMA) and Inferior Pancreaticoduodenal Artery (IPDA). PV=Portal Vein. **b.** Intraoperative finding, showing mobilization of Superior Mesenteric Vein (SMV) and dissection of the connective, neural, and lymphatic tissue surrounding Superior Mesenteric Artery (SMA) and the Inferior Pancreaticoduodenal Artery (IPDA). P = Pancreatic Stump; PV = Portal Vien. **c.** Operative findings after extended PD: general view. HA = Hepatic Artery; GA = Left Gastric Artery; SA = Splenic Artery; SV = Splenic Vien; SMV = Superior Mesenteric Vien; P = Pancreatic Stump; VC = Vena Cava; and A = Aotra. **d.** Operative findings after extended pancreaticoduodenectomy, focused on the dissection among the Vena Cava (VC), the Aorta, (A) the Left Renal Vein (LRV) and the Superior Mesenteric Artery (SMA). (PV = Portal Vien; P = Pancreatic Stump; HA = Hepatic Artery; GA = Left Gastric Artery, SA = Splenic Artery, SV= Splenic Vein).

dissected and resected from the superior margin of celiac axis down to inferior mesenteric artery (Fig. 1c–d).

Standard and extended PDs were compared with respect to survival. As assignment to either treatment was not randomized, the occurrence of a priori differences between patients undergoing standard or extended PD cannot be ruled out. To enhance the comparability between the two groups, extended PDs (n=9) were compared with standard PDs performed *approximately* over the same period, i.e., between 1990 and 2001 (n=16). In this analysis, operative (n=1) and in-hospital mortality for complications of surgery (n=1) were also considered.

Prognostic Factors

All the pathological specimens were grossly and microscopically reviewed to confirm diagnosis, size, macroscopic aspect (ulcerated or nonulcerated tumor) and to precisely establish the local tumor spread (T-stage 1: intraductal tumors restricted to muscle of Oddi; T-stage 2: infiltration of duodenal submucosa; T-stage 3: involvement of muscolaris propria, and T-Stage 4: infiltration of the periduodenal fat and pancreas, according to Yamaguchi and Enjoji[14]), lymph node status and tumor grading (low, grade 1; moderate, grade 2; high, grade 3).

In 42 patients operated on, the morphological examination was supplemented with a molecular investigation aimed at identifying factors predictive of prognosis. Molecular analysis included the occurrence of microsatellite instability (MSI) and of allelic losses (LOH) at chromosome 17p and 18q at sites where TP53 and DPC4 genes, involved in the pathogenesis of ampullary cancer, are located, respectively. The analysis was performed as previously described.[15,16]

Statistical Analysis

Follow up information was collected through clinical visits or telephone interviews at least twice a year. None of the patients was lost to follow-up. One death from lung cancer was considered as censored observations at the time of death (150 months). Postoperative mortality was considered

when comparing standard and extended PD to reproduce the by-intention-to-treat approach routinely used in clinical trials, whereas it was excluded when evaluating the prognostic significance of clinical, pathological, and molecular factors.

The probability of tumor-related survival was calculated according to the Kaplan–Meier method, and survival curves were compared by the log-rank test for each prognostic factor.

The following prognostic factors were evaluated by a Cox regression model in 59 subjects: sex, age, depth of invasion (T4 vs T1-T2-T3), macroscopic aspect (ulcerated vs nonulcerated), grading of tumor differentiation (poor versus well/moderate), nodal involvement (N+ versus N0). Another Cox regression model was performed in the 42 subjects with information on molecular status, considering the following variables: depth of invasion (T4 vs T1-T2-T3), grading of differentiation (poor vs well/moderate), chromosome 17p and 18q status (loss vs retention). It was not possible to include MSI status in the Cox model, as no event was observed in MSI-positive patients. Significance of differences was evaluated by the likelihood ratio test and the hazard ratio for the continuous variable (age) was calculated on the basis of an increase in the values of 1 SD. The assumptions of proportional hazard over time made in the Cox model were met for all the variables tested according to graphical methods.[17]

Differences in baseline characteristics between patients undergoing standard and extended PD were evaluated by Fisher exact test for dichotomous variables (sex, ASA score, gross aspect, grading, node metastasis, jaundice, 17p and 18q allelic status), by chi-square for trend for ordinal variables (T stage), by *t* test for normally distributed continuous variables (age, size) and by Mann–Whitney test for asymmetrically distributed continuous variables (number of positive nodes). Disease-related survival curves after standard or extend PD were compared by the log-rank test. Significance level was set at $P<0.05$.

Results

Median follow-up time of surviving patients was 130 months (range 7–248). Median survival time in the overall series amounted to 31 months, and 5- and 10-year survivals were 46% (95% CI 32.7–60.7%) and 33% (95% CI 19.7–48.7%), respectively.

The main clinical and demographic characteristics of the cohort under study are shown in Table 2. Most patients were men and age was 57.3±9.9 years (mean±SD). Tumor size was 2.3±1.1 cm and about half of the tumors were ulcerated. Grade of tumor differentiation was moderate or poor in 90% of the cases, and nearly 50% of cancers were T4. Nodal metastases were present in 37% of patients.

Table 2 Main Demographic and Clinical Characteristics of the Series Under Investigation (59 Patients with Ampullary Adenocarcinoma)

	N=59	Deceased	Mean 5-year survival, mos.	*P* value
Sex				
Men	42 (71.2)	28/42 (66.7)	40.2	0.195
Women	17 (28.8)	8/17 (47.1)	63.1	
Age				
<52 years	20 (33.9)	13/20 (65.0)	40.0	0.954
52–62 years	20 (33.9)	12/20 (60.0)	54.0	
>62 years	19 (32.2)	11/19 (57.9)	44.8	
Size (cm)				
<2 cm	17 (28.8)	7/17 (41.2)	61.5	0.160
≥2 cm	42 (71.2)	29/42 (69.0)	41.1	
Gross aspect				
Not ulcerated	27 (45.8)	14/27 (51.9)	59.7	0.051
Ulcerated	32 (54.2)	22/32 (68.8)	35.9	
Grading				
Well	6 (10.2)	2/6 (33.3)	100.0	0.008
Moderate	30 (50.8)	16/30 (53.3)	53.7	
Poor	23 (39.0)	18/23 (78.3)	23.4	
T stage				
1	2 (3.4)	0/2 (0.0)	–*	<0.001
2	14 (23.7)	6/14 (42.9)	71.4	
3	14 (23.7)	8/14 (57.1)	52.8	
4	29 (49.2)	22/29 (75.9)	26.5	
Node metastasis				
N0	37 (62.7)	19/37 (51.4)	61.3	0.004
N+	22 (37.3)	17/22 (77.3)	19.4	
Jaundice				
Absent				0.205
Present	43 (72.9)	29/43 (67.4)	40.7	
Chromosome 17p allele**				
Retention				<0.001
Loss	23 (54.8)	21/23 (91.3)	8.7	
Chromosome 18q allele**				
Retention	27 (64.3)	14/27 (51.9)	55.6	0.002
Loss	15 (35.7)	14/15 (93.3)	6.7	
MSI status**				
Positive	5 (11.9)	0/5 (0.0)	100	0.009
Negative	37 (88.1)	28/37 (75.7)	29.7	

Variables are reported as absolute frequency with percent frequency in brackets, Five-years survival was computed by the Kaplan-Meier method and significance of differences in survival was assessed by the log-rank test.

* The two T1 subjects are censored at 36 and 38 months respectively. To compute significance of differences, these subjects were grouped together with T2 patients.

MSI=MicroSatellite Instability.

**Available in 42 subjects.

Chromosome 17p and 18q LOH were detected in 23 (55%) and 15 cases (36%), respectively, and coexisted in 12 cases (29%). MSI-positive was identified in five cases (12%).

As shown in Fig. 2, clinico-pathological factors associated with poor survival in univariate analysis were: T-stage 4 ($P<0.001$), poor differentiation ($p=0.003$), node metastases ($P=0.004$), and ulceration of the cancer ($P=0.051$). In

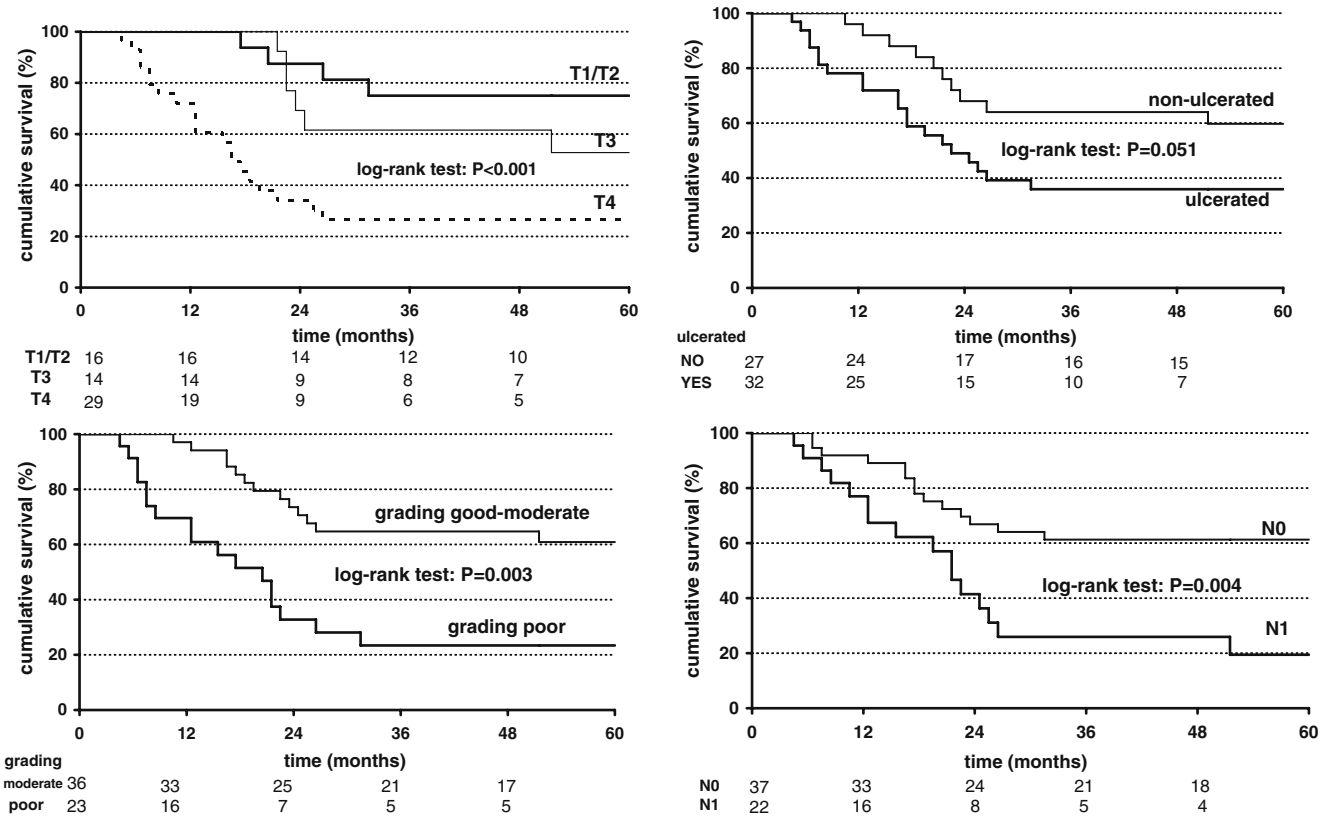

Fig. 2 Kaplan–Meier survival curves, as a function of T-stage (A), ulcerated and nonulcerated form (B), grade of differentiation (C), and lymph node status (D) in ampullary adenocarcinoma.

multivariate analysis, only depth of invasion (P=0.003) and grading (P=0.013) emerged as independent prognostic factors (Table 3).

Molecular factors were not associated with tumor size, gross aspect, and grading (Table 4). On the other hand, 17p and 18q LOH were significantly more common in node-positive tumors. The five patients with MSI-positive cancers were all in T-stages 2 and 3 and without node metastases.

In univariate analysis, all the molecular factors considered appeared as important predictors of survival (Fig. 3). All MSI-positive patients were long-survivors, whereas 5- and 10-year survivals in the patients with MSI-negative cancers were 30 and 24%, respectively. Chromosome 17p and 18q LOH were associated with poor prognosis (Fig. 3). In multivariate analysis, T-stage (Hazard Ratio of T-stage 4 vs 1-2-3 =3.87, 95% CI: 1.61–9.29, P=0.002) and chromosome 17p LOH (Hazard Ratio loss vs retention = 5.00, 95% CI: 1.81–13.79, P=0.001) were independent predictors of survival, whereas grading of tumor differentiation and chromosome 18q LOH lost their significance (Table 5).

Whichever the stage, the loss of chromosome 17p identified patients with worse prognosis. Among patients with T-stage 1- 2-3 disease, those with retention of chromosome 17p had a much better outcome (100% at 5-year survival) than those with chromosome 17p allelic loss (18% at 5-year survival). Similarly, patients with T-stage 4 cancers had a 5-year survival of 38% when retaining 17p alleles, whereas none of those with chromosome 17p allelic loss survived more than 26 months after surgery. As shown in Fig. 4, T stage was more important in the first 20 months of follow-up, whereas chromosome 17p LOH became more important thereafter. Indeed, when considering only the first 20 months of follow-up, statistical significance was retained by T stage (P<0.001), but not by chromosome 17p LOH (P=0.060); the reverse pattern was observed when excluding the first 20 months of follow-up (P=0.542 for T stage and P<0.001 for chromosome 17p LOH).

Table 3 Relative Risks of Death from Ampullary Adenocarcinoma as a Function of Clinic and Pathologic Factors, Computed by Multivariate Survival Analysis

	HR (95% CI)	P value
Sex	1.43 (0.55–3.71)	0.473
Age (SD=9.9 years)	1.31 (0.84–2.04)	0.236
Depth of invasion (T4 vs T1-T2-T3)	3.61 (1.48–8.80)	0.003
Gross aspect (ulcerated vs nonulcerated)	1.18 (0.57–2.44)	0.648
Grading (poor vs well/moderate)	3.27 (1.29–8.31)	0.013
Node metastasis (N+ vs N0)	1.20 (0.52–2.80)	0.666

Hazard ratios (HR) and P values (likelihood ratio test) were derived from Cox regression model, controlling for all other variables. n=59.

Table 4 Relation Between Molecular and Pathological Factors

	Size (cm)	Gross aspect: ulcerated	Grading: Poor	T stage: 4	Nodal status: N^+
Chromosome 17p allelic status					
Retention (n=19)	2.2±1.2	8 (42%)	8 (42%)	8 (42%)	4 (21%)
Loss (n=23)	2.5±1.2	14 (61%)	10 (44%)	12 (52%)	13 (57%)
P value	0.377	0.352	1	0.551	0.029
Chromosome 18q allelic staus					
Retention (n=27)	2.2±1.0	12 (44%)	12 (44%)	10 (37%)	6 (22%)
Loss (n=15)	2.8±1.5	10 (67%)	6 (40%)	10 (67%)	11 (73%)
P value	0.133	0.209	1	0.107	0.003
MSI status					
Positive (n=5)	2.1±1.2	3 (60%)	2 (40%)	0 (0%)	0 (0%)
Negative (n=37)	2.4±1.2	19 (51%)	16 (43%)	20 (54%)	17 (46%)
P value	0.567	1	1	0.049	0.070

Results are presented as mean±SD for continuous variable (size) and as absolute frequency with percent frequency in brackets for categorical variables. Significance of differences was evaluated by Fisher exact test for categorical variables (gross aspect, grading, T stage, node metastasis), and by t test for the continuous variable (size).
MSI=MicroSatellite Instability.

The data of the two groups of patients, undergoing standard (16 patients) or extended PD (9 patients) between 1990 and 2001, are summarized in Table 6. The mean number of excised nodes was 12.9±4.7 after standard PD and 33.1±10.7 after extended PD. The two groups were homogeneous with respect to all other clinical and pathological factors considered, with the only exceptions were age and calendar year of surgery, as patients undergoing extended resection were significantly older, and extended PD was introduced in 1994. Of note, no patient was excluded because of advanced age. The number of patients with node metastases as well as the number of positive nodes did not differ significantly

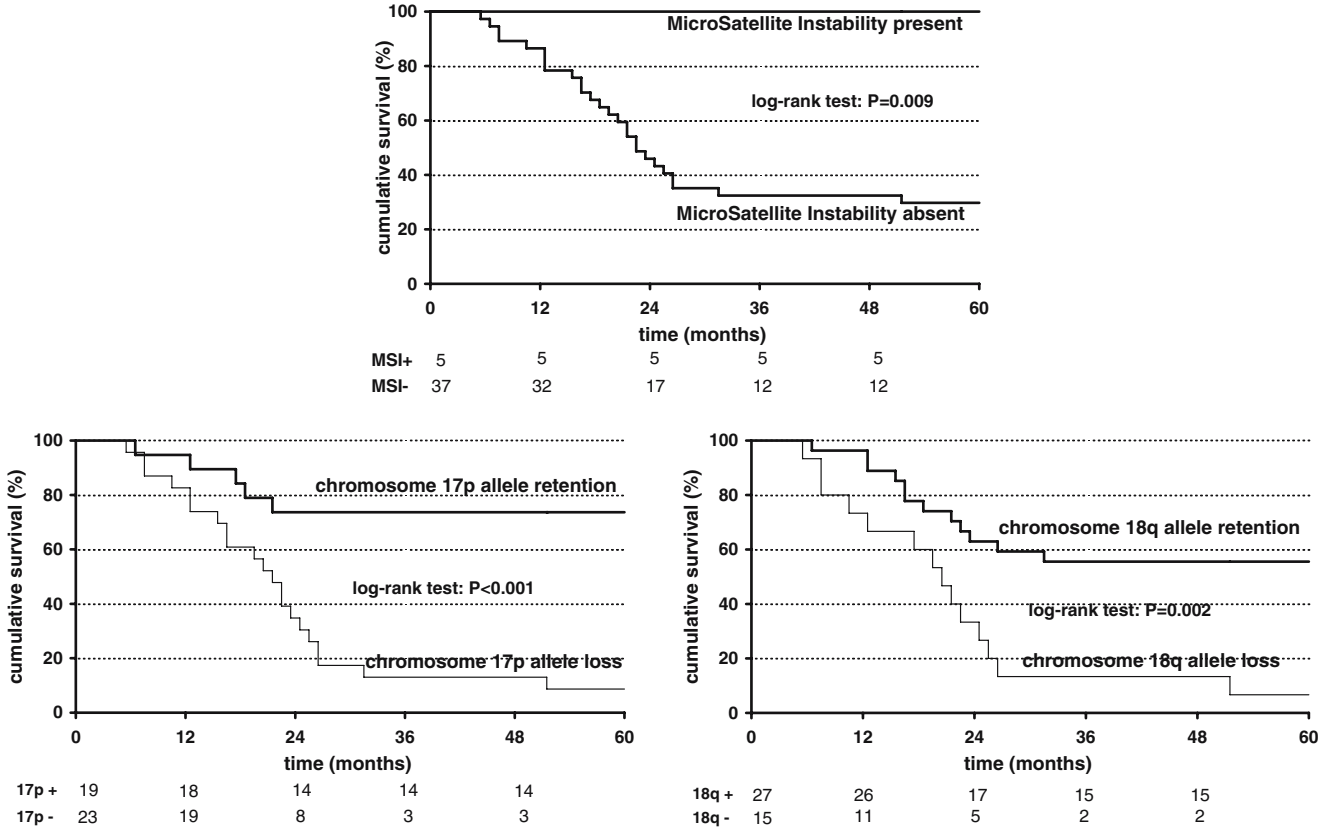

Fig. 3 Kaplan–Meier survival curves, as a function of MicroSatellite Instability (MSI) phenotype (A), chromosome 17p allelic status (B), and chromosome 18q allelic status (C) in ampullary adenocarcinoma. MSI+=MSI-positive; MSI-=MSI-negative; 17p-=chromosome 17p allelic loss; 17p+=chromosome 17p allelic retention; 18q-=chromosome 18q allelic loss; 18q+=chromosome 18q allelic retention.

Table 5 Relative Risks of Death From Ampullary Adenocarcinoma as a Function of Pathologic and Molecular Factors, Computed by Multivariate Survival Analysis

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Depth of invasion (T4 vs T1-T2-T3)	4.04 (1.85–8.79)	<0.001	3.87 (1.61–9.29)	0.002
Grading (poor vs well/moderate)	1.83 (0.87–3.84)	0.114	1.79 (0.84–3.83)	0.136
Chromosome 17p (loss vs retention)	4.71 (1.93–11.49)	<0.001	5.00 (1.81–13.79)	0.001
Chromosome 18q (loss vs retention)	3.18 (1.48–6.84)	0.004	1.32 (0.53–3.29)	0.549

Hazard ratios (HR) and *P* values (likelihood ratio test) were derived from Cox regression model, either considering each variable separately (univariate analysis) or controlling for all other variables (multivariate analysis). n=42.

between the two groups. Of note, three patients undergoing extended PD presented metastases to superior mesenteric artery nodes (number 14 of the Japanese Classification). Three-year disease-related survival for extended pancreaticoduodenectomy was remarkably higher (83%) than that for standard resection (31%, P=0.018; Fig. 5).

Discussion

The main results of the present study are:

1) Among clinical and pathologic factors depth of invasion (T stage) and, to a lower extent, grading of tumor differentiation emerged as independent prognostic factors.
2) Among molecular variables, chromosome 17p LOH, but not 18q LOH, appeared as an independent prognostic factor: 17p status allowed to improve prognostic definition even within the same T stage. In multivariate survival analysis depth of invasion was the most important prognostic variable in the first 2 years of follow-up, whereas 17p LOH became the most important variable thereafter.
3) All patients with MSI-positive cancers were long survivors, whereas 5- and 10-year survivals in the patients with MSI-negative cancers were 30 and 24%, respectively.
4) Extended PD was associated with a better survival than the standard procedure. This result should be interpreted with caution, as the study was not randomized.

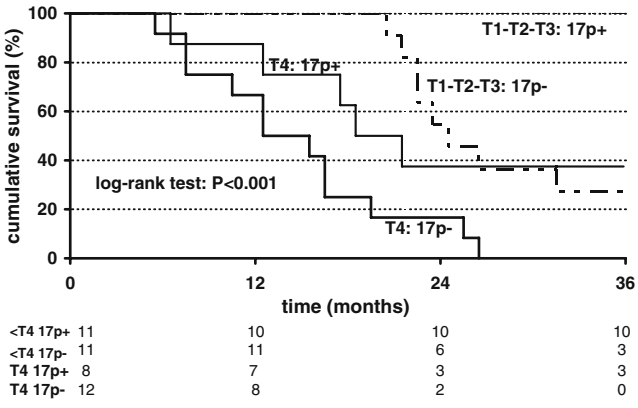

Fig. 4 Kaplan–Meier survival curves, according to both T-stage and chromosome 17p allelic status in ampullary adenocarcinoma. 17p-= chromosome 17p allelic loss; 17p+=chromosome 17p allelic retention.

The prognostic significance of risk factors in ampullary carcinoma is a matter of controversy, with the only exception of local tumor spread (T stage). Patients affected by this type of cancer experience a longer survival, compared to those with pancreatic ductal adenocarcinoma, and the longer natural history has favored the proposal of many prognostic factors. Some of these have been derived from studies on ductal adenocarcinoma or colorectal cancer, which presents morphological and time-course affinities with ampullary carcinoma. The most frequently proposed prognostic variables are macroscopic appearance, pathological characteristics, tumor grading, local invasiveness (T factor), and lymph node status.

A nonulcerated appearance of tumors suggests a better prognosis than the ulcerated and scirrhous forms.[14,18,19] Many authors claim that tumors with papillary histotype present a better prognosis.[20,21] According to various reports, a high-grade tumor carries a very poor prognosis, with no patient surviving at 5 years,[22–25] whereas satisfactory survival rates (48–62%) are reported for resections of low-grade carcinomas.[21,24]

Local tumor spread seems to be the most important prognostic factor[4,26]. A number of authors believe that infiltration of the pancreas is associated with no 5-year survival even in the absence of other negative prognostic factors[27,28] and assume that these tumors have the same poor prognosis of pancreatic ductal carcinoma.[29–32] Accordingly, Yamaguchi and Enjoi[14], on the basis of a series of 109 patients, stated that the main prognostic factor is whether the tumor has spread beyond the sphincter of Oddi.

Tumor size had been considered as an important predictor of mortality, with a poor prognosis expected for tumors exceeding 2 cm. However, this criterion was shown not to be an independent prognostic factor, as it is no longer significant when T stage is taken into account.[33,34] The size criterion may

Table 6 Main Demographic and Clinical Characteristics of the 25 Patients with Ampullary Adenocarcinoma who Underwent Pancreaticoduodenectomy During the 1990s, According to the Type of Surgical Intervention (Standard or Extended)

	Pancreaticoduodenectomy		
	Standard (n=16)	Extended (n=9)	P value
Sex			
Men	10 (62.5)	7 (78)	0.661
Women	6 (37.5)	2 (22)	
Age (years)	56.0±11.3	65.3±7.6	0.038
ASA score			
I	7 (44)	3 (33)	0.691
II	8 (50)	6 (67)	
III	1 (6)	–	
Year of surgery			
1990–1993	13 (81)	–	0.001
1994–2001	3 (19)	9 (100)	
Size (cm)	2.2±0.9	1.9±0.9	0.405
Gross aspect			
Not ulcerated	5 (31)	6 (67)	0.115
Ulcerated	11 (69)	3 (33)	
Grading			
Well–moderate	9 (56)	7 (78)	0.401
Poor	7 (44)	2 (22)	
T stage			
1 and 2	7 (44)	2 (22)	0.336
3	4 (25)	3 (33)	
4	5 (31)	4 (44)	
Node metastasis			
N0	10 (62.5)	4 (44)	0.434
N+	6 (37.5)	5 (56)	
Number of excised nodes			
Median (range)	12 (8–23)	32 (20–49)	–
Mean±SD	12.9±4.7	33.1±10.7	
Number of involved nodes per node-positive patients			
Median (range)	2 (1–7)	3 (1–6)	0.792
Mean ±SD	3.17±2.71	3.20±1.98	
Jaundice			
Absent	5 (31)	5 (56)	0.397
Present	11 (69)	4 (44)	

Categorical variables are reported as absolute frequency with percent frequency in parentheses, continuous variables (age and tumor size) as mean±SD. Median and range are also shown for asymmetrically distributed variables (number of excised or involved nodes).
Significance of differences was assessed by Fisher exact test for dichotomous variables, by chi-square for trend for ordinal variables (T stage), and by t test (age, size) or Mann–Whitney U test (year of surgery, number of involved nodes) for continuous variables. ASA score = American Society of Anesthesiology score. For statistical purpose, the patient with an ASA score III was considered together with patients with an ASA score II.
ASA score=American Society of Anesthesiologists score.

simply be a marker of other risk factors, given that larger size is correlated with a greater frequency of pancreatic, vascular, perineural, and lymph-node invasion.[24,29,35,36]

Lymph node infiltration has been taken into consideration by all authors addressing survival in this type of malignancy. Most of them reported a significant higher survival in patients undergoing resection without nodal metastases.[22,25,31–40]. In some cases, patients with nodal metastases[27,28] undergoing resection were reported to experience the same survival rate as patients undergoing only palliative treatment, with no survivor at 5 years. A few authors do not attribute any prognostic significance to lymph node metastases.[4,24,41–45]

In the present series, the following factors were found to have an adverse effect on survival in univariate analysis: ulceration of the tumor, poor differentiation, T-stage 4, and lymph node metastases. At multivariate analysis, however, only T stage and grading of tumor differentiation proved to be independent prognostic factors, in agreement with the current literature.

As most clinical and pathological factors did not prove to be reliable prognostic criteria, research has been focused on factors correlated with DNA anomalies of neoplastic cells. Recently, molecular investigation has come up with new criteria based on chromosome deletions,[16] oncogenes[46,47] and suppressor gene abnormalities,[16,48] and microsatellite instability (MSI).[15,48]

Familial colorectal cancer, polypoid and nonpolypoid,[49,50] and sporadic colorectal cancer have offered a whole range of gene mutations, related to inactivation of oncosuppressor genes (APC, DCC, p53), activation of oncogenes (K-ras), and MSI phenotype. Reports of such mutations in carcinoma of the ampulla are relatively recent,[15,16] but the early results seemed promising in improving assessment of prognosis.

In the present study, we tested for deletion of chromosomes 17p and 18q and for MSI. At multivariate analysis, deletion of chromosome 17p proved to be an independent prognostic factor. Five-year survival rate was 74% in patients with preserved chromosome 17p versus 9% in patients with chromosome 17p deletion. The adverse effect of chromosome 17p loss was independent of local tumor spread: in the present series, in those cases with preserved chromosome 17p, T stage 1-2-3 tumors and T stage 4 tumors had 5-year survival rates of 100 and 38%, respectively, whereas in those

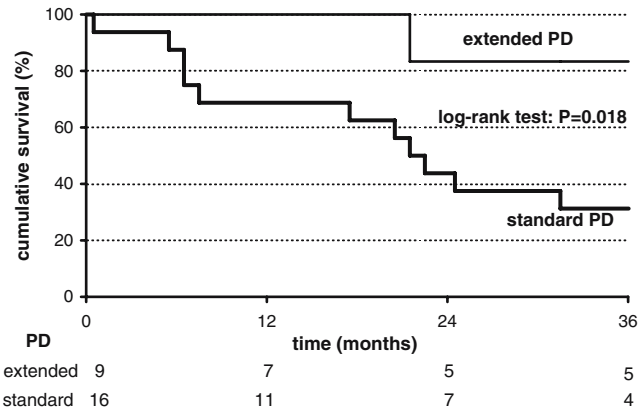

Fig. 5 Kaplan–Meier survival curves after standard or extended pancreaticoduodenectomy (PD) for ampullary adenocarcinoma.

cases with chromosome deletion survival dropped sharply to 18 and 0%, respectively.

Only five patients out of 42 (12%) were MSI-positive and these were all alive after 12 years, unlike the MSI-negative subjects who presented 5- and 10-year survival rates of 30 and 24%, respectively. It should be noted that patients with MSI-positive cancer were all in T-stages 2 and 3 and did not present nodal metastases. Moreover, it is of interest that one patient with a T-stage 4 MSI-positive colloid ampullary carcinoma, not considered for the present analysis, was a long survivor (≥14 years). Similarly, three MSI-positive cases described by Scarpa et al.[16] encountered a favorable prognosis even when presenting an advanced tumor (N1 and/or T4).

Recently, Nakata et al.[51] reported similar results for pancreatic cancer, as MSI-positive cancer, which was observed in a minority of patients (17%, eight out of 46), was associated with a more favorable prognosis (hazard rate [HR] of MSI-negative vs MSI-positive=5.577; P=0.007). As tumor-infiltrating leukocyte intensity was larger in MSI-positive than MSI-negative tumors, the authors[51] proposed that the stronger immunoreaction elicited by MSI-positive cancer could partly explain the better prognosis.

The molecular markers are not only prognostic factors, but may also serve as indicators of the therapeutic strategy. Indeed, in those cases where gene investigation reveals deletion of chromosome 17p, one may expect that surgical therapy alone will fail to prove curative and hence adjuvant treatments should be considered. On the contrary, surgical treatment alone seems adequate in MSI-positive patients.

At present, adjuvant treatments are based on the use of chemotherapy and radiotherapy, the results of which appear anything but encouraging.[1,26,29] However, the benefits of adjuvant treatments will probably improve when molecular markers will allow to identify those patients who can really benefit from these treatments.

Pancreaticoduodenectomy is the treatment of choice for carcinoma of the ampulla of Vater. The 5-year survival with this procedure ranges from 24 to 60%,[18,22,29,30,31,37,41,42,52–55] with mean values around 35–46%.[1,2,4,38,43,56,57]

Ampullectomy had been advocated mainly by French surgeons,[58,59] as it seemed associated with lower postoperative morbidity and mortality without worsening of long-term survival.[36,41,60,61] However, later studies showed high incidence of postoperative complications and recurrence,[24,62] so that its indications have been restricted to high-risk patients or to T-stage 1 tumors.[63,64]

Recently extended lymphadenectomy was introduced in the treatment of pancreatic and periampullary tumors, but the results have been discordant and the procedure is still a matter of some controversy.[5,13,65,66]

The lymph node stations most frequently involved in ampullary carcinoma after extended pancreaticoduodenectomy were the posterior and anterior pancreaticoduodenal lymph nodes, the nodes of the inferior pancreaticoduodenal and mesenteric arteries, and less frequently the para-aortic lymph nodes, pericholedochal and retroportal lymph nodes, whereas the pyloric, coeliac, medio-colic, and hepatic artery lymph-node stations were never invaded.[3,7,18,30,67,68] In particular, the involvement of perimesenteric lymph nodes in the present study (33%) was in the upper limit of the range reported in the current literature, which varies from 11–17%[18,30,68] to 30%[7,8] of cases. This percentage remarkably increases when one considers only node-positive patients (57–59%).[7,8] Of note, nearly all the patients with multiple-node metastases (10 out of 11) in the series of Fernandez-Cruz[7] had positive nodes around the superior mesenteric artery, which are not usually resected with the standard technique. Also, nodes along the inferior pancreaticoduodenal artery (Fig. 1 A–B) should be resected as they are often positive, in up to 57% of cases.[8]. A lower involvement is observed in para-aortic lymph nodes, as they are metastatic in 6–8% of cases.[7,8] However, it should be reminded that this site is not always surgically explored;[8] for instance, in only five out of 39 (13%) in the series of Shirai.[8] In our nine cases, no metastasis to para-aortic lymph nodes was detected.

Few studies[7,8,30,67–69] exist on extended pancreaticoduodenectomy (PD) in ampullary tumors, and only one was a randomized clinical trial that recruited 294 periampullary tumors, including 62 ascertained ampullary tumors.[69] No difference in survival was detected between patients undergoing standard or extended PD. However, 62 cases, although representing a quite large sample size from a clinical point of view, are rather few from a statistical point of view. Our study does not add much to this debate, being based on an even smaller number of patients (n=25). Moreover, the study was not randomized, and thus the influence of unknown prognostic factors, unevenly distributed among the two groups, cannot be ruled out. Anyway, we found that extended PD was associated with a remarkably higher survival than standard PD. It should also be reminded that the surgical technique we adopted for extended PD was slightly different in the present study compared to the above-mentioned trial.

The proportion of N+ positive patients was slightly higher after extended PD (five out of nine) than after standard PD (six out of 16), and this could reflect a better staging after the more radical intervention. However, the numbers are too small to draw a definite conclusion (p=0.43).

There are other oncological reasons to believe that the combined clearance of perimesenteric lymphatic vessel and nodes and plexus, performed during pancreaticoduodenectomy, can improve prognosis after radical resection by suppressing two major pathways for tumor spread, namely, the lymphatic and the perineural pathways.[8,12,68] Two

recent studies[70,71] support this hypothesis, as they showed that perineural invasion[70] and lymphatic vessel invasion[71] were independent predictors of poor survival. Of note, perineural infiltration has been reported in 16 to 56% of ampullary carcinoma.[67,72]

Conclusion

In conclusion, the mainstay in the management of carcinoma of the ampulla of Vater firmly remains pancreaticoduodenectomy, in our opinion, associated with extended lymphadenectomy including the posterior and anterior pancreaticoduodenal, pericholedochal, and, above all, the perimesenteric and para-aortic nodes.

The degree of local invasion of the tumor and a number of molecular markers (deletion of chromosome 17p, microsatellite instability, or MSI-phenotype) are factors for prognostic assessment and for planning multimodality treatment: in our series, MSI-positive phenotype identified long survivors after surgical removal of cancer, whereas allelic losses of chromosome 17p identified aggressive cancers in which adjuvant therapy might have been useful to improve survival.

When comparing standard and extended pancreaticoduodenectomies, it should be reminded that the study was not randomized and the series was rather small. However, the recorded difference in survival was so large that a beneficial effect of the extended intervention seems a likely explanation.

As ampullary carcinoma is rather rare, a conclusive clinical evidence can be achieved only through a multicentric randomized trial to achieve an adequate number of cases.

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Prognosis and Results After Resection of Very Large (≥10 cm) Hepatocellular Carcinoma

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Abstract
Introduction Few potentially curative treatment options exist besides resection for patients with very large (≥10 cm) hepatocellular carcinoma (HCC). We sought to examine the outcomes and risk factors for recurrence after resection of ≥10 cm HCC.
Methods Perioperative and long-term outcomes were examined for 189 consecutive patients from 1993 to 2004 who underwent potentially curative resection of HCC ≥10 cm (n=24; 13%) vs. those with HCC <10 cm (n=165; 87%). Disease-free survival (DFS) and overall survival (OS) were determined by Kaplan–Meier analysis and patient, tumor, and treatment characteristics were compared using univariate and multivariate analysis.
Results Median follow-up was 34 months. Tumors ≥10 cm were more likely to be symptomatic, of poorer grade, and have vascular invasion (p<0.05). Twelve patients (50%) underwent an extended resection of more than four hepatic segments or resection of adjacent organs for oncologic clearance (diaphragm-2, inferior vena cava (IVC)-2, median sternotomy-1). Postoperative complications were more common after resection of >10 cm HCC (12/24, 50% vs. 35/165, 21%; p=0.04). Median DFS was significantly shorter in patients with large HCC (≥10 cm) group compared to patients with smaller HCC (8.4 vs. 38 months; p=0.001), but overall survival was not different between the two groups (5-year survival 54% vs. 53%; p=0.43). Seventeen patients (71%) with very large HCC developed recurrences (12 intrahepatic, five systemic); eight of these patients (47%) underwent additional therapy (resection-4, TACE-3, RFA-1). Pathological positive margins and vascular invasion were significant determinants of DFS in tumors ≥10 cm (p<0.05), but only vascular invasion was an independent risk factor for recurrence after multivariate analysis (HR 0.17; 95% CI: 0.04–0.8). Median OS after recurrence was 24 months.
Conclusion Surgical resection is the optimal therapy for very large (≥10 cm) HCC. Although recurrences are common after resection of these tumors, overall survival was not significantly different from patients after resection of smaller HCC in this series.

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Keywords Hepatocellular carcinoma · Resection · Vascular invasion · Recurrence · Ablation · Liver transplant

The rising incidence of hepatocellular carcinoma (HCC) in North America is well documented, with an increase of 75% over the past decade in the United States alone.[1] As the hepatitis C epidemic grows, the ability to screen all patients at risk for HCC may diminish, leading to more patients presenting with a larger tumor burden.[2] Current trends from other parts of the world would suggest that unless screening modalities and access to care for patients

with viral hepatitis improve, some societies will be faced with many patients with very large HCC for which surgical resection may not be considered.[3,4]

Treatment options for very large (≥10 cm) HCC are limited. Larger tumors are more likely to recur,[5,6] harbor unrecognized small vessel tumor invasion,[7] and may portend toward worse biological behavior from genetic factors that are currently unknown.[8] Initial experience with liver transplantation (LT) for HCC of all sizes, particularly large, unresectable tumors, led to poor results until Mazzaferro[9] published outstanding outcomes with LT specifically for small (<5 cm) HCC. Local therapy such as ablation is not effective for larger tumors.[10] Transarterial chemoembolization (TACE) is an attractive option for large HCC, but the response rate has generally been poor and long-term outcomes are not well known.[11] Resection has been and still appears to be the best option in patients with HCC ≥10 cm because it is potentially curative, can be performed safely with acceptable morbidity and does not compete for the scarce supply of donor livers.[12–16] The purpose of this study was to determine and analyze the outcomes of patients who have undergone curative resection of ≥10 cm HCC by comparing them to patients with HCC <10 cm, and to determine the factors that are independently associated with the recurrence of HCC.

Methods

Patient Selection

A retrospective cohort study spanning the 11-year period from 1993 to 2004 was performed. One hundred eighty-nine consecutive patients who underwent attempted curative liver resection for HCC at the Toronto General Hospital, University Health Network of the University of Toronto were identified. Patients were divided into those that underwent resection for very large (≥10 cm) HCC and for smaller (<10 cm) HCC based on preoperative computed tomography (CT). Institutional Review Board approval for this studied was obtained from the University Health Network of the University of Toronto. All patients in this study had a confirmed diagnosis of HCC at surgical pathology. Patients with fibrolamellar HCC or those who underwent resection with noncurative intent (e.g., exploratory laparotomy or biopsy only) were omitted from the analysis. Patients who underwent ablation, either radio frequency or percutaneous ethanol injection, as the sole therapy of their tumor were not included in this analysis. Surgical mortality was considered as death occurring within 90 days after surgery. Morbidity was defined as any complication requiring an intervention during the perioperative period.

Preoperative Assessment

All patients were managed with liver resection as the initial treatment for their primary liver tumor. Patients were offered surgical resection if their tumor(s) were resectable with adequate margin based on cross-sectional imaging, if they had adequate estimated postresection hepatic function, and preoperative and intraoperative absence of extrahepatic tumor. All patients underwent preoperative viral serology testing, laboratory assessment of liver function, triple-phase CT, and transabdominal ultrasound (US) to evaluate for cirrhosis and tumor characteristics. A hypervascular lesion with rapid washout in the background of cirrhosis was the most common imaging characteristic of HCC. Preoperative vascular invasion was defined as the presence of tumor thrombus in any major hepatic or portal vein branches on CT or US. Hepatic reserve was assessed using Child-Pugh classification[17] (protime, bilirubin, albumin, ascites, and encephalopathy), plus platelet count. Since 1999, indocyanine green (ICG) clearance was routinely used to assess hepatocellular function with retention at 15 min less than 15% considered adequate reserve.[18] Routine biopsy of the lesion was not performed before resection if the lesion(s) had typical imaging features of HCC.[19]

Surgical Technique

Intraoperative ultrasound (IOUS) was used at laparotomy to confirm the anatomic characteristics of the tumor, and to evaluate the remnant liver for additional tumors. In the early years of the study (1993–2002), parenchymal transection of the liver was achieved with crush clamp technique or use of the ultrasonic aspirator (CUSA, Valleylab, Boulder, CO). Since 2002, a precision water-jet dissection system has been used for most cases (Hydro-Jet Dissector, ERBE, Tubingen, Germany).

Follow-up and Analysis

Postoperatively, patients were followed with physical exam, serial CT scans, or US and alpha-fetoprotein levels (AFP), if elevated preoperatively, at 3-month intervals for the first year, and then every 6 months. All patients in this analysis had a minimum 6-month follow-up with median follow-up of 34 months (range 6–149). Recurrence of HCC was identified by new or growing lesions on imaging with appearances typical of HCC or a rising AFP. Lesions not typical of HCC were confirmed by biopsy.

Pathologic specimens were reviewed for tumor characteristics including: number and size of tumors, tumor grade, vascular invasion, and microscopic margins. A margin of ≥1 mm was considered a negative margin. The analysis was also done with margins ≥10 mm considered as a negative

margin with no difference in results. Postoperative pathologic vascular invasion was defined as histological involvement of lobar or segmental branches of portal or hepatic veins or gross invasion of the right or left main branches of the portal or hepatic veins.

Patient demographics, tumor, operative, and treatment characteristics were evaluated. The after variables were analyzed: age, gender, Child-Pugh classification, AFP, hepatitis serology, and extent of resection. Model for End-Stage Liver Disease (MELD) score was calculated in an attempt to predict mortality after liver resection. Patients were pathologically staged according to the sixth edition of the American Joint Commission on Cancer (AJCC)[20].

Table 1 Clinical and Histopathological Features of Patients Who Underwent Resection for HCC

Characteristic	HCC ≥10 cm (*n*=24)	HCC <10 cm (n=165)	*p* value
Mean Age (years)	57±15	62±14	0.54
Viral Hepatitis			0.21
HBV	9 (38%)	73 (44%)	
HCV	1 (4%)	36 (22%)	
Symptoms	20 (83%)	40 (24%)	0.01
Child-Pugh class			
A	24 (100%)	145 (92%)	0.41
B	0	14 (8%)	
C	0	0	
MELD score	7.2±3.1	8.1±5.8	0.32
Mean ICG15	9.3±4.1	9.8±3.7	0.72
Preoperative vascular invasion	8 (33%)	13 (8%)	0.001
Median AFP	1010 (5-303,000)	35 (2 - 320,000)	0.03
Tumor number	1.4±1.7	1.7±2.0	0.68
Tumor size (cm)	13.1±2.9	4.7±2.2	<0.001
Grade			0.05
Well	3 (13%)	39 (24%)	
Moderate	13 (54%)	96 (58%)	
Poor	8 (33%)	21 (13%)	
n/a	0	9 (5%)	
Pathological vascular invasion	13 (54%)	43 (26%)	0.004
Positive margins	9 (37%)	6 (4%)	0.04
AJCC T category			0.09
T1	5 (21%)	105 (64%)	
T2	6 (25%)	39 (24%)	
T3	10 (42%)	19 (11%)	
T4	3 (12%)	2 (1%)	

Tumor size: largest diameter of largest tumor in cm. Positive margins: pathological assessment of tumor <1 mm from resection margin.
HBV = Hepatitis B Virus; HCV = Hepatitis C Virus; n/a = data not available; MELD = Model for End Stage Liver Disease;
p value: comparisons between groups were performed using the Chi-square test for categorical variables and the Student's *t* test for continuous variables.

Terminology with respect to liver resection is that proposed by the International Hepato-Pancreatico-Biliary Association (IHPBA), also known as the Brisbane terminology (http://www.ihpba.org).

Comparisons between groups were performed using the Chi-square test for categorical variables and the student *t* test for continuous variables. Time to recurrence (disease-free survival, DFS) and time to death were determined by Kaplan-Meier analysis and results for subgroups of patients were compared with log-rank test (SPSS software version 13.0; SPSS Inc., Chicago, IL). All variables that appeared to be significantly associated with survival ($p<0.1$) were entered into a backward stepwise Cox proportional hazards model to test for significant effects while adjusting for multiple factors simultaneously. A p value less than 0.05 (two-tailed) was considered to be statistically significant.

Results

Patient and Tumor Characteristics

The clinical and histopathological characteristics of the entire cohort, divided into patients who underwent resection of either HCC <10 cm or ≥10 cm, is shown on Table 1.

Most patients who underwent resection for HCC had Child-Pugh class A cirrhosis. There was no difference in MELD scores between the two groups. Tumors ≥10 cm were more likely to be symptomatic ($p=0.01$), higher AFP ($p=0.03$), poorer grade ($p=0.05$), positive margins ($p=0.04$), and vascular invasion (preoperative: $p=0.001$; pathological: $p=0.04$). Specifically, 58% (14/24 patients) presented with abdominal pain as the most common presenting symptom in ≥10 cm HCC group compared to only 12% (20/165) in <10 cm HCC group. Poor tumor differentiation was more common in the ≥10 cm group (33 vs. 13%). Vascular invasion, both preoperative radiological invasion and pathological small vessel invasion, was also more common in ≥10 cm group with more than half of patients with vascular invasion on explant analysis. More than half (54%) of patients with ≥10 cm HCC were AJCC stage T3 or T4 largely because of vascular invasion of major portal or hepatic veins.

Perioperative Outcomes

Twelve patients with HCC ≥10 cm (50%) underwent resection of more than four hepatic segments. En bloc resection of adjacent organs included diaphragm in two patients, resection of the inferior vena cava (IVC) in two patients, and median sternotomy for exposure in one patient. Postoperative complications were more common after resection of ≥10 cm HCC (12/24, 50% vs. 35/165, 21%;

Table 2 Postoperative Complications After Resection for HCC

Complication	HCC >10 cm (n=24)	HCC <10 cm (n=165)
Aspiration	1	1
Bile leak	3	7
Hepatic insufficiency	4	3
Myocardial infarction	1	1
Stroke	1	0
Other	0	15
Death	2	7
Total	12 (50%)	35 (21%)

p=0.04; Table 2) and included hepatic insufficiency (17 vs 2%) bile leak (13% vs 4%) and stroke or MI (8% vs 1%). The perioperative mortalities were similar in the two groups (8% vs. 4%, p=0.7).

Recurrence and Survival Outcomes

The median follow-up was 34 months. The median DFS was significantly shorter in patients with very large (≥10 cm) HCC group compared to patients with smaller (<10 cm) HCC (8.4 vs. 38 months; p=0.001, Fig. 1). However, overall survival was not different between the two groups; the 5-year survivals were 54% vs. 53% for the very large tumors compared to the smaller HCC (p=0.43; Fig. 2). At the close of this study, 46% (13/28) of patients remain disease-free. The cause of death in the very large HCC group was metastatic HCC in all ten patients (36%), whereas HCC was the cause of death in 41% (67/164) of patients with smaller HCC. Five patients (3%) died of other causes in the smaller HCC group.

Predictors of recurrent HCC in tumors ≥10 cm by univariate analysis were positive margins (p=0.03) and major vascular invasion (p=0.01), but only vascular invasion (HR 0.17; 95% CI: 0.04–0.81) proved to be an independent risk factor for recurrence by multivariate analysis (Table 3).

Recurrent disease occurred in 17 of the HCC ≥10 cm patients (71%) of which 12 were intrahepatic and five were systemic; eight of these patients (47%) underwent additional therapy for recurrence including re-resection (n=4), TACE (n=3), and ablation (n=1). Of the intrahepatic recurrences, five lesions were focal solitary lesions amenable to further intervention. The remaining seven recurrences were multifocal lesions. The recurrence pattern appeared to be a new primary in the majority of cases (n=8; 75%) similar to smaller HCC. Median OS after recurrence for patients with resection of ≥10 cm HCC was 24 months.

Discussion

Surgical resection has been considered the optimal therapy for very large (≥10 cm) HCC. In this analysis, we found

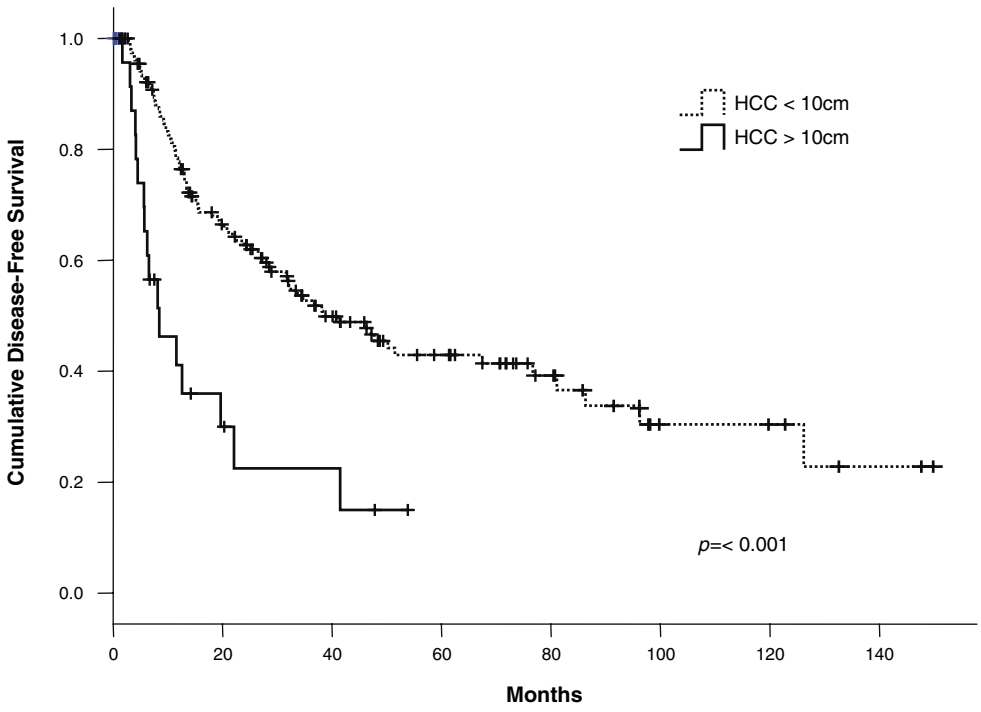

Figure 1 Disease-free survival comparing very large (≥10 cm) and small (<10 cm) HCC.

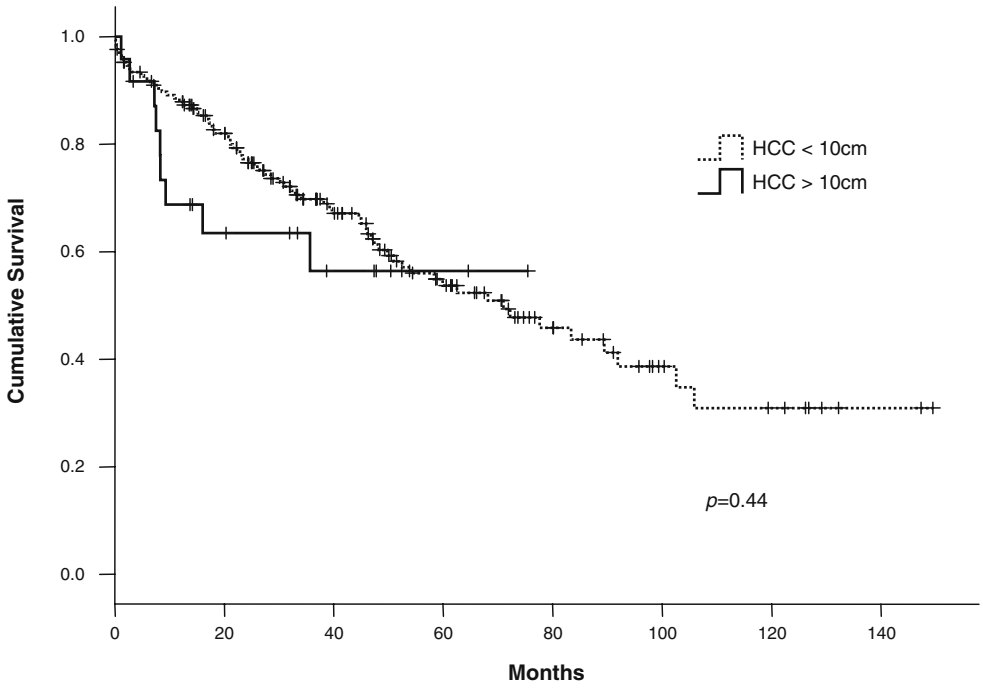

Figure 2 Overall survival comparing very large (≥10 cm) and small (<10 cm) HCC.

the overall survival after resection for very large HCC (54% vs. 53% at 5 years) was not significantly different than after similar resections for smaller HCC. These results differ from other series, which have reported lower survival after resection of large HCC with 5-year survival ranging from 25 to 40%.[13,20,20–22] This study suggests that hepatic resection should be performed in patients with resectable HCC with adequate hepatic reserve and minimal comorbidities regardless of tumor size. This study is among the first to document similar survival after potentially curative resection of both very large tumors ≥ 10 cm and smaller HCC.

With recent advances in preoperative planning, risk assessment, and better surgical techniques, liver resection for very large HCC can be performed safely with low morbidity in high-volume centers.[23,24] Similar to other series and our experience with smaller tumors, we achieved morbidity and mortality rates of 42% and 8%, respectively, after resection of ≥10 cm HCC.[12,13] Our current assessment algorithm of resectability of HCC patients includes assessment of liver function, triple-phase liver CT, contrast-enhanced US, ICG clearance and consideration of eligibility for LT. Laboratory evaluation of liver function is best confirmed with a normal protime and platelet count >100,000. The combination of triple-phase CT and contrast-enhanced US assesses the liver for nodularity and fibrotic characteristics, potential tumor vascular invasion, and identifies potential synchronous lesions. As most patients with ≥10 cm HCC require major liver resections of >4 segments, adequate hepatic reserve is a priority. ICG clearance has been a useful adjunct to quantify hepatic reserve in patients with HCC at our institution since 1999.[18] Inadequate ICG clearance, defined as >15% at 15 min (ICGR15), has not only altered operative strategies, but has also steered the discussion for palliative therapy such as external radiation, chemotherapy, or TACE. Appropriate patient selection for aggressive resection is the most important factor in achieving acceptable rates with these large tumors.

Although the incidence of major complications were significantly more common after resection of very large HCC, the perioperative mortality was similar and did not appear to have an affect on OS comparisons with the smaller HCC group. Hepatic insufficiency was seen in four patients out of 28 (14%), and probably reflects extensive resections that were undertaken in this group. The large number of complications can be expected not only because of the nature of resection, but also because the comorbid

Table 3 Univariate and Multivariate Analysis of Prognostic Factors

Variable	*p* value	HR (95% CI)
Symptoms	0.11	
Preoperative vascular invasion	0.01	0.81 (0.18–3.6)
Tumor number	0.36	
Tumor size	0.19	
Grade	0.10	1.2 (0.32–4.1)
Pathological vascular invasion	0.02	0.17 (0.04–0.8)
Positive margins	0.03	1.5 (0.39–5.7)

factors and presenting symptoms of the patients appeared to be worse in the larger HCC group.

The current study identified a high recurrence rate (>70%) after resection of very large HCC. Although vascular invasion and positive margins were significant variables for recurrence on univariate analysis, pathological vascular invasion was the only independent factor of recurrence in this series after multivariate analysis. Larger tumors have been shown to harbor microvessel tumor invasion, poorer degree of differentiation, and a propensity for multinodular lesions and subsequent recurrence.[7, 12] Liau et al. found that intraoperative blood loss >2 l and vascular invasion predicted survival after resection of >10 cm HCC.[13] We did not account for blood loss as a variable in our database because of the inaccuracy and degree of estimation used to calculate it after cases. The International Cooperative Study Group on Hepatocellular Carcinoma found that 54 and 41% of lesions larger than 5 cm were high-grade tumors and harbored vascular invasion, respectively.[7] In another study from this group, they reported that tumor size and clinical factors do not predict survival after resection of 10 cm tumors.[20] In this series, our results also corroborate these findings; in selected patients tumor size and symptoms do appear to affect recurrence after resection. Unlike other studies, we were not able to demonstrate the use of AFP as a prognostic indicator after resection probably because of a small sample size in this study.[20] Future studies focusing on the genetic and molecular factors involved with tumor growth may lead to a better understanding of HCC recurrence after resection or LT.[25]

Most liver surgeons consider the presence of tumor thrombus in the inferior vena cava or main portal vein a contraindication for hepatic resection. Tumor invasion of major vessels is a poor prognostic factor, but resection may be justified in selected cases because of more favorable results compared with nonsurgical treatment.[22,26] Two patients in this series had known tumor thrombus in the IVC preoperatively. One patient underwent a median sternotomy and anterior approach to hepatectomy for optimal visualization and exposure of the hepatic venous confluence and control of the IVC (Fig. 2). After gaining control of the IVC and visualizing the clot with the assistance of intraoperative US and transesophageal echocardiography, opening the right hepatic vein and removing free-floating thrombus can be achieved in a well-controlled fashion. This approach has been advocated by other groups for large HCC that present with floating tumor thrombus in the IVC.[27] Lui and colleagues in Hong Kong have advocated an anterior approach to large right-sided tumors. Their group has demonstrated less blood loss and lower hospital mortality with this approach rather than mobilizing the right lobe with a bulky tumor off of the retroperitoneum and IVC.[28]

What is the role of transplantation for very large HCC and how does it compare with resection? Initial studies of LT for these larger tumors revealed high recurrence rates and treatment failures until it was established that only smaller HCC fare well after transplantation.[9] Recently, some authors have suggested that these guidelines may be too rigid and some have advocated LT for larger tumors.[29–31] Further multicenter trials with the use of pretransplant therapies such as TACE or ablation may help determine the role of liver transplantation for large (≥10 cm) HCC.

Is there a role for neoadjuvant therapy before resection of very large HCC? To date, neoadjuvant strategies before liver resection, such as portal vein embolization (PVE) and TACE have failed to demonstrate any survival benefit.[32–34] The use of these therapies may be appealing to surgeons in the neoadjuvant setting of resectable tumors if prognostic histopathological characteristics of the tumor, such as microvascular invasion or tumor grade, are altered before resection. The effect of PVE on hypertrophy in cirrhotic livers is still uncertain with variable results.[35–38] Our results suggest that PVE may not have a role in overall survival as very few patients in this study underwent PVE.

As advocated in the current AJCC staging classification, these data emphasize that morphologic criteria such as tumor size do not accurately predict outcome after resection of HCC. Hepatic resection can be performed safely for ≥10 cm HCC and in selected patients, can lead to long-term survival. Further studies are necessary to determine which patients in this subgroup would benefit from resection, but at the present time, resection appears to remain as the most favorable option.

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Prognostic Value of Preoperative Peripheral Blood Monocyte Count in Patients with Colorectal Liver Metastasis after Liver Resection

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Abstract Prognostic values of leukocyte subset counts in peripheral blood of cancer patients have not yet been fully investigated. We retrospectively examined the relation between preoperative absolute counts of peripheral blood leukocyte subsets and clinicopathologic factors and long-term prognosis in 97 patients with liver metastasis from colorectal cancer who underwent hepatic resection. Median preoperative peripheral blood leukocyte subset counts were as follows: neutrophils 3148/mm^3; lymphocytes 1574/mm^3; monocytes 380/mm^3. Univariate analysis indicated significantly worse 5-year cancer-related survival for patients with a peripheral blood monocyte count >300/mm^3 (67.5%) than for patients with a count ≤300/mm^3 (36.8%). Multivariate analysis showed a preoperative peripheral blood monocyte count >300/mm^3 and preoperative CEA level (>10 ng/ml) to be independent predictive factors for cancer-related survival after hepatic resection. The preoperative peripheral monocyte count correlated positively with white blood cell and neutrophil counts, but not with the tumor number, interval between colorectal and hepatic surgery, or preoperative serum CEA level. Our findings indicate that a preoperative absolute peripheral blood monocyte count >300/mm^3 is an independent predictive factor for cancer-related survival of patients with colorectal liver metastasis who have undergone hepatic resection.

Keywords Colorectal cancer · Liver metastasis · Peripheral blood · Monocyte count · Prognosis

Introduction

The incidence of colorectal carcinoma is increasing worldwide. Approximately 30–50% of patients with colorectal cancer suffer recurrence after curative colorectal resection.[1] The organ that most frequently contains metastatic deposits from colorectal cancer is the liver, followed by the lung, bone, and peritoneum. Hepatic resection is considered the most effective therapy for colorectal liver metastasis, and the reported overall survival rate after hepatic resection is 26–51%.[2–5] Several clinicopathologic factors that influence patient survival after hepatic resection have been identified; these include the interval between colorectal and hepatic surgeries,[2,4,5] the number of hepatic metastases,[3,4–6] size of the liver tumor,[4] the preoperative serum carcinoembryonic antigen (CEA) level,[3,5,7] lymphatic invasion in the liver,[8] and nodal metastasis in the hepatic hilum.[2] Most investigators agree that the interval between colorectal and hepatic surgeries, the number of hepatic tumors, and the preoperative serum CEA level are the most important determinants of long-term survival after hepatic resection.

A few studies of peripheral blood cells in cancer patients have indicated that a decreased lymphocyte count or increased monocyte and/or neutrophil count in the peripheral blood is a predictor of a poor prognosis in cancer patients.[9–12] Moreover, the relation between preoperative

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inflammatory status and prognosis after treatment for patients with malignant tumors has been investigated. Some investigators identified preoperative C-reactive protein (CRP) elevation as an independent predictive factor for short-term survival of patients with colorectal cancer.[13–15] It was speculated that progressive tumor destroys surrounding tissue and leads to a nonspecific inflammatory reaction or that CRP is upregulated by proinflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor.[13,14] In addition, several authors have shown that some patients with advanced malignant tumor fall into immunosuppressive status, which might contribute to a poor prognosis in patients who undergo curative resection.[16,17]

Dendritic cells (DCs) identify antigen-presenting cells and mature DCs lead to activation of antigen-specific cytotoxic lymphocytes.[18] DCs can derive from peripheral monocytes. Immunotherapy based on DCs has been performed recently in patients with various types of malignant tumor; however, the results have not been satisfactory.[19–22] Several investigators suggested poor function of peripheral blood DCs obtained from cancer patients, especially those with advanced-stage disease.[23] To the contrary, some investigators have shown that DCs with regulatory function (regulatory DCs) cause immunosuppression by activated and differentiated regulatory T cells in patients with malignant tumor.[24] The DCs may be included in the peripheral blood monocyte subset or may differentiate from peripheral monocytes.

To date, the prognostic value of preoperative leukocyte subset counts in peripheral blood has not been investigated in patients with colorectal liver metastasis. Therefore, the aim of this study was to clarify the prognostic value of preoperative peripheral blood leukocyte subset counts, especially the absolute monocyte count, in patients with colorectal cancer who have undergone hepatic resection.

Materials and Methods

During the period January 1985 through March 2004, 132 patients with colorectal liver metastases underwent hepatic resection at the Department of Surgery I, Oita University Faculty of Medicine. Thirty-five of these patients were excluded from the study: three (2.3%), who died of postoperative complications within 30 days; two who had obvious residual tumor at the time of surgery; 25 whose preoperative peripheral leukocyte subset counts were not obtained; one whose hepatic tumor had fallen into complete necrosis, and four whose clinicopathologic data were not certain. Thus, 97 patients who underwent hepatic resection with a curative intent were included in this study. All patients underwent regular follow-up examinations at our outpatient clinic and were monitored for recurrence by assessment of serum tumor markers every 2 months and by ultrasonography or contrast computed tomography study every 4–6 months. We defined cancer recurrences when metastatic tumors were identified by radiologic examinations, such as computed tomography, ultrasonography, and bone scintigraphy.

Upon admission to our hospital, a complete blood count and blood chemistry profile were routinely obtained for each patient. The absolute count of peripheral blood leukocytes (normal count 2950–8970/mm^3) and of each subset were included. Leukocytes were divided into neutrophil (normal percentage 42.2–74.7%), lymphocyte (normal percentage 17.7–46.5%), monocyte (normal percentage 1.3–8.0%), eosinophil (normal percentage 0–8.4%), and basophil (normal percentage 0–1.1%) subsets, and the absolute counts of neutrophils, lymphocytes, and monocytes were determined. The serum biochemistry data included the carcinoembryonic antigen (CEA) level (normal <5 ng/ml).

We investigated 13 clinicopathologic variables, i.e., sex, age, interval between colorectal and hepatic resection, number of hepatic metastases, diameter of hepatic tumor, preoperative CEA level, site of primary tumor, grade of primary cancer, status of nodal metastasis of primary colorectal cancer, leukocyte (neutrophil, lymphocyte, and monocyte) subset counts, and extent of hepatic resection (Table 1). The extent of hepatic resection was defined according to Couinaud's classification system, with minor hepatic resection defined as resection of fewer than two segments and major hepatic resection as resection of two or more segments.

Patient outcomes were determined on the basis of clinical data obtained from patients' medical records as of June 30, 2006. The mean and median follow-up periods of surviving patients after hepatic resection were 44.2 and 30 months, respectively. The prognostic significance of clinicopathologic factors in relation to cancer-related survival was investigated by univariate and multivariate analyses. Data were censored in the analysis of cancer-related survival if a patient was living or had died of unrelated disease and in the analysis of disease-free survival if a patient was living or had died of unrelated disease without recurrent colorectal carcinoma. Survival rates were calculated by the Kaplan–Meier method, and differences were analyzed by univariate log-rank analysis. In the comparisons of clinicopathologic factors and leukocyte counts, continuous variables were analyzed by Kruskal–Wallis test, and nominal variables were analyzed by Fisher's exact probability test. Variables with a P value of <0.1 in univariate analysis were used in subsequent multivariate analysis based on the Cox proportional hazards model. P value <0.05 was considered significant in all

Table 1 Results of Univariate Analysis of Cancer-Related Survival after Hepatic Resection

Clinical Variable	No. of Patients	5-Year Cancer-related Survival Rate (%)	*P* value
Sex			
Male	60	40.9	0.88
Female	37	51.9	
Age (years)			
≤60	32	46.2	0.70
>60	65	45.1	
Interval (months)			
<12	64	38.3	0.10
≥12	33	56.9	
CEA (ng/ml)			
≤10	41	64.1	<0.01
>10	56	31.7	
Tumor size (mm)			
<50	74	49.8	0.18
≥50	23	34.8	
Tumor grade			
Well-differentiated	54	49.0	0.17
Moderately/poorly	43	38.5	
Primary organ			
Colon	65	43.9	0.39
Rectum	31	49.6	
Colon+rectum	1	0	
Tumor number			
<4	87	48.1	0.07
≥4	10	0	
Primary nodal metastasis			
Absent	41	44.1	0.72
Present	56	48.1	
Lymphocyte count (/mm^3)			
≤1500	43	45.6	0.63
>1500	54	44.3	
Neutrophil count (/mm^3)			
≤3000	44	58.2	
>3000	53	35.5	
Monocyte count (/mm^3)			
≤300	22	67.5	0.04
>300	75	36.8	
Extent of hepatic resection			
Major	38	45.7	0.97
Minor	59	44.4	

Interval means period between colorectal and hepatic surgeries. CEA = carcinoembryonic antigen; moderately/poorly, moderately or poorly differentiated.

analyses. Statistical analysis was performed with JMP software (JMP, SAS Institute Inc, Cary, NC).

Results

Patient Characteristics

The 97 patients who underwent hepatic resection with a curative intent comprised 60 men and 37 women with a mean age of 62.6 years. The mean and median intervals between colorectal and hepatic surgery were 12.2 and 7 months, respectively. The mean number and size of hepatic tumors were 1.8 (range 1–8) and 39.1 mm (range 10–130 mm), respectively. Patients had the following number of metastatic liver tumors: 1 (n=55), 2 (n=18), 3 (n=10), 4 (n=4), 5 (n=3), 6 (n=2), and 8 (n=1). The mean preoperative serum CEA level was 49.9 ng/ml (range 0–915.0 ng/ml; median 13.8 ng/ml). The primary tumor was located in the colon in 65 patients, in the rectum in 31 patients, and in both the colon and rectum in 1 patient. According to Dukes' classification system, 41 of the primary tumors were at stage A or B tumors, and 56 were stage C tumors. Metastatic liver tumors were graded

as well differentiated (n=54) and moderately to poorly differentiated (n=43). Thirty-eight patients underwent major hepatic resection, and 59 underwent minor hepatic resection.

Mean and median peripheral blood cell counts were as follow: leukocytes 5748.6/mm^3 and 5510/mm^3, respectively (range 2300–10,410/mm^3); neutrophils 3391.8/mm^3 and 3148/mm^3 (range 1079–6966/mm^3); lymphocytes 1657.4/mm^3 and 1574/mm^3 (range 589–3662/mm^3); and monocytes 419.7/mm^3 and 380/mm^3 (range 136–1183/mm^3). The patients were stratified according to absolute counts of each peripheral blood leukocyte subset as follows: ≤3000/mm^3 (n=44) and >3000/mm^3 (n=53) for neutrophils; ≤1500/mm^3 (n=43) and >1500/mm^3 (n=54) for lymphocytes; and ≤300/mm^3 (n=22) and >300/mm^3 (n=75) for monocytes.

Survival

Of the 97 patients who underwent hepatic resection for colorectal liver metastasis, 47 (48.5%) had died by June 30, 2006. The causes of death were as follows: colorectal cancer (n=43) and unrelated diseases (n=4; liver cirrhosis in one, necrotizing myositis in one, acute myocardial infarction in one, and pneumonia in one). The 5-year cancer-related survival and disease-free survival rates were 44.9 and 31.4%, respectively.

Recurrent disease was found in 14 of the 22 (63.6%) patients with a monocyte count ≤300/mm^3 and in 49 of the 75 (65.3%) patients with a monocyte count >300/mm^3. Five-year cancer-related and disease-free survival rates after hepatic resection were 67.5 and 37.5%, respectively, for patients with a peripheral blood monocyte count ≤300/mm^3 and 36.8 and 29.6%, respectively, for those with a count >300/mm^3. Univariate analysis of the 13 clinicopathologic factors examined in relation to cancer-related survival after hepatic resection revealed that the cancer-related survival rate after hepatic resection was significantly worse for patients with a monocyte count >300/mm^3 than for those with a monocyte count ≤300/mm^3 (P=0.04; Table 1 and Fig. 1) as well as for those with an elevated preoperative serum CEA level (≥10 ng/ml) or large number (≥4) of liver tumors. The disease-free survival rate did not differ between groups stratified according to monocyte counts (P=0.24; Fig. 2). There was no statistical relation between cancer-related survival or disease-free survival and the absolute peripheral blood lymphocyte or neutrophil count. Multivariate analysis of the two significant factors revealed that a peripheral blood monocyte count >300/mm^3; relative risk (RR), 1.55; confidence interval (CI), 1.04–2.54; and preoperative serum CEA elevation (>10 ng/ml) (RR, 2.70; CI, 1.36–5.84) negatively influenced cancer-related survival after hepatic resection (Table 2).

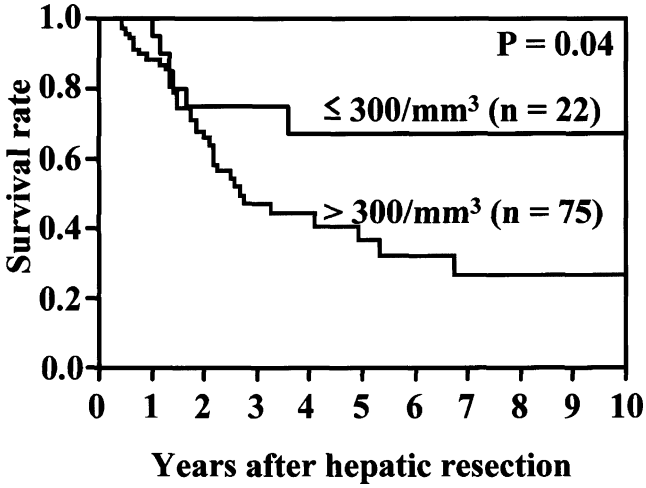

Figure 1 Cancer-related survival curves according to absolute count of preoperative peripheral blood monocyte count. Cancer-related survival rate is significantly better for patients with a count ≤300/mm^3 than for patients with a count >300/mm^3 (P<0.04).

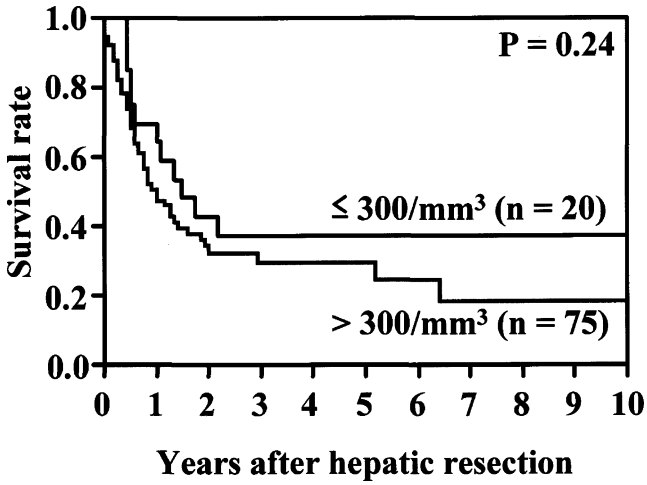

Figure 2 Disease-free survival curves according to absolute preoperative peripheral blood monocyte count. Disease-free survival rates do not differ between patients stratified according to peripheral blood monocyte counts (P=0.24).

Relation Between Peripheral Blood Monocyte Count and Clinicopathologic Factors in Patients with Colorectal Liver Metastasis

We analyzed 13 clinicopathologic factors in relation to peripheral blood monocyte counts, as shown in Table 3. The peripheral blood monocyte count was positively related to the preoperative peripheral blood leukocyte and neutrophil counts. However, other factors, including those reflective of tumor progression (interval between colorectal

Table 2 Results of Multivariate Analyses of Cancer-Related and Disease-free Survival after Hepatic Resection

Clinical Variable	Cancer-related Survival	
	RR (CI)	*P* value
CEA (ng/mL)		
≤10	1.00	< 0.01
>10	2.70 (1.36–5.84)	
Tumor number		
<4	1.00	0.27
≥4	1.71 (0.63–3.88)	
Monocyte count (/mm^3)		
≤300	1.00	0.03
>300	1.55 (1.04–2.54)	

CEA = carcinoembryonic antigen; RR = relative risk; CI = confidence interval

and hepatic surgery, preoperative serum CEA level, and the number and size of hepatic tumors), did not relate to the preoperative peripheral blood monocyte count.

Discussion

Risk factors for a poor outcome after hepatic resection for patients with colorectal liver metastases have been investigated. Several investigators agree that the short period between colorectal and hepatic surgeries, large number of metastatic liver tumors, preoperative serum CEA elevation, and presence of extrahepatic metastasis were strong predictive factors for survival after hepatic resection.[2–6] We previously investigated preoperative risk factors that affect survival of patients with colorectal liver metastasis and showed that the interval between colorectal and hepatic surgeries, number of liver tumors, and preoperative serum CEA level are independent risk factors influencing cancer-

Table 3 Clinicopathologic Factors in Relation to Peripheral Blood Monocyte Count

Variable	No. of Patients	Peripheral Blood Monocytes (/mm3)		*P* value
		<300	≥300	
Sex				
Male	60	11	49	0.20
Female	37	11	26	
Age (years)	97	62.7	62.6	0.73
Interval (months)*				0.08
<12	64	11	53	
≥12	33	11	22	
CEA (ng/ml)	97	41.6	52.3	0.22
Primary organ				
Colon	65	14	51	0.69
Rectum	31	8	23	
Colon+rectum	1	0	1	
Tumor number	97	1.55	1.89	0.09
Tumor size (mm)	97	35.4	40.2	0.77
Tumor grade				
Well-differentiated	54	11	43	0.54
Moderately/Poorly	43	11	32	
Primary nodal metastasis				
Absent	41	9	32	0.88
Present	56	13	43	
WBC count (/mm^3)	97	4704.1	6054.9	<0.01
Neutrophil count (/mm^3)	97	2623.7	3617.1	<0.01
Lymphocyte count (/mm^3)	97	1574.3	1681.8	0.24
Extent of hepatic resection				
Major	38	9	29	0.85
Minor	59	13	46	

CEA, carcinoembryonic antigen; moderately/poorly, moderately or poorly differentiated; WBC, white blood cell. *Interval means period between colorectal and hepatic surgeries. Continuous variable is expressed by mean value.

related survival after hepatic resection.[5] However, only a small number of clinicopathologic factors predictive of long-tem survival after hepatic resection have been reported. In the present study, we clarified that the absolute peripheral blood monocytes count and the serum CEA level are independent preoperative prognostic factors. The absolute peripheral blood monocyte count might be available to patient selection for hepatic resection or indication for postoperative adjuvant chemotherapy in patients with colorectal liver metastasis.

Several investigators have reported a relation between preoperative peripheral blood leukocyte subset counts and prognosis in patients with malignant tumors, such as carcinoma of the stomach,[9] neck and head,[10] and other organs.[11,12] Some investigators have reported that preoperative leukocyte subset counts in peripheral blood can be indicative of tumor progression or of prognosis in cancer patients. Bruckner et al.[9] showed that a pretreatment absolute neutrophil count <6000/mm^3, lymphocyte count >1500/mm^3, and monocyte count 300–900/mm^3 were independent indicators of a good prognosis for patients with metastatic gastric cancer. Elias et al.[10] analyzed mononuclear cell percentages in 55 patients with epidermoid carcinoma of the head and neck and found that high lymphocyte (≥30%) and low monocyte percentages (<10%) correlated with early-stage disease and were associated with a good prognosis. Riesco reported that cancer curability correlated positively with pretreatment peripheral leukocyte count and negatively with the pretreatment neutrophil count in patients with various types of cancer.[11] Recently, we reported that an increased preoperative peripheral blood monocyte count (>300/mm^3) correlated negatively with disease-free survival after hepatic resection in patients with hepatocellular carcinoma (HCC).[12] However, there have been no reports describing the prognostic significance of preoperative leukocyte subset counts in patients with colorectal cancer.

Several investigators have reported cut-off values for the preoperative peripheral blood monocyte count as it pertains to survival analysis. Bruckner et al.[9] reported that a monocyte count of 300–900/mm^3 was an independent indicator of a good outcome in gastric cancer patients with ambulatory status. In our previous investigation in HCC patients, we used a cut-off value of 300/mm^3 for peripheral blood monocytes.[12] Although the normal peripheral blood monocyte count is described by our institution as a percentage of the white blood cell count (1.3–8.0%), we decided upon a cut-off value of 300/mm^3 in the present study, according to the previous reports.

The mechanism explaining the relation between an increase in the number of peripheral blood monocytes and decrease survival remains unclear. Some studies have indicated that preoperative systemic inflammation as determined by the serum CRP level adversely affects survival after curative resection in patients with colorectal cancer.[13–15] Because the serum CRP level was shown to correlate with tumor stage, it was speculated that tumor progression might destroy tissue surrounding the tumor.[14] In our previous study of HCC patients, the absolute peripheral blood monocyte count also correlated with tumor progression.[12] However, in the present study, the peripheral blood monocyte count did not relate to tumor progression, i.e., to the interval between colorectal and hepatic surgery, the size and number of hepatic tumors, or the preoperative serum CEA level. Moreover, the preoperative peripheral blood monocyte count was significantly related to cancer-related survival but not to disease-free survival after hepatic resection. These findings suggest that growth of a recurrent tumor is more rapid in patients with a high monocyte count than in those with a low monocyte count. Thus, proinflammatory cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor, produced by the increased number of peripheral blood monocytes, might stimulate cancer cell growth,[13,14] or DCs derived from peripheral blood monocytes might have regulatory function for host immunity against the tumor.

The monocyte subset in peripheral blood includes the DC population. DCs, which are antigen-presenting cells and which activate the anti-tumor immune response of the tumor-bearing host, have been used for immunotherapy for malignant tumor.[20–22] Recently, several investigators showed that regulatory DCs in the peripheral blood might induce proliferation of $CD4^+CD25^+$ regulatory T cells, which inhibits the proliferation or activation of $CD4^+CD25^-$ or $CD8^+CD25^-$ T cells and suppresses host anti-tumor immunity.[24,25] We did not investigate functions of DCs and natural killer cells in peripheral blood. Our results, however, support the theory that impairment of the antigen-presenting function of DCs or increasing regulatory DCs holds patients with colorectal liver metastasis in immunosuppressive state and thus leads to a poor outcome after hepatic resection. Several investigators have suggested reducing the number of impaired nonregulatory DCs[23] or inactivating the function of regulatory DCs by immunosuppressive drugs might be necessary to impair DC immunotherapy.[26,27] Functional analyses of the peripheral blood monocyte subset should be performed in the future.

In conclusion, the absolute number of preoperative peripheral blood monocytes is an independent factor that influences cancer-related survival after hepatic resection for patients with liver metastasis from colorectal cancer, and it may be related to tumor growth. The function of DCs in patients with an increased preoperative peripheral blood monocyte count may be impaired, and a new strategy to induce DC maturation may be necessary for DC-based immunotherapy to be effective in these patients.

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Pathologic Correlation Study of Microwave Coagulation Therapy for Hepatic Malignancies Using a Three-Ring Probe

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Abstract Microwave coagulation therapy (MCT) for the ablation of unresectable hepatic malignancies is a promising alternative to radiofrequency and cryoablation techniques. There are few data on the clinical effectiveness of MCT. In vivo pathologic evaluation of ablated tumor tissue is not well described for the three-ring microwave probe. The study design was a prospective trial enrolling patients with resectable hepatic malignancies. Lesions underwent in vivo MCT with the three-ring probe prior to liver resection. Gross and histologic evaluations of the tumor were performed, including nicotinamide adenine dinucleotide (NADH) vital staining. A total of nine patients with metastatic colon cancer were enrolled and had NADH stains performed of their pathologic specimens. The median size of the metastasis being ablated was 3.5 cm (range, 1.5–12.3). Fifty-six percent of the tumors demonstrated evidence of spontaneous coagulative necrosis on immediate histologic examination. The median dimensions of the ablation zones were 5 cm (range, 3–7)×4.5 cm (range, 2.5–5.2)×4.2 cm (range, 2–5) with a 5-min ablation at 60 W. The median ablation volume was 50.6 cm^3 (range, 9–78). NADH vital staining was performed of the ablation zones with 100% absence of staining in the tumor tissue and in benign hepatic parenchyma, which is consistent with irreversible cellular damage. In conclusion, in vivo MCT of hepatic malignancies with the three-ring probe produces nonviable tumor cells after a 5-min ablation. The ablation time is significantly shorter than other available ablative techniques. Immediate histologic exam produces some evidence of coagulative necrosis. Further study of this promising technology is warranted.

Keywords Liver · Microwave · Ablation · Pathology · Malignancies

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Abbreviations

MCT	microwave coagulation therapy
NADH	nicotinamide adenine dinucleotide
RFA	radiofrequency ablation
CT	computed tomography

Introduction

Hepatic resection is the primary treatment modality for primary liver malignancies as well as isolated liver metastases from colorectal cancer and other selected metastatic solid tumors.[1,2] Unfortunately, only approximately 10–20% of patients with primary and secondary hepatic malignancies are candidates for surgery either due to the extent and/or location of liver tumors or the patient's underlying liver disease or systemic comorbidities.[3,4]

The application of ablative technologies provides patients who have unresectable hepatic malignancies a local treatment option that appears to have some clinical benefits with lower complication and mortality rates than conventional surgery.[5] Today, radiofrequency ablation (RFA) is the most commonly used technique, with many reports showing that it can be applied safely to provide local control in patients with inoperable hepatic tumors.[6] However, multiple studies of RFA have demonstrated that the larger the lesion, the higher the rates of local recurrence, especially for tumors greater than 3 cm.[5] The radiofrequency energy can only produce a limited ablation zone, and if the tissue becomes charred, there may be incomplete tissue ablation between the tines of the RFA probes.[7] The initial RFA devices required median ablation times of 60 min for lesions greater than 3 cm, and although the technology has improved, ablating a volume of tissue 5 cm in diameter still takes approximately 14 min with the latest probes.[8] RFA does not affect medium- to large-size blood vessels because the blood flow creates a heat sink that dissipates the thermal energy.[9] This has the potential to provide incomplete ablation of perivascular tissue.

Similar to RF ablation, microwave coagulation therapy (MCT) uses heat to destroy tumor cells. MCT does not use electrical current and therefore does not require the use of grounding pads on the patient. MCT produces thermal energy via a local microwave field that causes coagulative necrosis resulting in tissue destruction.[10] This energy is passed down an antenna probe that has been placed in the center or around a liver tumor. It produces direct heating of tissue which is not limited by increased impedance caused by charring like RFA.[7] This has the potential to result in larger and more complete zones of ablation in a shorter period of time. It is currently approved by the Food and Drug Administration for thermocoagulation of human tissue.

To date, microwave ablation has been performed in animal and human subjects using straight antennae or dual-ring probes.[7,11–13] The straight probes produced an elliptical zone of ablation that required overlapping applications to treat a spherical tumor.[14] Additionally, these probes require direct puncture of the tumor, which has the potential to cause seeding of the needle tract. Dual-ring probes have been shown to produce larger ablation zones in a single application compared to the straight probes.[12,13] The ring probes encircle the tumor instead of penetrating it, which may decrease the risk of needle tract seeding and facilitate more accurate probe placement.

This study examined the effectiveness of a three-ring MCT probe to produce a zone of ablation and cause tissue destruction. Patients with hepatic malignancies already undergoing resection were treated, and the tissue underwent pathologic and immunohistochemical analyses to determine the characteristics of the ablation zones and tissue viability. The hypothesis for this study was that the three-ring MCT probe is able to produce a zone of ablation in both normal liver parenchyma and tumor tissue that leads to tissue destruction and nonviable cells.

Methods

This protocol was approved by the Wake Forest University Baptist Medical Center Institutional Review Board. Inclusion criteria consisted of either a primary or metastatic tumor of the liver that could undergo a potentially curative resection. The cancer had to be biopsy-proven which could either be done preoperatively or intraoperatively prior to microwave ablation and confirmed by frozen section. Patients could not have significant extrahepatic disease; those with minimal extrahepatic disease with limited liver lesions could undergo surgery at the discretion of the attending surgeon. All patients had normal liver function and could not have any significant comorbidities that would preclude general anesthesia and major abdominal surgery. Between November 2003 and December 2004, 10 patients were enrolled in this study. However, one patient did not have any vital stains performed due to an error in pathologic processing and therefore was removed from analysis.

Surgical Approach

All patients underwent preoperative imaging with either computed tomography (CT) scan or magnetic resonance imaging of the abdomen and pelvis. Depending on the tumor type and primary site, additional imaging was obtained which included CT of the chest or positron emission tomography scan. All liver resections were performed by two surgical oncologists (P.S. or E.A.L.). An extended right subcostal or midline incision was made depending on the location of the hepatic tumor or if an extrahepatic procedure was also to be performed. Intraoperative ultrasound was performed on all subjects to look for undetected lesions, assist with placement of the microwave ablation probe, monitor the ablation, and define anatomical landmarks and tumor boundaries to facilitate resection. Early in the study, hepatic parenchymal transection was performed with a combination of electrocautery, Tissuelink™ saline-cooled coagulation dissector, and linear endoscopic vascular staplers. Later on the Tissuelink™ device was replaced by the harmonic scalpel. Postoperatively, patients were monitored either in the intensive care unit or acute care unit for 1–2 days prior to transfer to the floor.

Microwave Coagulation Therapy

The microwave generator (Vivant Medical, Mountain View, CA, USA) is a proprietary system which produces energy at a frequency of 2.45 GHz. The power could be continuously varied. Microwave energy was transmitted along a coaxial cable to the three-ring probe device (VivaRing™ ATOM™). The probe consists of three 13-gauge needles from which 24-gauge ring antennas (3.0 cm in diameter) are deployed (Fig. 1). Each needle probe is positioned at an equidistant triangular point, and all three rings intersect to form a spherical cage. To assist in the operation of the three-ring device, a conventional surgical electrocautery device (model E-8006; Valley Laboratories, Boulder, CO, USA) was attached to the microwave probe. During deployment of the three rings, 60–70 W of continuous power was applied to assist the ring in cutting through liver parenchyma. This prevented any distortion of the three-ring configuration by allowing the rings to form with minimal resistance.

All probe placements were confirmed by intraoperative ultrasound, and all ablations were monitored in real time using an Aloka (Wallingford, CT, USA) Side Fire "T" 7.5 MHz intraoperative transducer. Because the aim of the study was to determine the effect of MCT using the three-ring probe on both normal hepatic parenchyma and tumor tissue, no attempt was made to fully ablate the liver lesions. In fact, the probes were placed toward the edge of mass so that the tumor and normal liver would both be treated. MCT was performed using 60 W of applied power for 5 min in all cases. A temperature probe was placed in the center of the ablation zone to document the temperature achieved with MCT. No vascular inflow control was used during any of the ablations.

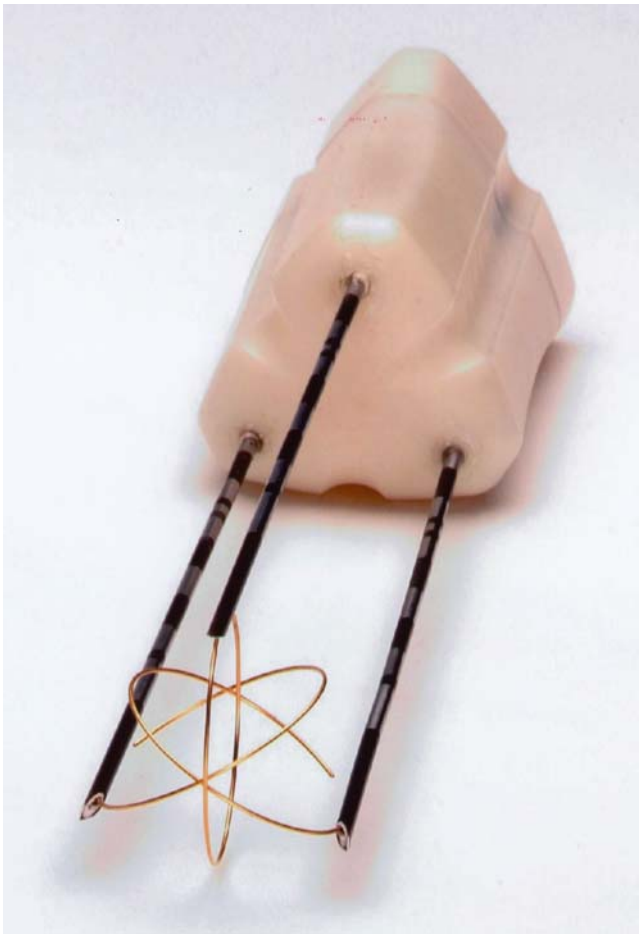

Figure 1 Three-ring microwave ablation probe (VivaRing™ ATOM™).

Pathologic Analysis

The study pathologist (K.R.G.) described the extent of tumor necrosis and surrounding histologic changes. The unfixed liver specimen was sliced at 5 mm intervals in a perpendicular axis to the resection margin. The slices were numbered consecutively and photographed to document the gross extent of the tumor and ablation zone shape. Measurements were taken of the tumor and ablation zone for volume calculations. Tissue cores were taken in all cases at the following sites: (1) center of the tumor, (2) center of the ablation, (3) edge of the ablation, and (4) 5 mm outside the ablation (zone of hyperemia). Sites for the tissue cores were determined by selecting best slice with relatively sharp tumor/benign interface. The center of the tumor was biopsied to provide an example of malignant tissue, the center of the ablation zone was biopsied to examine an area of maximal ablation, the edge of the ablation zone would provide an example of the ablated/unablated tissue interface, and the area 5 mm outside the ablation zone was biopsied to demonstrate untreated tissue.

Nicotinamide adenine dinucleotide (NADH) staining was performed on these samples to assess metabolic activity and therefore determine tissue viability. Representative samples of the tumor and normal liver were frozen in liquid nitrogen for the tumor bank. The specimen was subsequently fixed in formalin. Tissue blocks for histological examination were then selected from the slices and processed for hematoxylin and eosin staining. The pathologist recorded tumor and ablation zone dimensions, presence of coagulative necrosis, microscopic alterations in the tumor and normal liver other than necrosis, and areas of tumor and normal liver not staining for NADH.

The ablation zones were measured in three dimensions—length, width, and depth. Qualitative evaluation of the shape of the ablation zone was noted. Each lesion was inspected for the extent of the ablation zone and degree of both normal liver and tumor involvement. Volume calculations were based on the formula for a prolate ellipse ($[l \times w \times h]/2$).

Results

Patient Demographics

Nine patients were evaluable in the study—all of them had a diagnosis of hepatic colorectal metastases (Table 1). The median tumor size was 3.5 cm (range, 1.5–12.3). The first five patients had received systemic therapy for their metastatic disease prior to hepatic resection. Seven of the nine patients had additional procedures performed besides resection of the lesion undergoing MCT. These included hepatic arterial infusion pump placement, venous access device placement, colon resection, ileostomy takedown, resection of retroperitoneal mass, additional liver resections, and RFA of additional liver lesions.

There were no complications associated with the use of MCT in these lesions, either in the operating room or postoperatively. Intraoperative ultrasound easily demonstrated all hepatic tumors. The ablation formed a hyperechoic zone with gas bubbling. At the end of the ablation, the exact demarcation between ablation zone and untreated tissue was not clearly seen by ultrasound. Removal of the three-ring probe from the liver resulted in bleeding which was easily controlled with electrocautery or argon beam coagulator. Temperature probes were inserted in the center of the ablation zones, and these demonstrated temperatures of 100°C during the active phase of ablation.

Ablation Data

After the ablated lesions were resected, gross photos were taken of representative sections (Fig. 2). Ablation zones had a mottled whitish appearance with adjacent tumor exhibiting a more reddish-tan hue. Table 2 describes the ablation zone characteristics. Fifty-six percent of the ablation zones demonstrated immediate histologic evidence of coagulative necrosis (Fig. 3). These heat-induced morphologic changes included accentuation of nuclear elongation, an apparent fusion of adjacent nuclei, and a blurring of the chromatin. The median dimensions of the ablation zones were 5 cm (range, 3–7)×4.5 cm (range, 2.5–5.2)×4.2 cm (range, 2–5), with a median volume of 50.6 cm^3 (range, 9–78). The ratio of the largest to the smallest diameter of each ablation zone was calculated. Two patients had ratios of ≥2, indicating a more ellipsoid shape. NADH vital staining of the ablation zones demonstrated absence of staining in tumor tissue and in benign hepatic parenchyma in all patients, which is consistent with irreversible cellular damage.

Table 3 presents the results of the NADH staining at four selected sites on the surgical specimen. Cores biopsies were taken from the center of the tumor, center of the ablation, edge of the ablation, and 5 mm from the edge of the ablation. In addition to routine histology, the cores underwent NADH staining. In the center of the tumor, five of the eight patients demonstrated nonviable tumor tissue based on NADH staining, whereas patient #6 had tumor tissue which stained positive for NADH—this was due to the large size of the tumor and the ablation zone not reaching the center of it. Patients #4 and #7 had only benign nonviable hepatic parenchyma in the biopsy and no metastatic adenocarcinoma. The distal aspect of the tumor center core biopsy from patient #5 had viable normal liver parenchyma. Core biopsies of the center of the ablation demonstrated lack of NADH staining in all available core specimens. In patient #5, the ablation zone was centered over the tumor, and therefore, the same core specimen represented the center of the tumor and the center of the ablation. Patient #9 did have less than 100 cells in the biopsy that demonstrated moderate staining for NADH. At the edge of the ablation, seven of the nine patients demonstrated both nonviable and viable tissue based on NADH staining. Patient #6 had both viable and nonviable tumor tissue (Fig. 4). Patients #1 and #7 had only normal

Table 1 Patient Tumor and Procedural Information

Patient	Primary	Tumor (cm)	Location (segment)	Previous Chemotherapy	Procedure	Additional Procedures
1	CRC	1.5	5	No	Segment 5 resection	
2	CRC	3.5	5/6	No	Bisegmentectomy (5/6)	
3	CRC	6	2/3	No	L lateral segmentectomy	Hepatic arterial infusion pump
4	CRC	6.5	5	No	Segment 5 resection	R hemicolectomy
5	CRC	3	4B	No	L hepatic wedge resection	Hepatic arterial infusion pump; R venous access device
6	CRC	12.3	4	Yes	L hepatic lobectomy	Hepatic arterial infusion pump
7	CRC	2.5	6	Yes	R hepatic lobectomy	L hepatic wedge resection; RFA segment 4×2; hepatic arterial infusion pump
8	CRC	5.5	3	Yes	L hepatic lobectomy	Segment 6 resection; hepatic arterial infusion pump
9	CRC	2.7	4A	Yes	L hepatic lobectomy	R hepatic wedge resection × 2

CRC Colorectal carcinoma, *RFA* Radiofrequency ablation

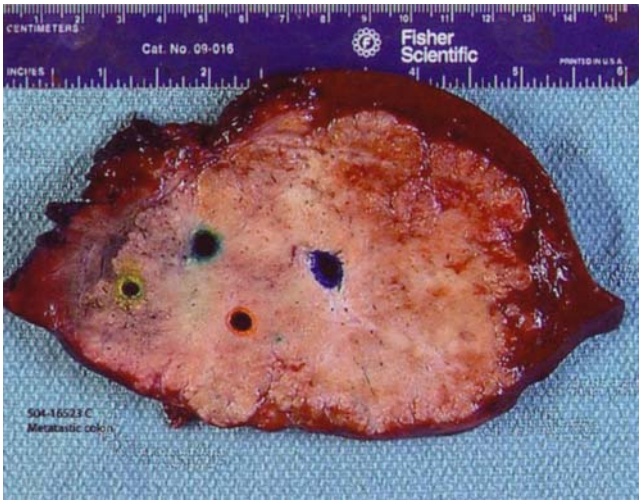

Figure 2 Gross photo of ablation zone with tumor (blue—center of tumor, orange—center of ablation, green—edge of ablation, yellow—zone of hyperemia).

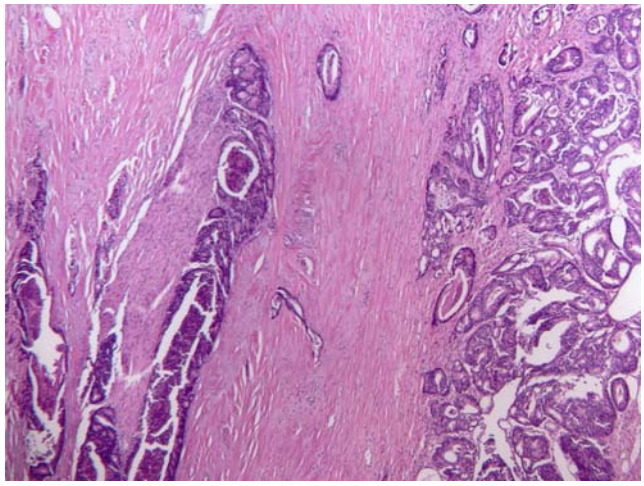

Figure 3 Photomicrograph of ablated tumor tissue (left) and viable tumor tissue (right). Hematoxylin and eosin stain (4×A).

liver tissue that was positive for NADH. The core specimens taken 5 mm outside the ablation zone demonstrated viable normal liver parenchyma in eight patients. Patients #6 and #7 also had evidence of nonviable tissue.

Discussion

This study represents an examination of the latest generation MCT probe being used in human liver tissue with pathologic and immunohistochemical correlation. Table 4 reviews previous series examining the results of earlier versions of MCT probes being studied in porcine and human liver tissue with a comparison to the current series. Early reports discussed using single antenna probes with relatively small ablation diameters and volumes.[7,14] One paper from Wright et al.[7] presented data using multiple single antenna probes placed simultaneously with either sequential or simultaneous ablations being performed. The simultaneous ablation approach produced significantly larger ablation zone volumes than the sequential method.[7] After this, another report presented data using either one-ring, two-ring parallel, and two-ring orthogonal MCT probes in a porcine liver model.[13] Although the orthogonal two-ring probe had a slightly smaller ablation zone volume compared to the parallel two-ring probe, the orthogonal probe achieved the highest in vivo temperatures and on pathologic analysis was found to produce a more spherical lesion with more complete tissue destruction in the ablation zone compared to the other probe configurations. The single antenna probe was compared to RFA by Wright et al.[11] in a pig liver model in which local blood-vessel deflection was measured at the periphery of the ablation zone as a method to assess heat-sink effect. Local blood vessels caused 3.5 and 26.2% deflection at MCT ablation and RFA zones, respectively ($p<0.05$), suggesting that MCT may be less affected by the heat dissipation effect of blood vessels that may play a factor in local recurrence after RFA. In a follow-up paper, the two-ring orthogonal probe was used in human liver tumors scheduled to undergo resection.[12] The average ablation volume was 27.7 cm^3, and immunohistochemical staining confirmed loss of tumor viability after MCT.

The three-ring probe in the current study is designed to build upon the properties of the two-ring orthogonal probe. The rings have been designed to possess a broad field of power density—up to 2 cm surrounding each antenna—which should allow for larger zones of active heating compared to previous MCT probes. Using the same wattage and time of ablation, the three-ring probe produced a median ablation volume of 50.6 cm^3, which is almost twice that of the two-ring orthogonal probe. These determinations were made from measurements of the pathologic speci-

Table 2 Ablation Zone Characteristics

Patient	Coagulative Necrosis	Largest–smallest diameter ratio	Ablation Zone Dimensions (cm)			Ablation Zone Volume (cm^3)
1	No	1.2	5.5	4.8	4.5	59.4
2	No	1.3	5.8	4.7	4.5	61.3
3	Yes	2.1	7	5	3.3	57.8
4	Yes	1.2	6	5.2	5	78
5	No	1.5	3	3	2	9
6	No	1.1	4.8	4.5	4.2	45.3
7	Yes	1.4	4.9	4.5	3.5	38.6
8	Yes	2	5	4	2.5	25
9	Yes	1.1	5	4.5	4.5	50.6
Median		1.3	5	4.5	4.2	50.6

Table 3 Ablation Core NADH Staining Characteristics by Location

Patient	Center of Tumor				Center of Ablation				Edge of Ablation				5 mm Outside of Ablation			
	Liver		Tumor		Liver		Tumor		Liver		Tumor		Liver		Tumor	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
1				X	NA				X				X			
2				X				X	X	X			X			
3				X		X		X	X	X			X			
4		X				X		X	X	X			X			
5	X			X	X			X	X			X	NA			
6			X					X			X	X	X			X
7		X						X	X				X	X		X
8	NA					X		X	X	X			X			
9				X	X	X			X	X			X			

NA Not available

mens. Similar to RFA, intraoperative ultrasound was unable to clearly distinguish the border between ablated and nonablated tissue. In this current study, the objective was to document the ability of the three-ring probe to produce tissue destruction and nonviable cells in an in vivo model. There was no intent to evaluate the heat-sink effect, and therefore, the exact relationship of the ablation zone to blood vessels was not examined.

In Table 2, patients #5 and #8 had ablation volumes of 9 and 25 cm^3, respectively, which are much smaller than the other patients. Patient #5 had a 3-cm lesion on the surface of the liver, and this may have limited the thickness of the ablation zone. However, the dimensions of 3×3 cm are still smaller than other ablation zones. If the rings were not completely below the surface of the liver, this could potentially lead to an incomplete ablation zone. Exact positioning of the probe is not available from the operative note. Patient #8 had a 5.5-cm lesion in segment 3 of the liver, which was a recurrence after percutaneous RFA. It is possible that previous charring of hepatic parenchyma may have limited the size of the microwave ablation zone. In fact, this was a patient in which the largest to smallest diameter ratio was 2, producing an ellipsoid lesion. Patient #3 also had a similar ratio. Both patients had lesions in the left lateral segment located close (<5 mm) to the left hepatic vein on imaging. Previous reports have demonstrated the tendency for MCT lesions to travel along the blood vessels, which may have caused elongation of the ablation zones.[7]

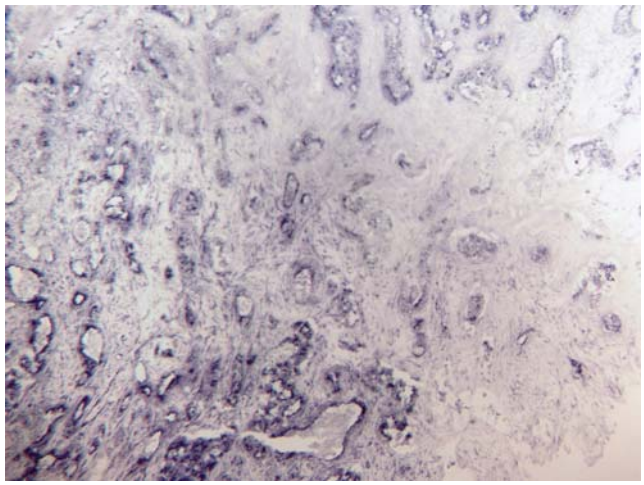

Figure 4 Photomicrograph of viable tumor tissue (lower left) and nonviable ablated tumor tissue (upper right). NADH stain. (4×A).

The first five patients in our series had received systemic chemotherapy for their metastatic disease prior to their hepatic resection, whereas the last four patients had not. In order to determine if the chemotherapy had any effect on the MCT, we compared ablation zone volumes between patients #1–#5 and #6–#9 to see if there were any significant differences. Using the Wilcoxon rank-sum test, the $p=0.19$. It is interesting to note that when patient #5 was removed from the analysis—comparing patients #1–#4 versus #6–#9—there was a significant difference ($p=0.03$).

A previous report from Shock et al.[13] reported the degree of ablation outside the loop as being approximately 0.4–0.8 cm. The three-ring probe had a median minimum and maximum diameter of 4.2 cm (range, 2–5) and 5 cm (range, 3–7), which would produce a median ablation zone of 0.6–1.5 cm outside the 3-cm rings, assuming symmetrical ablation outside the loops. This larger zone of ablation probably results from the addition of another heating antenna as well as larger rings of 3 cm compared to the two-ring orthogonal probe which had 2.7-cm rings.[12] Their study used 60–70 W of power for all ablations, whereas this current study used 60 W. Therefore, it does not appear that any variation in power played a role in the differences in ablation zone dimensions. All ablations were only performed for 5 min under the protocol. The previous report on the two-ring probe in human liver tumors performed one ablation out of the six using a 7-min ablation, and after seeing no change

Table 4 Previous Pathologic Correlation Studies of Microwave Coagulation Therapy Probes

Author/Date	Number of Subjects	Probe Type	Power (W)	Ablation Time (min)	Tissue	Temperature (°C)	Max Median Ablation Diameter (cm)	Median Ablation Volume (cm^3)	Path Evaluation	Stain Performed
Shibata/2000[14]	4	Single antenna	40	2.5	Porcine liver	43	2	NA	Yes	No
Wright/2003[7]	11	Single antenna	40	10	Porcine liver	112.5	2.1	7.4	Yes	No
Wright/2003[7]	11	Single antenna, multiple sequential	40	10	Porcine liver	NA	2.9	14.6	Yes	No
Wright/2003[2]	13	Single antenna, multiple simultaneous	40	10	Porcine liver	109.6	4.8	43.1	Yes	No
Shock/2004[13]	7	One-ring probe	60	7	Porcine liver	60	3.4	6.4	Yes	Yes
Shock/2004[13]	9	Two-ring probe, parallel	60	7	Porcine liver	91.9	4.6	32.3	Yes	Yes
Shock/2004[13]	9	Two-ring probe, orthogonal	60	7	Porcine liver	97.2	4.3	29.5	Yes	Yes
Wright/2005[11]	9	Single antenna	40	10	Porcine liver	NA	6.8	13.9	Yes	No
Meredith/2005[12]	6	Two-ring probe	60	5	Human liver tumor	NA	NA	27.7	Yes	Yes
Shen/2006	9	Three-ring probe	60	5	Human liver tumor	100	5	50.6	Yes	Yes

NA Not available

in ablation size, all subsequent ablations were performed for 5 min only.[12] Further study is warranted to determine if longer ablation times or greater power can produce larger zones of ablation using the three-ring probe.

Histologic examination of the ablation zones demonstrated evidence of coagulative necrosis in 56%. The lack of coagulative necrosis in all specimens is consistent with a previous study from Goldberg et al.[15] in which 11 hepatic tumors were treated intraoperatively with RFA and then immediately resected for examination, and another 12 lesions were treated percutaneously with RFA and then resected 3–7 days later. Tissues immediately resected did not show definitive evidence of coagulative necrosis, whereas those tumors which were ablated and then removed in a delayed fashion all possessed histologic findings consistent with coagulative necrosis. The ablated tissues were stained to detect cytosolic lactate dehydrogenase and mitochondrial enzyme activity; these were absent in all pathologic specimens, which were consistent with reports that hepatocytes require 24–36 h after ischemic injury to display histologic evidence of necrosis. NADH staining allows immediate assessment of tissue viability when histologic findings are inconclusive. This is an extremely accurate method to determine tissue viability, which has been demonstrated in previous reports to be effective in the evaluation of ablated tissue.[16,17]

All ablation zones in the current study underwent NADH staining to determine metabolic activity and tissue viability. As the ablation was meant to encompass tumor and normal hepatic parenchyma, the center of the tumor did not correlate necessarily with the center of the ablation. Some of the core biopsies could not be located at the time of data analysis. Cores taken from the center of the tumor demonstrated 88% (7/8) of specimens with no viable tissue by NADH staining. The patient with the viable tumor center biopsy had a 12.3-cm lesion, and because the three-ring probe is placed at the tumor–liver interface, the center of the lesion was not ablated. In patient #5, the presence of viable liver parenchyma could reflect a biopsy taken from a specimen slice too close to the tumor edge that inadvertently caught the tumor/liver interface. All available core biopsies taken from the center of the ablation zone demonstrated lack of NADH staining. In three patients, the biopsies demonstrated both liver and tumor tissue, likely reflecting the fact that the ablation was centered over the tumor/liver interface. Patient #9 appeared to have evidence of an incomplete ablation because a small number of cells remained viable. The same core specimen from patient #5 was used for center of tumor and center of ablation. Cores taken from the edge of the ablation mostly had evidence of both viable and nonviable hepatic parenchyma, which is to be expected. In the cores taken 5 mm outside of the ablation zone, eight cores demonstrated normal liver tissue which stained positive for NADH. One patient did not have a core taken from this region because of the small size of the specimen. These biopsies

clearly demonstrate the ability of the three-ring MCT probe to produce nonviable cells in both normal hepatic parenchyma and malignant hepatic colorectal metastases.

This study has several limitations. The first is the loss of data from one patient who was entered into the study and never had his core biopsies analyzed with the NADH stain, as well as the individual core biopsy specimens that never underwent immunohistochemical examination. Inconsistencies in the histologic findings from the core biopsy specimens suggest that there may have been some quality control issues with the location from where they were supposedly taken. Two patients had core biopsies of the tumor center but only benign liver tissue was found. Pathologic descriptions of these two sites as well as rereview of the slides demonstrate that the histology was markedly altered by the ablation, and accurate evaluation was difficult, raising the possibility that this was actually tumor tissue. Lastly, identification and notation of blood vessels in relation to probe placement were not evaluated in this study. Therefore no comment can be made about the effect of the heat-sink phenomenon on ablation zones generated by the three-ring MCT probe.

Initial use of the three-ring probe in human liver tumors brought up several technical issues. A theoretical advantage of the three-ring configuration is more accurate placement of the antenna in relationship to the lesion. Formation of a cage by the three rings around a hepatic mass would imply that the entire lesion should be completely ablated by the probe. However, the three-ring design is bulky, and because the liver rests under the ribcage, tumors located in the superior and posterior segments of the liver could be difficult to ablate due to positioning considerations. Most of the lesions in this study were located in the anterior and inferior aspects of the liver, and there were no problems encountered with probe placement. Also, unlike RFA probes, the three-ring MCT probe does not have a tract ablation feature, so when the needle antenna was removed from the liver parenchyma, there was bleeding albeit this was easily controlled with electrocautery. However, once the three-ring probe is deployed, the cage formed by the rings keeps the probe firmly in position and there is no movement, unlike needle probes which can slip in and out at the insertion site. The addition of a conventional electrocautery device to provide cutting power to the rings during deployment is advantageous in preventing any distortion of the cage when used in cirrhotic livers or with tumors which are dense or fibrotic. There was no evidence of image distortion under ultrasound when the rings were being extended with cutting power.

MCT has been used clinically in Japan for several years.[18,19] MCT has not been widely adopted in the US, because in the past MCT could only produce relatively small ablation zones. The three-ring MCT probe represents the latest generation device that offers the potential for faster ablation times with larger ablation zones compared to RFA. The cage configuration of the three rings allows for easier lesion targeting. Pathologic and immunohistochemical analyses with NADH staining of ablated liver tumor specimens have documented lack of tissue viability and in some cases histologic evidence of coagulative necrosis. Although not tested in this study, MCT also has the advantage of allowing the placement of multiple probes at the same time, which would decrease the ablation time for multiple hepatic lesions.[7]

Based on this study and previous reports of earlier probes in porcine and human liver models, phase-II treatment studies of this promising technology in unresectable hepatic malignancies are ongoing. Issues regarding local recurrence rates, complications, and clinical outcomes remain to be answered. Future versions of multiple-ring MCT probes will need to address concerns about the ease of application for tumors deep in the liver and a tract ablation feature to prevent bleeding from puncture sites. As methods of tissue ablation improve, future research will also have to focus on better imaging modalities to assess the true extent of ablation zones at the time they are being produced.

Conclusion

MCT for unresectable hepatic malignancies is a promising alternative to RFA. The three-ring probe has been shown in this pathologic correlation study to produce tissue destruction of both tumor tissue and normal hepatic parenchyma in an in vivo model with larger volumes of ablation than previous MCT devices. Phase-II clinical trials are currently open to study the effectiveness of this technology in patients with unresectable primary and secondary hepatic malignancies.

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A Study Into the Risk of Exacerbation of Chronic Hepatitis B After Liver Resection for Hepatocellular Carcinoma

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Abstract Liver resection is commonly performed for solitary hepatocellular carcinoma (HCC) in well-compensated cirrhotic and noncirrhotic patients. Data concerning exacerbation of chronic hepatitis B (ECHB) post-liver resection are scant. To determine the incidence, risk factors, and clinical outcomes of ECHB in patients who underwent hepatic resection for HCC. The methods consisted of a retrospective review of consecutive patients with chronic hepatitis B virus (HBV) infection who had undergone liver resection for HCC from January 2002 to December 2004. Seventy-seven patients underwent 82 liver resections; the mean age was 58.0±12.1 years; 87% male; 20% hepatitis B e-antigen positive. Incidence of all causes of postoperative hepatitis was 25.6% (n=21), and ECHB was 8.5% (n=7). Both groups had their peak alanine aminotransferases, 231.0 IU/L (74–1,400) and 312 IU/L (147–1,400), respectively, observed at day 84 postresection. Three patients died as a result of ECHB within 4 months postsurgery. One- and 2-year survival rates were poorest for the ECHB group at 42.9 and 21.4%, compared with those with postoperative hepatitis due to other causes at 60.3 and 45.2% and those without postoperative hepatitis at 87.7 and 73.5% (p<0.001). Liver resection for HCC in patients with chronic HBV infection carries a risk for ECHB, and affected patients have poorer clinical outcomes. There is a need for close monitoring of these patients preoperatively and in the early postoperative period.

Keywords Liver cancer · Decompensation · Liver failure · Reactivation

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Introduction

Hepatitis B virus (HBV) infection is a risk factor for hepatocellular carcinoma (HCC). The annual incidence of HCC among all patients with chronic HBV infection was estimated to be 0.5%, and among those complicated by cirrhosis, to be 2.5%, per year[1]. In patients with decompensated cirrhosis and for those with HCCs that meet the Milano criteria[2], the optimal treatment would be liver transplantation. However, for solitary tumors in well-compensated cirrhotic patients, the best management strategy is still contentious, although in countries where liver transplantation resources are limited, liver resections are commonly performed[3]. In noncirrhotic patients who can tolerate major resection with minimal morbidity, liver resection for HCC remains the treatment of choice[4].

Exacerbation of chronic hepatitis B (ECHB) secondary to reactivation of HBV replication is a well established complication in cancer patients who receive cytotoxic or immunosuppressive therapy. In the setting of liver resection for HCC patients, the repercussions of ECHB can be fatal

given their potential limited hepatic reserves. Assessment of risk factors for postoperative ECHB in patients with chronic HBV infection is important for preemptive clinical management. Unfortunately, data concerning ECHB occurring in patients after hepatic resection are scant.

The aim of this study was to determine the incidence, risk factors, and clinical outcomes of ECHB in patients who underwent hepatic resection for HCC.

Material and Methods

Patients

The surgical department HCC registry was accessed for all cases of resections performed for HCC in the period from January 2002 to December 2004. Patients who were positive for hepatitis B antigen and were proven to have HCC in the resected liver tumor were studied. Patients with concomitant hepatitis C infection or human immunodeficiency infection or use of immunosuppressive or cytotoxic agents, including chemo-lipoidization 4 weeks prior to Surgery, were excluded. A total of 126 patients underwent resections for HCC during the study period and 77 patients who fulfilled the above criteria were included in the study.

Data were systematically collected to determine baseline demographics, type of hepatic Surgery performed, clinical outcomes pertaining to HCC recurrence, hepatic decompensation, and mortality. Serial liver function test, prothrombin time, hepatitis B e antigen (HBeAg) and antibody profile and HBV DNA levels (Bayer Versant HBV DNA assay v1.0 for the period from January 2002 to March 2004 and Bayer Versant HBV DNA assay v3.0 for April 2004 to December 2004) taken perioperatively were documented up to 24 weeks postsurgery. Results of the above tests obtained within 4 weeks prior to Surgery were taken as baseline values. Postoperative hepatitis was defined arbitrarily as a serum alanine aminotransferase (ALT) level more than twice the baseline value or ALT> 200 IU/L (ALT reference range: 7–36 IU/L) between 2 and 24 weeks postresection. We disregarded ALT elevation during the early postoperative period as transient ALT elevations were commonly observed among our patients (92%) in the few days immediately after liver resection Surgery. ECHB was defined as postoperative hepatitis associated with a detectable HBV DNA level, in the absence of other causes like tumor progression, other viral infections, ischemic injury, hepatotoxic drugs, HBV mutant-associated hepatitis flares, recent discontinuation of antiviral treatment, and sepsis.

Hepatic decompensation was defined as a new onset of encephalopathy or ascites, increase in prothrombin time by >3 s of the preoperative level or an increase in bilirubin level to twice the normal upper limit (bilirubin reference range: 3–24 μmol/L) if initially normal, or twice the baseline level if initially abnormal[5].

Statistical Analyses

The Student's *t* test, Chi-square test (or Fisher's exact test) were used to compare means and categorical outcomes, respectively. Analyses were performed to determine factors for postoperative hepatitis and ECHB. Cumulative survival rates were calculated by the Kaplan–Meier method with significance of difference in survival analyzed by log-rank test. Univariate analysis using Cox regression was used to ascertain predictors of survival following Surgery. A two-tailed p value <0.05 was considered statistically significant. All results were expressed as median values unless otherwise stated. Data were analyzed with SPSS version 12.0.

Table 1 Baseline Characteristics

Characteristic	
Number of patients	77
Repeat surgeries	5
Gender[a]	
Male	72 (88)
Female	10 (12)
Mean age at surgery (years)	58 (30–82)
Type of resection[a]	
Segmentectomy/partial resection	58 (70)
Hemihepatectomy	24 (30)
HBeAg status (n=46, 60% tested)[a]	
Positive	9 (20)
Negative	37 (80)
Preoperative HBV DNA (n=23, 30% tested)[a]	
Detectable	13 (57)
Undetectable	10 (43)
Child-Pugh status[a]	
A	77 (94)
B	5 (6)
Albumin (g/L, range)	38 (23–48)
Bilirubin (μmol/L, range)	16 (4–201)
Prothrombin time (s, range)	12.4 (10.5–15.0)
ALT (IU/L, range)	41.5 (10–187)
Proportion with preoperative ALT	
≥1×ULN	45 (55)
≥2×ULN	18 (22)
≥3×ULN	9 (11)

ALT <37 IU/L. Values are median (ranges) unless indicated otherwise. *HBeAg*=Hepatitis B e antigen, *HBV DNA*=Hepatitis B virus deoxyribonucleic acid, *ALT*=Alanine aminotransferase, *ULN*=Upper limit of normal.

[a] Values in parenthesis are percentages

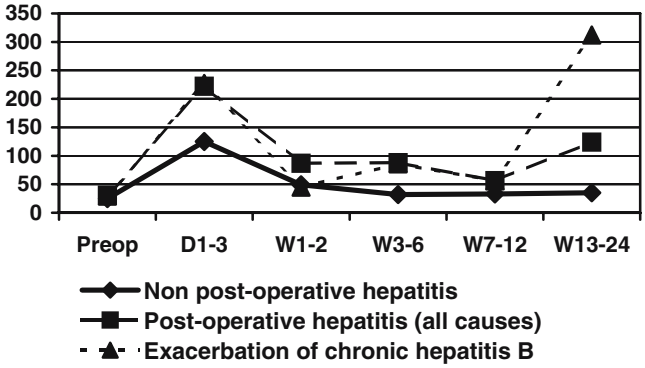


Figure 1 Pattern of alanine aminotransferase (ALT) elevation (mean).

Results

Study Population

Our study population was comprised of 77 patients who underwent 82 liver resections, of which, five patients had repeat liver resections for recurrent HCC during the period of study. The types of hepatic resections performed were partial resection and segmentectomies in 70% and major hemihepatectomy in 30%. The mean age of patients was 58.0± 12.1 years (range: 30–82) with a male predominance of 87%. Hepatitis B e antigen status was tested in 46 patients, and 20% of those tested were positive. Of the 23 patients tested for HBV DNA preoperatively, 13 patients had detectable HBV DNA. The use of antiviral treatment preoperatively was found in 18.3% of patients. The mean period of follow-up was 20.2±11.8 months (range: 2–44). Table 1 summarizes the baseline characteristics of our patients.

Incidence of Postoperative Hepatitis and ECHB

Transient elevation of serum ALT in the first week after resection occurred in 92% of cases and resolved by the second week. The pattern of ALT elevation is as shown in Fig. 1. The peak serum ALT was 222.0 IU/L and declined by week 2 postresection. The incidence of postoperative hepatitis was 25.6% (21/82), with peak ALT 231.0 IU/L (range: 74–1,400) observed at day 84 (range: 15–217) postresection. The majority of postoperative hepatitis patients, 66.7% (14/21), developed ALT elevation within the first 12 weeks after Surgery. The incidence of ECHB was 8.5% (7/82), with peak ALT 312 IU/L (range: 147–1,400) at day 84 (range: 15–145) postresection. Four of the seven patients (57.1%) with ECHB developed ALT elevations within the first 12 weeks after Surgery. Within the postoperative hepatitis group, ECHB accounted for 33.3% (7/21) of the cases. In this ECHB group, three cases were detected via routine liver function test postsurgery, and the remaining four cases were symptomatic at the point of presentation. The other causes of postoperative hepatitis were sepsis (n=2), metastatic HCC (n=2), withdrawal of antiviral treatment (n=2), tyrosine–methionine–aspartate–aspartate mutant flare (n=2), drugs (n=1), and combined sepsis and drugs (n=1). The causes of postoperative hepatitis were undetermined in four cases (19%), as HBV DNA test was not performed as part of the evaluation. However, ALT elevation in these four cases resolved spontaneously and was not associated with hepatic decompensation or mortality.

Risk Factors for Postoperative Hepatitis and ECHB

Age groups ≤40 and >40 years, gender, type of liver resection, HBeAg status, preoperative presence of HBV DNA, and preoperative usage of antiviral treatment were not significantly associated with the risk of postoperative hepatitis or ECHB. We analyzed preoperative ALT elevation stratified to 1× upper limit of normal (ULN), 2× ULN, and 3× ULN as possible risk factors for postoperative hepatitis and ECHB. Only preoperative ALT level ≥3× ULN was found to be a significant risk factor for postoperative hepatitis (p=0.04) but not for ECHB. In the group that experienced postoperative hepatitis due to other causes, 9/14 (64.3%) had preoperative ALT ≥3× ULN, whereas all seven patients with ECHB had preoperative ALT <1.5× ULN.

Table 2 Comparison of Hepatic Decompensation Between Exacerbation of Chronic Hepatitis B (ECHB) vs Postoperative Hepatitis Due To Other Causes

Groups With Postop Hepatitis	Incidence of Decompensation	Mean Bilirubin[a] (μmol/L)	Mean Prothrombin time[a] (s)	Mean Alanine Transferase[a] (IU/L)
ECHB (n=7)	85.7% (6/7)	282.9 (13–553)	25.7 (11.2–53.7)	494.1 (147–1,400)
Other causes of postoperative hepatitis (n=14)	42.9% (6/14)	94.0 (9–308)	16.6 (11.4–38.6)	301.9 (74–848)
p value	0.160	0.013[b]	0.008[b]	0.060

[a] Values in parenthesis denote range
[b] Significant p value

Table 3 Clinical Features of Patients with Exacerbation of Chronic Hepatitis B (ECHB)

Patient	Sex/ Age	Baseline				At Time of ECHB				Development of Hepatic Decompensation	Time Length Between Dx and Lamivudine Tx (days)	HCC Recurrence	Outcome (Time After ECHB)
		HepBe Ag	ALT/ × ULN	TB	HBV DNA ($\times 10^6$)	Onset after Surgery (days)	HepBeAg	ALT/ TB (peak)	HBV DNA ($\times 10^6$)				
1	M/66	+	24/0.6	32	<0.7[a]	45	NT	231/32	5.4[a]	No	7	Yes	Died (11 mo)
2	M/77	−	48/1.3	17	NT	112	−	873/405	78.6[a]	Yes	2	No	Died[b] (20 d)
3	M/63	−	21/0.6	12	NT	36	−	147/13	18.1[c]	Yes	1	No	Alive (9 mo)
4	M/42	+	43/1.2	34	NT	58	+	312/475	95.7[c]	Yes	2	Yes	Died[b] (12 d)
5	M/73	−	31/0.9	16	<0.7[a]	103	NT	339/553	23.5[a]	Yes	14	No	Died (4 mo)
6	M/60	−	33/0.9	10	NT	15	−	157/180	1.2[a]	Yes	4	Yes	Alive (20 mo)
7	M/64	−	29/0.8	13	NT	105	−	1,400/ 322	>100[a]	Yes	2	No	Died[b] (14 d)

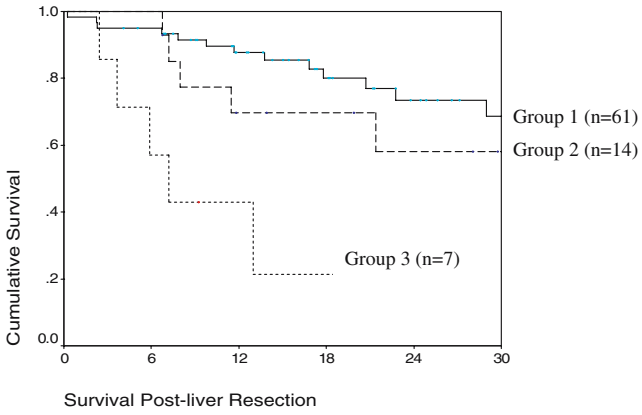
ALT <37 IU/L

Pt = patient, mo = months, d = days, NT = not tested, Dx = diagnosis, Tx = treatment, TB = total bilirubin (<24 μmol/L), *ALT*=Alanine aminotransferase, *ULN*=Upper limit of normal, *HBV DNA*=Hepatitis B virus deoxyribonucleic acid, *HCC*=Hepatocellular carcinoma.

[a] HBV DNA lowest limit of detection $<0.7\times 10^6$ cop/ml

[b] Patient died of hepatic failure secondary to ECHB

[c] HBV DNA lowest limit <2,000 cop/ml



Patients at risk

Group 1	56	42	29	20	14
Group 2	14	8	7	5	3
Group 3	4	2	1	0	0

Figure 2 Survival after liver resection according to postoperative hepatitis and ECHB ($p<0.001$; log rank test). *Group 1*, without postoperative hepatitis; *group 2*, postoperative hepatitis due to other causes; *group 3*, ECHB.

Clinical Outcomes in Postoperative Hepatitis and ECHB

Within the postoperative hepatitis group, 6/7 (85.7%) with ECHB experienced hepatic decompensation, compared with 6/14 (42.9%) in those with postoperative hepatitis due to other causes ($p=0.160$). The degree of hepatic decompensation in terms of prothrombin time prolongation and bilirubin elevation were significantly worse for those with ECHB, as shown in Table 2. Three patients died as a result of ECHB resulting in liver failure, despite prompt institution of lamivudine treatment. The clinical features of patients who experienced ECHB are shown in Table 3.

Overall survival rates for the study population postsurgery were 80.6% at 1 year and 66.2% at 2 years. Survival rates at 1 and 2 years for patients without postoperative hepatitis ($n=61$) were 87.7 and 73.5%, respectively, whereas those of patients with postoperative hepatitis due to other causes ($n=14$) were 69.6 and 58.0%, and those with ECHB ($n=7$) were 42.9 and 21.4%, respectively. The Kaplan–Meier survival plots after liver resection comparing those without postoperative hepatitis, those with postoperative hepatitis due to other causes, and ECHB are shown in Fig. 2. The ECHB group had the poorest survival among the three groups postsurgery ($p<0.001$). Those with HCC recurrence during the period of follow-up likewise experienced decreased survival rates of 74.6% at 1 year and 48.5% at 2 years ($p=0.002$). Univariate analysis for predictors of survival found ECHB and HCC recurrence to be the only two significant risk factors, as shown in Table 4. Multivariate analysis was not performed because of small numbers within the ECHB group.

Neither postoperative hepatitis nor ECHB influenced tumor recurrence rates. In the postoperative hepatitis group, 47.1% had HCC recurrence, compared with 42.1% of those without postoperative hepatitis ($p=0.717$). Although the ECHB group had a higher tumor recurrence rate of 75%, compared with 41.4% in those without ECHB, the difference was not statistically significant ($p=0.310$).

Discussion

Apart from a Japanese study by Kubo et al.[6], which described reactivation of HBV replication and hepatitis, there has not been any prior systematic study on ECHB after liver resection. ECHB after liver resection can have serious consequences including fatality in view of compromised liver reserves after Surgery.

In our study, we observed that postoperative hepatitis occurred in 25.6%, similar to the reported rate of Kubo et al. of 24% (13/55), although a shorter time frame for ALT elevation (within 3 weeks to 3 months after Surgery) was used in their study. They were able to prospectively analyze HBV DNA levels in 25 patients and found seven (28%) patients with reactivation of HBV replication. Our seemingly lower ECHB rate of 8.5% may be contributed by the

Table 4 Predictors of Survival Post Liver Resection

Factors	Univariate Cox Regression Analysis *p* Value	Hazard Ratio RR (95% CI)
ECHB	<0.001[a]	6.34 (2.26–17.80)
HCC recurrence	0.002[a]	9.78 (2.24–42.65)
Postoperative hepatitis due to other causes	0.621	
HBeAg status	0.149	
Type of Surgery	0.584	
Gender	0.956	
Age group ≤40 or >40	0.263	
Preoperative ALT group<3×ULN or ≥3×ULN	0.628	

ALT <37 IU/L. *ECHB*=Exacerbation of chronic hepatitis B, *HCC*=Hepatocellular carcinoma, *HBeAg*=Hepatitis B e antigen, *ALT*=Alanine aminotransferase, *ULN*=Upper limit of normal.
[a] Significant *p* value

retrospective nature of the study, which had infrequent monitoring of HBV DNA levels in many cases.

Exacerbations of chronic hepatitis B recognized by abrupt elevations of ALT levels can occur during the natural disease course of HBV infection and the cumulative probability of developing exacerbation in 1 year for those with ALT levels <200 IU/L has been reported to be 6.3%[7]. We believe that our ECHB rate of 8.5% was not a sporadic event, given the close chronological sequence of hepatitis and Surgery. The majority of ECHB occurred within 12 weeks of Surgery, with peak ALT occurring 15 to 145 days after Surgery. This time course of ECHB postsurgery is consistent with well established examples of immunologically mediated hepatitis flare as seen 4–6 weeks after steroid withdrawal, 8–12 weeks after interferon treatment, and 4–36 weeks after the initiation of chemotherapy[8,9].

The commonly described causes for exacerbation in chronic hepatitis B[8] are immunosuppressive medications, antiviral therapy such as interferon treatment, HBV genotypic variations such as precore mutant and HBV DNA polymerase mutant, and superimposed infections with other hepatotropic viruses. These causes were carefully excluded before ECHB was attributed to liver resection Surgery. In addition, clinical conditions that may result in elevation of ALT level, such as sepsis, drugs, ischemia and tumor infiltration of the liver, were likewise excluded.

In the natural disease course of chronic HBV infection, evidence of HBV replication characterized by high HBV DNA load and HBeAg positive status would often precede a hepatitis flare[10]. We did not observe HBeAg positive status, high preoperative ALT level, or high HBV DNA level to be significant risk factors for ECHB postsurgery. This is likely because of insufficient preoperative assessment of HBeAg and HBV DNA status in our study population, as well as the small number of patients experiencing ECHB, affecting the analyses for risk factors. Although preoperative ALT ≥3× ULN was statistically associated with risk of postoperative hepatitis, it is difficult to comment on the clinical relevance of this finding because of the heterogeneity of causes for postoperative hepatitis in this group. However, patients with preoperative ALT ≥3× ULN did not experience an increased all-cause mortality rate compared with patients with ALT <3× ULN (28.8% in group with ALT <3× ULN vs 22.2% in group with ALT ≥3× ULN, $p=0.680$).

The clinical outcomes in terms of overall survival rates and the degree of decompensation were significantly worse among those with ECHB compared with those with postoperative hepatitis due to other causes. The early death of three patients from hepatic failure within 3 weeks of ECHB onset suggests that the prompt institution of lamivudine treatment at the occurrence of ECHB event may not be sufficient to avert a potentially fatal outcome given the compromised liver reserves.

Because of the retrospective nature of our study, our main limitation lies with insufficient preoperative and postoperative HBV DNA testing. As such, we were not able to demonstrate reactivation of HBV replication prior to the onset of ECHB. Nevertheless, it is important to note that the majority of patients (71%) with ECHB had normal preoperative ALT levels before Surgery.

Altered host defense following major Surgery had been linked to the development of infectious complications and sepsis. Major Surgery had also been described to cause a severe defect in T-lymphocyte proliferation and cytokine secretions[11], and endogenous corticosteroids levels had been shown to increase remarkably after major Surgery[12]. The postulate for ECHB in post-liver resection is attributed to an immunosuppressive state associated with resection Surgery, which may enhance viral replication and increased hepatocyte infection by HBV. In the later postoperative period, restoration of immune function leads to destruction of HBV-infected hepatocytes, giving rise to ECHB.

Conclusion

In conclusion, the incidence of ECHB was 8.5% of patients with chronic HBV infection undergoing liver resection for HCC, and these patients experienced poorer clinical outcomes in terms of survival and liver-related morbidity. Hence, it would be prudent to carry out postoperative surveillance with regular liver function test in the first 6 months postsurgery to aid the early detection of ECHB. It may be worthwhile to consider checking patient's viral replicative status in terms of HBeAg status and HBV DNA level preoperatively to better stratify the patient's follow-up schedules. Larger prospective trials should be carried out to determine the predictive value of preoperative HBeAg status and HBV DNA level, as well as the role of preemptive antiviral treatment in patients who have active viral replication.

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Prognostic Factors After Resection for Hepatocellular Carcinoma in Nonfibrotic or Moderately Fibrotic Liver. A 116-Case European Series

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Abstract The purpose of this study was to identify factors influencing prognosis after resection for hepatocellular carcinoma in the noncirrhotic liver and to measure the impact of moderate fibrosis on presentation and prognosis. A series of 116 primary procedures were performed for hepatocellular carcinoma in the noncirrhotic liver. These cases accounted for 42% of hepatic resections performed for hepatocellular carcinoma during the study period (1987–2005). Seventy-seven cases (58%) occurred in patients with nonfibrotic livers (Metavir score F0). The mean age was 61 years. The sex ratio was 3.5, with a female predominance before 50 years. Hepatitis B virus (HBV) or hepatitis C virus infection was found in 30% of patients. Symptoms were present in 64% of cases. Elevated serum alpha fetoprotein levels were observed in 44% of cases. Procedures involved minor hepatectomy in 40 cases, major hepatectomy in 72 cases, and transplantation in 4 cases. Postoperative mortality was 6% and morbidity was 31%. Complete resection was achieved in 90% of cases. The tumor was isolated in 72% of cases. The mean tumor diameter was 10.6 cm. Vascular invasion was observed in 48% of cases. Hepatocellular carcinoma in the nonfibrotic liver was associated with younger age and female sex, but there was no difference with other hepatocellular carcinoma with regard to histological or prognostic features. With a median follow-up of 79 months, overall survival was 40% for a median of 41 months. Multivariate analysis identified incomplete resection, vascular invasion, and HBV infection as independent factors of poor prognosis. In case of recurrence, repeat resection was feasible in 30% of cases with 69% survival at 5 years. Although hepatocellular carcinoma in the noncirrhotic liver is generally diagnosed at an advanced stage, its resectability remains high. As a result, hepatocellular carcinoma in the noncirrhotic liver accounts for a large proportion of cases in surgical series and has a better prognosis than hepatocellular carcinoma in the cirrhotic liver. Vascular invasion, incomplete resection, and HBV infection are independent factors of poor prognosis.

No competing interests declared.

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Keywords Hepatocellular carcinoma · Noncirrhotic liver · Nonfibrotic liver · Surgical resection · Prognostic factors

Abbreviations

HCC	Hepatocellular carcinoma
AFP	Alpha fetoprotein
HBV	Hepatitis B virus
HCV	Hepatitis C virus

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. In 80% of cases, HCC is a complication of cirrhosis[1] which is considered as a

precancerous state[2]. Because HCC in the noncirrhotic liver accounts for a minority of cases, much less information is available regarding its epidemiology, pathogenesis, and prognosis. The concept of noncirrhotic parenchyma covers a wide range of conditions ranging from strictly normal liver (Metavir score F0) to precirrhotic fibrotic liver (Metavir score F3)[3]. Underlying parenchymal status has important implications in therapeutic decision-making, i.e., resection for HCC in noncirrhotic liver vs transplantation for early HCC in cirrhotic liver[4]. The purpose of this study, which is a sequel to a previous report[5], was to identify factors influencing survival in patients undergoing surgical resection for HCC in noncirrhotic livers and to measure the impact of moderate fibrosis on presentation and prognosis.

Table 1 Epidemiologic, Clinical and Laboratory Findings

	Number of patients	%
Male/female (ratio)	90/26 (3.5)	–
Mean age (range)	61 years (21–81)	–
Diagnostic features		
Pain/asthenia	74	64
Asymptomatic	32	28
Follow-up screening	10	8
Abdominal mass	33	28
Serum AFP level (*n*:110)		
0–3×*N*	62	56
3–100×*N*	18	16
>100×*N*	30	27
Hepatitis virus infection (*n*:110)		
Negative hepatitis	77	70
Hepatitis B positive	20	18
Hepatitis C positive	13	12

Materials and Methods

Patients

From January 1987 to April 2005, we carried out 948 hepatic resections and 441 liver transplantations. The indication for 266 resections and 97 transplantations was HCC. One hundred and sixteen procedures, including 112 primary resections and four transplantations, involved patients without extensive fibrosis (F3) or cirrhosis (F4). These resections accounted for 42% of hepatectomies carried out for HCC during the study period. Treatments involving percutaneous radio-frequency ablation were not taken into account, including those performed for recurrence. Patients were of European origin in 92% of cases, African origin in 5%, and Asiatic origin in 3%. Mean patient age was 61±15 years, with a single peak distribution corresponding to the mean age. There were 90 men and 26 women that were unevenly distributed, with women accounting for 56% of patients in the under-50-years-old age group and only 12% of the over-50-years-old age group ($p<0.001$). Sixty-four percent of patients were symptomatic (Table 1). Risk factors for HCC included alcohol abuse in 22% of patients and hepatitis B virus (HBV) infection (defined as presence of any hepatitis B antigen or antibody) or hepatitis C virus (HCV) infection in 30%. Serum alpha fetoprotein (AFP) level was normal or less than three times normal in 56% of cases. Ultrasound and CT-scan imaging was performed in all patients. Other imaging modalities, i.e., magnetic resonance imaging, angiography, and bone scintigraphy, were not ordered routinely. Percutaneous biopsy was performed in 47% of cases. Biopsy findings were accurate and contributed to definitive diagnoses in 61% of cases, inaccurate in 9%, and not useful in 30%. Most fine-needle biopsies were performed prior to the patient's admission to our department. In a few cases, biopsy of the nontumoral liver was performed to detect cirrhosis.

Treatment

Partial hepatectomy was performed in 112 patients. According to Couinaud, hepatectomy was minor in 36% of cases and major in 64%. Minor procedures included tumorectomy or monosegmentectomy in 15 cases and bisegmentectomy in 25 cases. Major procedures included right hepatectomy in 27 cases, right hepatectomy extended to segment IV in 12 cases, left hepatectomy in 21 cases, left hepatectomy extended to segments V and VIII in three cases, central hepatectomy (segments IV, V and VIII) in seven cases, and other types of trisegmentectomy in two cases. Four young patients underwent liver transplantation due to the presence of unresectable bilobar sites associated with no detectable extrahepatic lesions. Postoperative mortality was calculated taking into account all deaths occurring during hospitalization. Criteria used to calculate morbidity have been described elsewhere[6]. No adjuvant treatment was indicated. Follow-up surveillance was based on imaging (ultrasound or CT scan) two to three times per year and measurement of serum AFP level in patients who had high levels prior to surgical treatment. In case of abdominal recurrence, repeat surgery was the preferred therapy if complete resection was considered feasible.

Pathology

Resections were considered as complete or curative (R0) if there was no macroscopic (R2) or microscopic (R1) evidence of residual tumor. Histological data included weight of the surgical specimen, number and size of

tumors, existence of satellite nodules, presence of microscopic or macroscopic vascular invasion, and Edmonson grade[7]. Tumor encapsulation status was not noted in all patients. Extent of hepatic fibrosis was classified according to the Metavir score[3] and slides from cases treated before the introduction of the Metavir score were reviewed. Patients were subdivided into two groups independently of serologic status, i.e., nonfibrotic liver group (Metavir score F0) and moderately fibrotic liver group (Metavir score F1–F2).

Statistical Analysis

A retrospective cohort was performed from prospectively collected data. Statistical analysis was performed with SPSS version 10.0. Comparisons were made using the chi-square test for proportions, the student *t* test for means, and the Mann–Whitney U test for nonparametric data. Actuarial survival was estimated using the Kaplan–Meier method and compared using the log-rank test. The study cutoff date was September 30, 2005. A Cox model was constructed for multivariate analysis of survival using significant data obtained in univariate analysis. A *p*-value less than 0.05 was considered as significant.

Results

Postoperative Course

Postoperative recovery was uneventful in 69% of cases. The postoperative mortality rate was 6%, with seven deaths due to hepatic or multiorgan failure (n=4), cardiopulmonary causes (n=2), and acute pancreatitis (n=1). The overall morbidity rate was 31%, including transient hepatocellular insufficiency in eight cases and transient biliary fistula in nine. Perioperative transfusion was required in 27% of patients. Seven patients required reoperation for intra-abdominal hemorrhage (n=3), bile duct injury (n=1), portal vein thrombosis (n=1), small bowel perforation (n=1), and possible intra-abdominal sepsis (n=1). The median duration of postoperative hospitalization was 12 days (range 5–54).

Histological Examination

Histological examination (Table 2) demonstrated conventional HCC in 108 cases, fibrolamellar HCC in five, and hepatocholangiocarcinoma in three. The tumor was isolated in 72% of cases and associated with daughter nodules in 56%. Microscopic or macroscopic vascular invasion was observed in 48% of cases. Surgical resection was complete (R0) in 90% of patients. The extratumoral liver was nonfibrotic (Metavir score F0) in 58% of cases and presented steatosis in 28%.

Table 2 Histological Data

	Number of patients	%
Mean tumor size (min–max)	10.6 cm (3–24)	
Mean specimen weight (min–max)	904 g (120–2,440)	
No. of tumor sites		
Isolated	83	72
Multiple	33	28
Daughter nodules	48	56
Histology		
Conventional HCC	108	93
Fibrolamellar HCC	5	4
Hepatocholangiocarcinoma	3	3
Surgical margins		
R0 (no evidence of involvement)	104	90
R1 (microscopic evidence of involvement)	3	3
R2 (macroscopic evidence of involvement)	9	8
Edmonson score		
Grade 1	10	9
Grade 2	74	67
Grades 3–4	26	24
Tumor capsule	54	66
Vascular invasion		
Total	54	48
Macroscopic portal vein	17	15
Macroscopic hepatic vein	4	3
Biliary tumor thrombus	5	4
Fibrosis (Metavir score)		
F0	67	58
F1	31	27
F2	18	15

Nonfibrotic Liver Versus Moderately Fibrotic Liver Groups

Tumors in nonfibrotic livers were associated with younger age (57 vs 66 years, p=0.001), female sex (sex ratio, 2.2 vs 8.8, p=0.007), and larger tumor size (11.5 vs 9.3 cm, p=0.013). HCV serology was more often negative in nonfibrotic liver than in moderately fibrotic liver (4 vs 20%), but there was no difference for HBV. Histological findings were not different in the nonfibrotic and moderately fibrotic liver groups (Table 3).

Surgical Outcome

No patient was lost to follow-up. Median follow-up was 79 months (range 5–218). Overall survival was 72% at 1 year, 54% at 3 years, 40% at 5 years, and 29% at 10 years. Recurrence-free survival was 60, 40, 33, and 15% at 1, 3, 5, and 10 years, respectively. In the group of four

Table 3 Comparison of Patients in Function of Fibrosis Status: Nonfibrotic (Metavir score F0) vs Moderately Fibrotic (Metavir score F1–F2)

	F0 (*n*:67)	F1–F2 (*n*:49)	*p*
Sex M/F	46/21	44/5	0.007
Mean age	57 years	66 years	0.001
Serum AFP level			
0–3×*N*	35	27	0.32
3–100×*N*	9	9	
>100×*N*	21	9	
Hepatitis virus infection			
Negative	48	29	0.029
Hepatitis B positive	12	8	
Hepatitis C positive	3	10	
Histology			
Conventional HCC	60	48	0.14
Fibrolamellar HCC	5	0	
Hepatocholangiocarcinoma	2	1	
Mean tumor size (SD)	11.5cm (4.5)	9.3 cm (3.9)	0.013
Edmonson score			
Grade 1	5	5	0.66
Grade 2	42	32	
Grades 3–4	15	11	
No. of tumor sites			
Isolated	46	37	0.42
Multiple	21	12	
Daughter nodules	33 (62%)	15 (47%)	0.17
Vascular invasion	31 (47%)	25 (49%)	0.84

patients who underwent transplantation, one was alive and recurrence-free after 34 months and three were dead; two died due to recurrence at 5 and 21 months and one due to causes unrelated to the tumor at 74 months.

Recurrences were observed in 64 of the 98 patients (65%) who underwent R0 resection and survived postoperatively. The mean interval for recurrence was 14 months (range 2–155). In 19 cases (30%), recurrence was treated by a second resection after a median interval of 35 months (range 9–122). Three patients required a third resection and one required a fourth resection followed by transplantation (not included in this study). Recurrence was intrahepatic in 15 cases and extrahepatic in seven. Extrahepatic locations included the peritoneum (n=2), adrenal gland (n=2), spleen (n=1), abdominal wall (n=1), and bone (n=1). Complete resection was achieved in 17 cases (77%). In the 15 patients undergoing repeat surgery for intrahepatic recurrence, hepatectomy was classified as major in four and minor in 11. Ten of the 19 patients treated for recurrence were alive at the end of the study, including seven who were recurrence-free, with a median follow-up of 100 months (range 20–171) from the date of the first operation. Actuarial survival in these 19 patients was 69% at 5 years from the first resection, and median survival was not reached.

Prognosis

Univariate analysis of survival identified six factors as being correlated with poor prognosis, i.e., serum AFP>3*N* (20 vs 54% at 5 years, $p<0.006$), HBV infection (10 vs 67% for HCV infection and 42% in the absence of any infection, $p=0.001$), multiple tumor sites (33 vs 43% for isolated tumors $p<0.025$), presence of daughter nodules (35 vs 50%, $p<0.012$), presence of vascular invasion (23 vs 53% without vascular invasion, $p<0.0001$), and R1–R2 resection (0 vs 45% with R0 resection, $p<0.0001$) (Fig. 1). Prognosis was not correlated with sex, age, transfusion, tumor size, tumor capsule, or extent of fibrosis (38% at 5 years for Metavir score F0 vs 43% for score F1–F2). Multivariate analysis (Table 4) demonstrated only three independent factors of poor prognosis, which were vascular invasion [hazard ratio (HR)=4.1, $p<0.001$], incomplete resection (HR=3.8, $p=0.008$), and HBV infection (HR=2.8, $p=0.004$).

Discussion

Although most HCCs occur in the cirrhotic liver, cases involving the noncirrhotic liver accounted for 42% of resection procedures for HCC in our experience and for 35 to 50% of resection procedures in recent surgical series, with no notable difference in the proportions observed in eastern[8–11] and western[12–16] series (Table 5). The main explanation for this high proportion in surgical series is that HCC in the noncirrhotic liver has a higher resectability rate than HCC in the cirrhotic liver[12]. In the series reported by Fong et al.[13], HCC in the noncirrhotic liver accounted for 25% of HCC and 35% of resections. Variations between series are due to differences in the incidence of viral infection and in the definition of the noncirrhotic. Regarding fibrosis, it should be pointed out that some therapeutic[17] or prognostic[18] studies have combined Metavir scores F3 and F4. In the study of Balzan et al.[18], Metavir scores F3–F4 fibrosis was an independent predictor of postoperative mortality. To clarify this issue, we did not include patients with Metavir score F3 from the noncirrhotic population. In our opinion, this also eliminated the confounding effects of a possible carcinogenic factor.

Comparison of the Metavir score F0 and F1–F2 groups in our series demonstrated that the F0 group contained more young patients, females, HCV infections, and large tumors than the F1–F2 group. However, there was no difference with regard to other tumor features or long-term survival (38 vs 43% at 5 years, respectively). This finding is inconsistent with the series of Shimada et al.[8] who reported that prognosis was better for HCC in nonfibrotic than fibrotic liver, although it is important to mention that

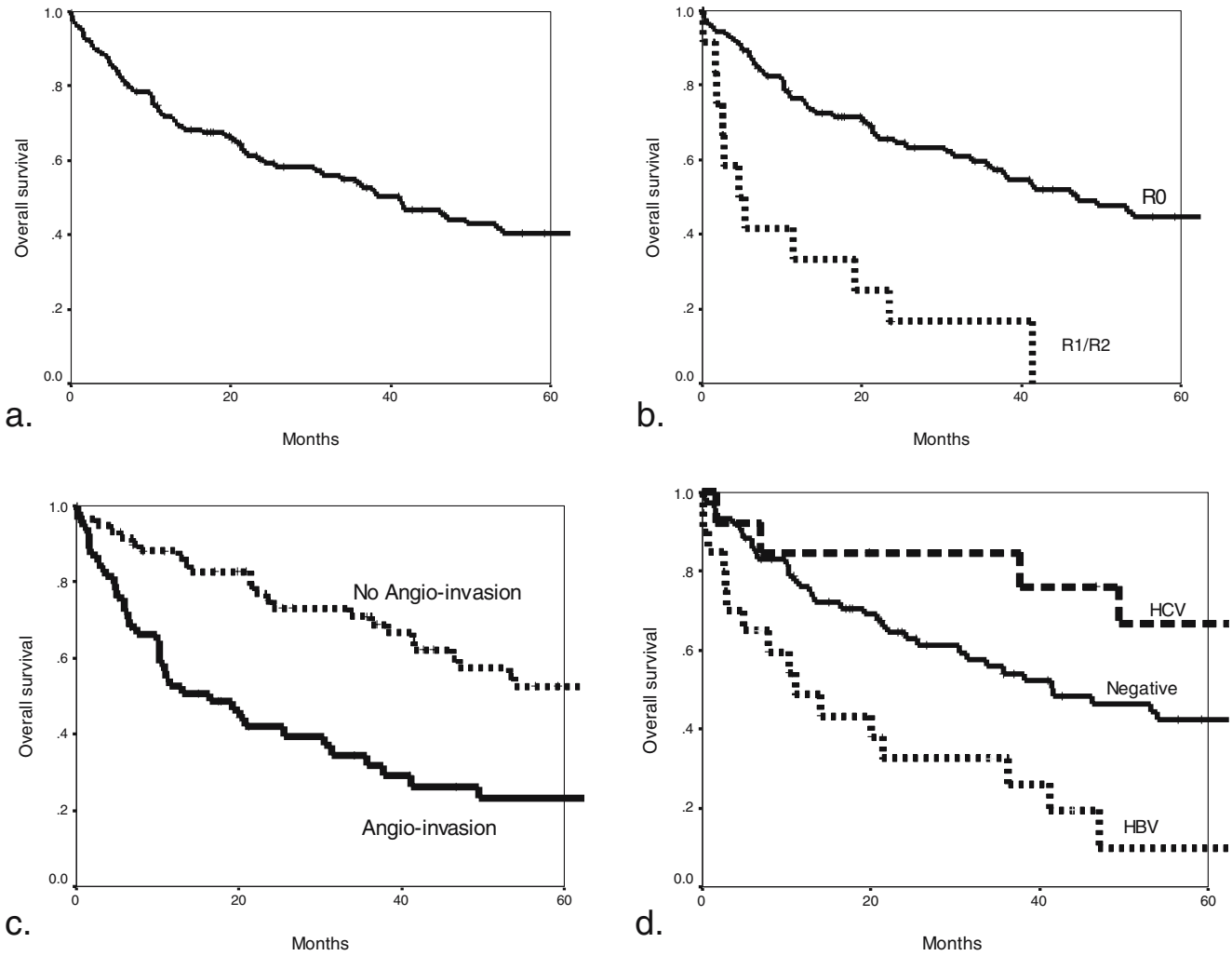

Figure 1 **a** Survival after hepatic resection for HCC in 116 patients. **b** Survival after hepatic resection according to the surgical margin. **c** Survival after hepatic resection in patients with or without vascular invasion. **d** Overall survival after hepatic resection according to virus infection status.

patients with cirrhosis were included in the fibrosis group. For comparable tumor size, the prognosis of HCC in the noncirrhotic liver was better than that of HCC in the cirrhotic liver. A multicenter study showed that 5-year survival after resection of HCCs larger than 10 cm was 27% in cirrhotic cases vs 40 to 50% in noncirrhotic cases[19].

Unlike the outcome of resection for HCC in the cirrhotic liver, which mainly depends on the status of the underlying parenchyma[20], the outcome of resection for HCC in the noncirrhotic liver is correlated mainly with tumor factors, i.e., tumor size; absence of tumor capsule; daughter nodules; and, above all, vascular invasion[9,13,14,16,21]. In our series, vascular invasion was the foremost prognostic factor (23 vs 53% survival at 5 years in the absence of vascular invasion). Microscopic vascular invasion is difficult to detect prior to surgery. In our opinion, macroscopic portal vein invasion does not contraindicate surgery[21] but it may indicate neoadjuvant intra-arterial chemo-embolization[22] or adjuvant iodine 131-iodized oil treatment[16]. Complete resection has consistently been identified as a prognostic factor when taken into account in multivariate analysis[9,10, 16]. Unlike reports from other teams, our study showed that HBV infection was an independent factor of poor prognosis after resection for HCC in the normal or moderately fibrotic livers (10 vs 42% survival at 5 years in the absence viral infection). HBV infection has been shown to have direct carcinogenic effects (insertional mutagenesis

Table 4 Factors Correlated with Overall Survival in Multivariate Analysis

	HR (95% CI)	*p* Value
Vascular invasion	4.1 (2–8.7)	<0.001
R1–R2 resection	3.8 (1.4–10.2)	0.008
HBV infection	2.83 (1.4–5.8)	0.004

R1/R2 microscopic/macroscopic evidence of residual tumor
CI = confidence interval

Table 5 Review of Recent Series of HCC in Noncirrhotic Liver

Author, year	*n* (% of resected CHC)	Liver parenchyma	% of hepatitis virus infection	5-year survival	Prognostic factors (multivariate analysis)
Eastern series					
Shimada, 2000	65 (13)	F0	51 (11 B, 22 C)	65%	–
Nagasue, 2001	100 (36)	Noncirrhotic	75 (33 B, 42 C)	50%[a]	Blood loss, R1–R2 resection, daughter nodules, vascular invasion, extent of resection
Chen, 2003	254 (42)	Noncirrhotic	89 (179 B, 39 C)	36%	Albumin, transfusion, R1–R2 resection, multiple tumors
Chang, 2004	223 (50)	Noncirrhotic	72 (181 B, 39 C)	53%	–
Western series					
Bismuth, 1995	68 (47)	Noncirrhotic	9 (B)	40%[a]	Tumor size >9 cm, no tumor capsule
Fong, 1999	54 (35)	Noncirrhotic	Virus excluded	42%	Tumor size, AFP >2,000, vascular invasion
Lang, 2005	33 (NA)	F0	Virus excluded	38%[b]	Vascular invasion
Laurent, 2005	108 (NA)	Noncirrhotic	20 (9 B, 12 C)	43%[a]	Transfusion, no tumor capsule, daughter nodules
Dupont-Bierre, 2005	84 (41)	Noncirrhotic	7 (4 B, 2 C)	44%	Multiples tumors, macroscopic vascular invasion, no adjuvant I^{131}
Present series	116 (42)	F0 to F2	30 (20 B, 13 C)	40%	R1–R2 resection, vascular invasion, HBV infection

[a] R0 resections only
[b] 3-year survival

and gene transactivation)[23] and to increase the risk for HCC 100-fold in comparison with patients without infection[24]. However, the implication of viral infection in prognosis after resection has not been demonstrated in eastern studies, in which the incidence of infection has been high. In two Taiwanese series with a comparable incidence of HBV infection, 5-year survival rates after resection for HCC in noncirrhotic liver varied from 36[10] to 53%[11]. In a Japanese series, recurrence-free survival was significantly higher in patients with HBV infection than with HCV infection (58 vs 6%)[9]. In our study, the overall survival was significantly higher in patients with HCV infection than with HBV infection. However, univariate analysis did not show a significant difference of survival between patients with HCV infection (n=13) and patients with no viral infection, and multivariate analysis identified only HBV infection as an independent factor of poor prognosis.

The survival rates in our series were comparable to those described elsewhere, especially with regard to outcome after complete resection: 45% at 5 years in our series vs 40 to 50% in recent series[9,12,15]. These findings clearly support complete resection of the HCC in noncirrhotic liver because these patients tolerate major resection with acceptable morbidity[12,14]. Another important discussion point involves resection of recurrence. Most recurrences are intrahepatic[12,15] and often resectable in noncirrhotic parenchyma. However, the incidence of repeat resection has varied widely in recent western reports. In the series of Lang et al.[14], the recurrence rate after complete resection was 31% (9/29), but repeat resection was never possible due to extrahepatic involvement. In the series of Dupont-Bierre et al.[16] and Laurent et al.[15], the recurrence rates were 41% (27/69) and 52% (56/108) and the reoperation rates were 11% (3/27) and 13% (7/56), respectively. However, it should be emphasized that the median follow-up time in these two series was only 25 months. In the series of Bismuth et al.[12], the recurrence rate was 59% (39/66) and the reoperation rate was 31% (12/39) with good long-term outcome. Our experience was comparable with a recurrence rate of 65% (64/98) within a median interval of 14 months after the first operation and a reoperation rate of 30% (19/64), with 69% survival at 5 years. It is interesting to note that the median interval from primary to secondary resection was 35 months (range 9–122). It is likely that a bias resulting from the selection of HCCs with less aggressive biological features explains not only the long interval for recurrence resection but also the remarkably long survival rate in this small group. In any case, these findings further confirm the value of prolonged postoperative follow-up and support aggressive management of recurrent HCC in patients with nonfibrotic or moderately fibrotic liver.

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Reducing Residual and Recurrent Stones by Hepatectomy for Hepatolithiasis

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Abstract The long-term outcomes of 97 consecutive patients with hepatolithiasis, who underwent treatment from January 1971 to June 2006, were analyzed. The short-term outcomes included the rate of residual stones and complications after treatment, whereas the long-term results included the stone recurrence rate. In 22 of the 97 (22.7%) patients, residual stones were found after treatment for hepatolithiasis. The incidence of residual stones was 0% in hepatectomy patients, 48.6% in cholangioenterostomy patients ($p<0.001$, compared with hepatectomy), 25.0% in T-tube drainage patients ($p=0.015$, compared with hepatectomy), and 10.0% in percutaneous transhepatic cholangioscopic lithotripsy (PTCSL) patients. In 15 of the 66 (22.7%) patients who were treated for hepatolithiasis, recurrent stones were found after intervals of 5 to 24 years. The incidence of recurrent stones was 13.9% in hepatectomy patients, 28.5% in cholangioenterostomy patients, 25.0% in T-tube drainage patients, and 50.0% in PTCSL patients ($p=0.021$, compared with hepatectomy). Hepatectomy appears to be the most effective treatment for selected patients with isolated left hepatolithiasis (L). In PTCSL procedures, favorable results have been obtained when the stones were completely cleared; however, the incidence of recurrent stones is high in patients after PTCSL.

Keywords Hepatolithiasis · Residual stones · Recurrent stones · Percutaneous transhepatic cholangioscopic lithotripsy (PTCSL) · Cholangiocarcinoma

Introduction

Hepatolithiasis is frequently found in patients with recurrent pyogenic cholangitis. It is a common disease in Southeast Asia, and the relative incidence of hepatolithiasis is 20% in China and Taiwan.[1,2] However, our study in Japan investigated 105,062 patients with cholelithiasis between 1989 and 1992; we found that 2,353 of these patients (2.24%) had hepatolithiasis.[3] The natural history is characterized by a progression of recurrent attacks of biliary sepsis. Hepatolithiasis can subsequently result in biliary cirrhosis and even cholangiocarcinoma. Resection of the liver lobe that contains strictures, atrophy, and multisegmental distribution of the stones has been effective in reducing residual stones or a recurrence. Furthermore, noninvasive treatments such as percutaneous transhepatic cholangioscopic lithotripsy (PTCSL)[4] and peroral cholangioscopy[5] have been established as effective.

However, postoperative residual and recurrent stones occurred in 20% of patients treated by hepatectomy, PTCSL, or other surgical methods.[1] To decrease the rate of residual and recurrent stones, it is necessary to accurately diagnose the complex pathology of hepatolithiasis and select the most effective treatment for each type of hepatolithiasis. A retrospective study was undertaken to analyze the rate of residual stones and complications of invasive and noninvasive treatments and procedures, as well as the long-term outcome including stone recurrence for these patients.

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Patients and Methods

Between January 1971 and June 2006, 2,660 patients with cholelithiasis were admitted to Wakayama Medical University Hospital (WMUH). Of these, cholecystolithiasis accounted for 79.2% of the cases, whereas hepatolithiasis accounted for only 97 patients (3.6%, 46 men and 50 women). In patients with hepatolithiasis, the ages ranged from 20 to 72 years with a mean age of 57 years. Forty-four (45%) of the patients had previously undergone one or more surgical procedures. We classified the 97 patients with hepatolithiasis by the lobe localization: L type: left-lobe type, R type: right-lobe type, and LR type: bilateral.

All patients underwent surgical or nonsurgical intervention, and the presence of hepatolithiasis was confirmed. In terms of the treatment procedures, 38 patients (39%) underwent hepatectomy, 37 patients (38%) underwent cholangioenterostomy (choledochojejunostomy or choledochoduodenostomy), 12 patients (12%) underwent T-tube insertion, and 10 patients (10%) underwent PTCSL. Intraoperative cholangioscopy (CHF-P20; external diameter, 4.9 mm: Olympus, Tokyo, Japan) was routinely used instead of intraoperative cholangiography for visualizing the residual stones, ductal strictures, and tumors. Postoperative cholangiography and cholangioscopy were routinely performed to detect residual stones. The indications for hepatectomy were as follows: stones localized in unilateral lobe, bile duct stricture associated with stones, atrophy of the affected liver segments or lobe, presence of liver abscess, and cholangiocarcinoma found or suspected clinically.

In cases treated by PTCSL, cholangioscopy was inserted through the percutaneous transhepatic cholangiodrainage fistula orifice, where grasping forceps could be inserted through the cholangioscopy to remove any stones. Giant and impacted stones were fragmented by introducing an electrohydraulic shock wave lithotripter probe or pulsed dye laser before 1997, and holmium YAG (Ho:YAG) lasers after 1998. We established the following treatment conditions: 0.8 J, 20 Hz, and 16 W.[6] Under these conditions, 1-cm stones can be pulverized in 10 s. Board-shaped stones can be sufficiently pulverized without inducing hemorrhage from the bile duct wall. In combination with other treatments such as PTCSL, extracorporeal shock wave lithotripsy (ESWL) was performed on patients with intractable hepatolithiasis, such as LR types, in whom hepatectomy would not be sufficient for complete remission. We analyzed chronological changes in treatment methods for patients with hepatolithiasis and investigated the most appropriate treatments for each disease type. Computed tomography (CT) or ultrasonography (US) follow-up was conducted every year, or whenever the patients presented with symptoms suggestive of cholangitis, to search for stone recurrence. Retrograde cholangiopancreatography, percutaneous transhepatic cholangiography, or magnetic resonance was performed to verify stone recurrence.

All data are expressed as mean±standard deviation. Statistical analysis was performed with a chi-square test and Student's *t* test. Probability differences of 0.05 or less were considered significant.

Table 1 Treatment Modalities for Hepatolithiasis According to the Location of Stones

Treatment		Stone Location		
		L	LR	R
Hepatectomy	(n=38)	33	3	2
Cholangioenterostomy	(n=37)	4	25	8
T-tube drainage	(n=12)	2	7	3
PTCSL	(n=10)	0	9	1
Total	(n=97)	39	44	14

Data in parentheses indicate the number of patients with residual stones.

L Left intrahepatic duct type, *LR* bilateral intrahepatic duct type, *R* right intrahepatic duct type, *PTCSL* percutaneous transhepatic cholangioscopic lithotripsy

Results

Table 1 shows the treatment for the three different L/R types. Hepatectomy was performed more frequently on patients with L-type hepatolithiasis (n=33 vs n=5). All lateral segmentectomies (n=18) were performed on patients with L-type hepatolithiasis, and left lobectomy was performed on those with L (n=12) or LR (n=3) type. Right lobectomy (n=2) was performed on patients with R-type

Table 2 Residual Stone Location after Treatment for Hepatolithiasis

Treatment	No. (%) of Patients with Residual Stone	Residual Stone Location		
		L	LR	R
Hepatectomy	0/38 (0)[a]	–	–	–
Cholangioenterostomy	18/37 (48.6)	4	9	5
T-tube drainage	3/12 (25.0)	1	1	1
PTCSL	1/10 (10.0)	0	0	1
	22/97 (22.7)	5	10	7

Data in parentheses indicate the rate of residual stones.

L Left intrahepatic duct, *LR* bilateral intrahepatic duct, *R* right intrahepatic duct, *PTCSL* percutaneous transhepatic cholangioscopic lithotripsy

[a] $p<0.001$ compared with cholangioenterostomy, $p=0.015$ compared with T-tube drainage

Table 3 Morbidity and Mortality after Treatment for Hepatolithiasis

	Hepatectomy (n=38)	Cholangioenterostomy (n=37)	T-Tube Drainage (n=12)	PTCSL (n=10)
Death	0	0	0	0
Liver failure	1	0	0	0
Cholangitis	0	2	0	3
Disturbance of liver function	4	3	3	2
Bile leakage	3	2	2	0
Wound infection	2	3	2	0
Intraabdominal abscess	2	1	0	0
Ileus	0	2	0	0
Pneumonia	1	0	0	0
Others	1[a]	1[b]	1[c]	0
Patients with complications, No.(%)	9 (23.7%)	9 (24.3%)	5 (41.7%)	4 (40.0%)

[a] Intraabdominal bleeding
[b] Acute gastric mucosal lesion
[c] Acute pancreatitis

hepatolithiasis. Cholangioenterostomy or PTCS was performed more frequently for R- or LR-type hepatolithiasis than L-type hepatolithiasis (n=43 vs n=4).

The rate of residual stones and the location of residual stones immediately after treatment are summarized in Table 2. The immediate stone clearance rate after hepatectomy was 100%, which was confirmed by cholangiography and cholangioscopy postoperatively. The rate of residual stones after hepatectomy was lower than after cholangioenterostomy (48.6%; p<0.001) or T-tube drainage (25.0%; p=0.015).

There were no surgical deaths among the 97 patients (Table 3). Immediate common complications after procedures included a disturbance of liver function (n=12), bile leakage (n=7), and wound infection (n=7). There was no significant difference of morbidity among these groups. Among the 38 patients who received hepatectomy for hepatolithiasis, 3 patients (7.9%) had coexisting cholangiocarcinoma: one diagnosed intraoperatively, and two incidentally discovered on a pathologic examination.

During a median follow-up of 108 months (range 62–288 months), 15 of the 66 patients (22.7%) who had no residual stones had developed recurrent stones as confirmed by CT scan or US (Table 4). The rate of recurrent stones after hepatectomy was lower than after a treatment of PTCSL (13.9 vs 50.0%, p=0.022). No patient who underwent hepatectomy had recurrent stones until after 5 years postoperatively, whereas all recurrent stones in other treatments occurred within an interval of 5 years. One patient developed an intrahepatic cholangiocarcinoma 17 months after PTCSL and received extended left hepatectomy. She died of tumor recurrence 2 years after the second operation (Table 4).

Four patients were recognized as having a stone recurrence after lateral segmentectomy (segment 2+3), including the biliary tree of segment 4 (three patients) and segment 6 (one patient), whereas no patients developed recurrent stones after a left hepatectomy (p=0.045) (Table 5). In addition to the three patients with coexisting cholangiocarcinoma at the time of hepatectomy, one patient died of tumor recurrence 18 months after a left hepatectomy, and two patients have been alive for more than 7 years. During a follow-up of 12 years, one patient who had received a right

Table 4 Stone Recurrence after Treatment for Hepatolithiasis

Treatment	No. of Patients with Stone Recurrence/ No. of Follow-up (Over 5 Years) Patients	Recurrence Interval After Treatment				
		~1 year	~2 years	5 years	~10 years	Over 10 years
Hepatectomy	5/36 (13.9)*	0	0	1	3	1
Cholangioenterostomy	4/14 (28.5)	1	2	1	0	0
T-Tube Drainage	2/8 (25.0)	0	1	1	0	0
PTCSL	4/8 (50.0)	2	1[a]	1	0	0
	15/66 (22.7)	3	4	3	3	1

Data in parentheses indicates the stone recurrence rate
*p=0.021 compared with PTCSL
[a] Complicate with cholangiocarcinoma

Table 5 Location of Stone Recurrence after Hepatectomy

Method of Hepatectomy	No. of Follow-up Patients	No. of Patients with Stone Recurrence	Location of Stone Recurrence
Lateral Segmentectomy	18	4*	Segment 4 (Three Patients) Segment 6 (One Patient)[a]
Left Hepatectomy	16	0	–
Right Hepatectomy	2	1	Segment 4
	36	5	

*p=0.045 compared with left hepatectomy

[a] Segment 6 was drained into the left main hepatic duct.

hepatectomy was diagnosed as having a cholangiocarcinoma and died of carcinomatous peritonitis.

Discussion

Hepatectomy is the most effective treatment for hepatolithiasis and removes not only all of the hepatic stones but also the associated pathological bile ducts including stricture, fibrosis, abscess, and carcinomatous bile ducts,[5] thus reducing the risk of recurrent intrahepatic stones. However, the indication of hepatectomy should be strictly considered. Indeed, in our hospital, this was relevant in only 40% of the patients in whom the stones were localized in the unilateral lobe.[6] Hepatectomy is most often indicated for the treatment of L-type hepatolithiasis, and lateral segment resection or left-lobe resection is performed to remove intrahepatic stones and pathologic bile ducts and is rarely indicated for patients with R-type hepatolithiasis.[7] Our data showed that left hepatic resection was performed more frequently than right hepatic lobectomy. The incidence of L-type hepatolithiasis is increasing in Japan, and hepatectomy now accounts for more than half of the surgical treatments performed for L-type hepatolithiasis.[6] Three patients who were treated by lateral segmentectomy required retreatment for repeated cholangitis or obstructive jaundice because of a stone recurrence in the left main branch or medial segment. In the 16 patients who underwent a left hepatectomy, the long-term results of the left hepatectomy were better than those of left lateral segmentectomy (segment 2+3), with lower rates of stone recurrence and stricture in the long-term follow-up.

With the recent advance of endoscopic and radiological intervention, PTCSL has become a well-established mode of treatment.[8] An attempt should be made to perform PTCS before other procedures to ascertain: the condition of the intrahepatic biliary tract, the location of stones, and the location and severity of biliary stricture or dilatation.[9] One of the reasons that multiple surgeries for patients with hepatolithiasis are often performed is because of an inappropriate initial treatment. PTCSL treatment is always recommended for patients with intrac hepatolithiasis, i.e., LR or R type, whereas hepatectomy is not indicated or recommended for patients who display liver atrophy or severe stricture of bile duct because cholangiocarcinoma rarely occurs.[8,9]

Residual stones are the most troublesome problem after treatment for hepatolithiasis.[9] The incidence of residual stones has been markedly reduced from 62.3 to 19.8%.[1,3,10] In our study, the residual stone rates after hepatectomy and PTCS for hepatolithiasis were 0 and 10%, respectively. Cholangiojejunostomy was one of the major treatment procedures for hepatolithiasis before 1985 in WMUH, and the rate of residual stones was 48.6%. However, after 1986, hepatectomy and noninvasive treatments including PTCSL have been performed more frequently, and the rate of residual stones has decreased to 2.1% after these treatments. Chen et al.[7] reported an only 9% recurrent stone rate after hepatectomy, and Jan et al.[11] reported stone recurrence rates after complete stone clearance for hepatolithiasis by hepatectomy and PTCSL of 9.5 and 36.4%, respectively. In another report of 19 patients who underwent complete lithotomy, calculi recurred in 4 (21%) patients, 3 of which recurred less than 1 year after PTCSL.[12] Huang et al.[4] reported a recurrence rate for hepatolithiasis, cholangitis, or both for 59% of patients after a successful PTCSL.[4] The rate of recurrent stones has been higher after PTCSL than after hepatectomy, the reason being that the structure of the bile duct remains unchanged even when initial therapy is successful in completely eliminating stones.[13]

Conclusion

Our results support the notion that hepatectomy should be considered when the stones are located in strictured bile ducts, especially within the unilateral lobe. Therefore, we recommend that hepatectomy should be considered when stones are localized in the unilateral lobe after PTCSL because we consider PTCSL to be a difficult treatment as a radical therapy for primary hepatolithiasis.

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Actual Recurrence Patterns and Risk Factors Influencing Recurrence After Curative Resection with Stage II Gallbladder Carcinoma

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Abstract Despite the advances in imaging techniques, most patients can only be diagnosed at advanced stage: The prognosis is very poor. Recent studies showed that aggressive radical resection for advanced gallbladder carcinoma can give an acceptable prognosis. However, recurrence frequently remains the main problem after curative resection of advanced gallbladder carcinoma. The aim of this study was to identify the patterns and risk factors of recurrence after curative resection for stage II gallbladder carcinoma. Between January 1991 and December 2003, 100 patients received radical curative resection for gallbladder carcinoma at Yonsei University Medical Center. Of these, 77 were defined with stage II gallbladder carcinoma according to the Union Internationale Contre Le Cancer classification (sixth edition). Of the 77 patients, 67 were reviewed for the predictors of tumor recurrence. Among the 67 patients, 38 (56.7%) suffered a recurrence. The mean length to the recurrence was 21.1±26.7 months, with the most common site being the intraabdominal organs: liver and aortocaval lymph nodes. Infiltrating and poorly differentiated types were identified as independent prognostic factors of recurrence after curative resection for stage II gallbladder carcinoma and it suggests that large multicenter randomized control trials are necessary to clarify the role of adjuvant chemotherapy in these patients.

Keywords Gallbladder · Carcinoma · Recurrence

Introduction

Gallbladder carcinoma is characterized by its aggressiveness in the course of disease. Despite the advances made in hepatobiliary imaging techniques, most patients can only be diagnosed at advanced stage: The prognosis is very poor. Nonetheless, recent studies showed that aggressive radical resection for advanced stage gallbladder carcinoma could give acceptable prognosis.[1–4]

Union Internationale Contre Le Cancer (UICC) classification (sixth edition) defines stage II as locally advanced and resectable, and stage III as locally unresectable. Many surgeons are trying to improve the clinical outcomes by aggressive radical resection for gallbladder carcinoma in stage II. But, tumor recurrence frequently becomes the main problem after curative resection of advanced gallbladder carcinoma and recurrences are often found in various forms or at more than one site simultaneously. Therefore, it is difficult to confirm the patterns of recurrence and to evaluate the risk factors of recurrence. There are many published studies that have investigated the clinicopathological aspect of gallbladder carcinoma, but most reports have focused only on prognosis of gallbladder carcinoma.[1–6]

The aim of this study is to identify the patterns of recurrence and factors affecting the recurrence after curative resection of gallbladder carcinoma at stage II.

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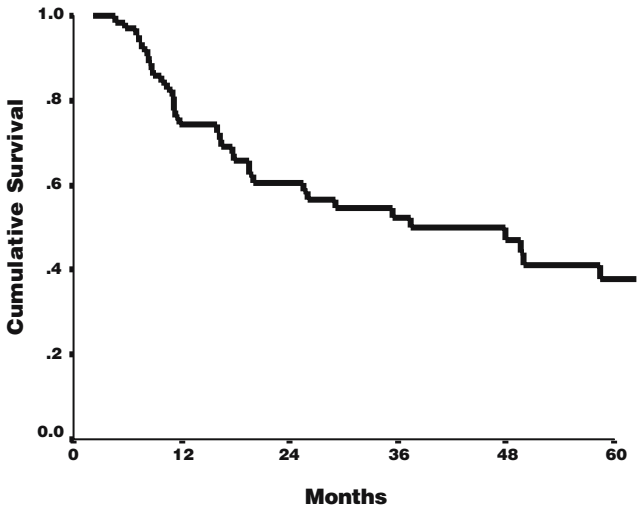

Figure 1 Cumulative survival rates in stage II gallbladder carcinoma.

Material and Method

Patients

Between January 1991 and December 2003, 100 patients received a radical resection for gallbladder carcinoma at Yonsei University Medical Center. Out of this group, Seventy-seven patients were defined as stage II gallbladder carcinoma according to the UICC classification (sixth edition). Ten patients were excluded due to incomplete clinicopathologic data or follow-up loss. As a result, sixty-seven patients who had undergone curative resection were retrospectively reviewed.

Follow-up Program and Judgment of Recurrence

The patients were followed closely until 31 May 2005. The follow-up duration ranged from 4.6 to 151.1 months, and the mean duration was 34.6 months. Routine follow-up program consisted of physical examination and laboratory tests, including estimation of carcinoembryonic antigen (CEA) and CA19-9 levels. The laboratory tests were performed monthly for the first 3 months, then once in 3 months for the next 2 years, and biannually for the following 2 years. Radiological examination was also performed as a part of routine follow-up program: This included chest radiography, abdominopelvic ultrasound, and computed tomography (CT). Abdominopelvic CT and whole body bone scan were performed biannually, beginning in the fifth week after the operation. Tumor recurrences were confirmed by radiological imaging techniques and pathological study of histological specimens wherein the majority were being made by radiological evaluation, including CT and ultrasonography.

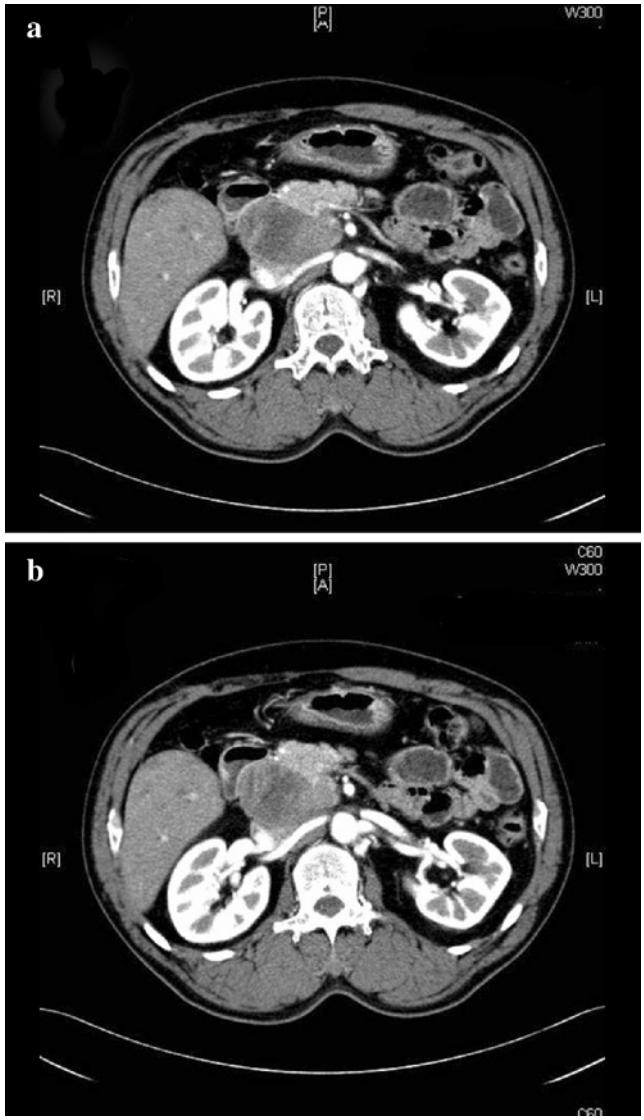

Figure 2 Imaging studies of a patient with aortocaval lymph node recurrence with increased serum CA 19-9 level. **a**, **b** Axial CT scans showing a huge aortocaval lymph node metastasis.

Table 1 Patterns of Recurrence in 38 Patients After Curative Resection

Site	No. of Patients
Intraabdominal recurrence	
Liver	14
Aortacaval lymph node	18
Trocar site	1
Peritoneal seeding	1
Extraabdominal recurrence	
Lung	1
Bone	1
Combined recurrence	
Liver+bone	1
Liver+aortocaval lymph node	1
Total	38

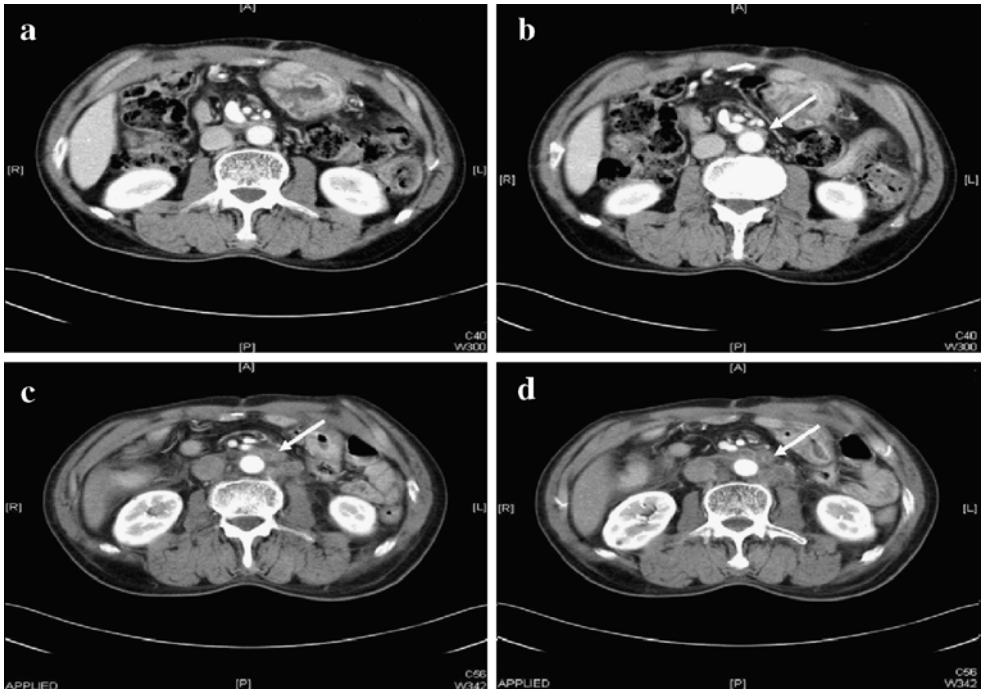

Figure 3 Imaging studies of a patient with aortocaval lymph node metastasis with normal initial serum CA 19-9 level, which eventually increased on the follow-up. **a**, **b** First CT scan showing questionable aortocaval lymph node recurrence with normal tumor marker level. **c**, **d** Three-month follow-up CT scan showing definite aortocaval lymph node recurrence with increased tumor marker level.

Statistical Analysis

Correlation of the variables was analyzed using Student *t*-test, chi-square test, and Fisher's exact test (SPSS Windows 11.0). Recurrence rate curves were constructed and compared by the Kaplan–Meier technique and the log-rank test, respectively. *P* values less than 0.05 were considered as statistically significant.

Results

Patients Characteristics

The total number of patients who received curative surgery for gallbladder carcinoma with stage II was 67 patients. Among 67 patients, 12 patients underwent laparoscopic cholecystectomy as initial operation and then according to

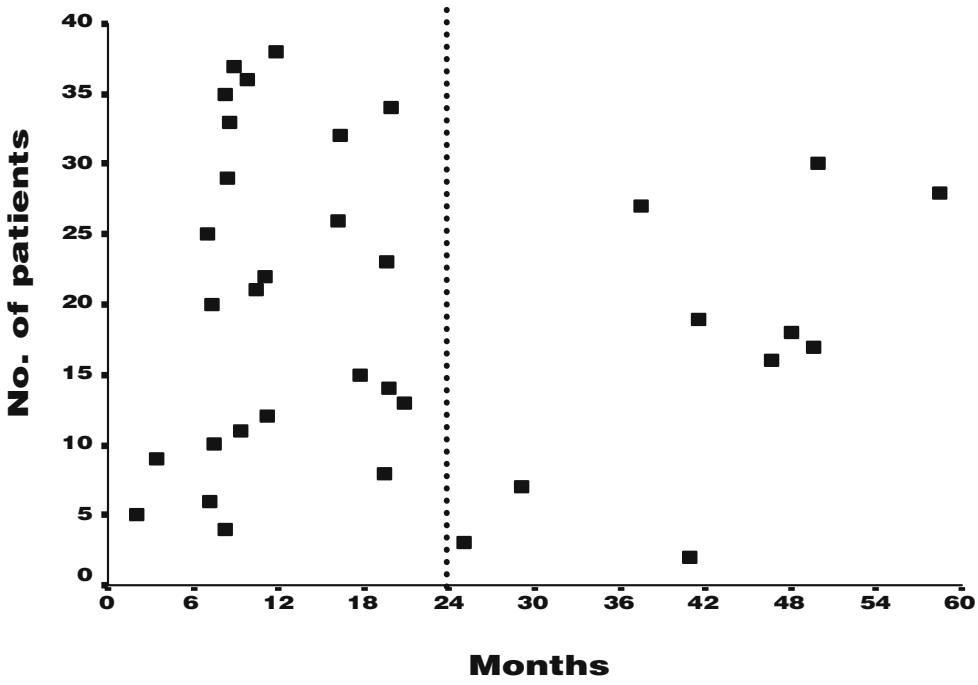

Figure 4 Time to recurrence after curative resection in 38 patients with stage II gallbladder carcinoma.

Table 2 Clinicopathological Features According to Recurrence Time

Variables	No. of Patients	Recurrence Time (months)		*P* Value
		Early (≤24 months)	Late (>24 months)	
Age (year)				0.031
≥60	22	12	10	
<60	16	14	2	
Sex				0.970
M	16	11	5	
F	22	15	7	
Size (cm)				0.652
≥4	8	6	2	
<4	30	20	10	
CA19-9 (U/ml)				0.305
≥40	9	7	2	
<40	9	4	0	
Site				0.169
Fundus/body	22	17	5	
Neck/whole	16	9	7	
Morphology				0.253
Infiltrating	24	18	6	
Nodular and papillary	14	8	6	
Differentiation				0.003
Well to mod differentiation	25	13	12	
Poor differentiation	13	13	0	
Invasion depth				0.850
T2	7	5	2	
T3	31	21	10	
Lymph node metastasis				0.510
Positive	25	18	7	
Negative	13	8	5	

the pathologic results, radical reoperation was performed. Ten patients underwent radical cholecystectomy with resection of liver bed; 36 patients underwent radical cholecystectomy with en bloc resection of liver segment 4b+5 without common bile duct (CBD) resection; 15 patients underwent en bloc resection of liver segment 4b+5 with CBD resection; 3 patients underwent radical cholecystectomy with resection of liver bed with pancreaticoduodenectomy. One patient underwent hepaticopancreaticoduodenectomy and two patients underwent radical cholecystectomy with CBD resection and Rt. hemicolectomy. All patients underwent dissection of the lymph nodes in the hepatoduodenal ligament, common hepatic artery, the celiac axis, and the posterior pancreatoduodenal nodes. According to the UICC classification (sixth edition), 67 patients were classified into 27 patients of stage IIa tumor (40.3%) and 40 patients of stage IIb tumor (59.7%).

Overall Survival Rate

Among the 67 patients who received radical resection for gallbladder carcinoma with stage II, the 3- and 5-year overall survival rates were 52.3 and 37.8%, respectively (Fig. 1).

Patterns of Recurrence

Among 67 patients, 38 patients (56.7%) had tumor recurrence confirmed by pathological study (n=6) or by

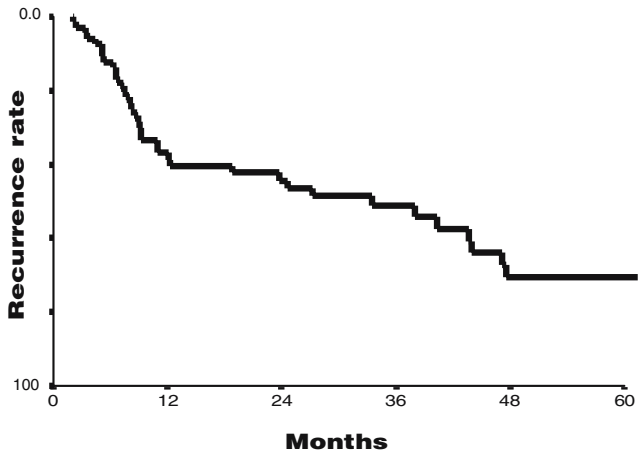

Figure 5 Cumulative recurrence rate in stage II gallbladder carcinoma.

Table 3 Recurrence Rate According to Prognostic Factors

Variables	No. of Patients	Recurrence Rates (%)		*P* Value
		1 Year	3 Years	
Age (year)				0.646
≥60	36	35.5	51.5	
<60	31	38.8	50.3	
Sex				0.185
M	22	52.7	64.5	
F	45	28.9	55.5	
Size (cm)				0.721
≥4	15	43.5	43.5	
<4	52	35.1	52.7	
CA19-9 (U/ml)				0.069
≥40	12	53.1	62.5	
<40	12	28.7	38.9	
Site				0.399
Fundus/body	46	33.6	48.6	
Neck/whole	21	42.9	55.0	
Morphology				0.026
Infiltrating	33	54.6	62.4	
Nodular and papillary	34	29.4	40.6	
Differentiation				0.027
Well to mod differentiation	47	27.3	42.3	
Poor differentiation	20	60.4	72.8	
Invasion depth				0.998
T2	13	35.4	50.6	
T3	54	41.7	53.3	
Lymph node metastasis				0.071
Positive	40	49.4	62.0	
Negative	27	18.4	36.2	

radiological examination (n=32). The average age of these 38 patients was 60.2 years (±10.0 years) and consisted of 16 men and 22 women.

The main patterns of recurrence in 38 patients are shown in Table 1. Thirty-six patients had only one recurrence site, one of them having multiple peritoneal seeding at the time of diagnosis and two of them each had solitary extra-abdominal recurrence at lung and bone without intra-abdominal recurrence. The other two patients (5.2%) had combined recurrence sites.

Aortocaval lymph nodes recurrence (47.4%) was observed most frequently (Figs. 2 and 3), followed by liver recurrence (36.8%).

Among locoregional recurrences, one patient had recurrence in umbilical port site on the 44 months after initial laparoscopic cholecystectomy. The patient survived for 12 months without any recurrence after wide excision of umbilical port site.

Length of Time to Recurrence and Factors Influencing Recurrence

The distribution of the interval from curative resection to the diagnosis of recurrence in recurred patients is as shown in Fig. 4. The mean length of time to recurrence was 21.1 months (±26.7 months). The patients were divided into two groups: early recurrence (≤24 months) and late recurrence (>24 months). In the early recurrence group, young age (<60 years) and poorly differentiated tumor were common in comparison with the late recurrence group (Table 2).

The 1- and 3-year recurrence rates in stage II gallbladder carcinoma were 36.9 and 51.2%, respectively (Fig. 5).

In univariate analysis, infiltrating type and poor differentiation had statistically significant factors as recurrence in stage II gallbladder carcinoma (Table 3). In a multivariate analysis, poor differentiation and infiltrating type were identified as independent prognostic factors of recurrence (Table 4).

Table 4 Risk Factors Influencing Recurrence of Stage II Gallbladder Carcinoma: Multivariate Analysis

Variables	*P* Value	Odds Ratio	Confidence Interval (95%)	
			Lower	Upper
Infiltrating type	0.034	0.447	0.913	1.064
Poor differentiation	0.028	2.289	1.095	4.355

Discussion

Gallbladder carcinoma is an aggressive malignancy. Despite the advances made in radiologic imaging techniques, most patients are diagnosed at advanced stage and the prognosis is very poor. Recent studies showed that aggressive surgical resection for advanced stage carcinoma can improve the prognosis.[1–4] Like other malignancies, tumor recurrence after radical resection for gallbladder carcinoma eventually leads to death. There are very few studies focused on the recurrence of gallbladder carcinoma after curative resection, despite its great impact on patient's outcome.

In this study, the analysis of the recurrence patterns was based on the clinical course or radiological examination, and there may be some underestimation of the exact sites of recurrence.

Liver metastasis and trocar site metastasis were confirmed easily by biopsy. However, as histological confirmation of aortocaval lymph node recurrence is difficult and entails morbidity for the patients, we followed guidelines to determine lymph node recurrence. This was detection of newly developed lymph nodes on follow-up CT that were not present before with increase in tumor marker levels, or not increasing tumor markers with enlarged lymph nodes as demonstrated by short-term follow-up CT.

But, our studies give some important suggestions about stage II gallbladder carcinoma recurrence. This study showed that intraabdominal recurrence was the most common pattern in the recurrence, and also that extraabdominal recurrence without intraabdominal recurrence was rare. Aramaki et al.[7] also reported that the most common site of recurrence was the intraabdominal organs, such as liver and aortocaval lymph nodes. Our study demonstrated that aortocaval lymph node metastasis was the most common site of recurrence in intraabdominal recurrence. We believed that it is necessary to determine the importance of paraaortic lymph node dissection in the prognosis of patients with gallbladder carcinoma at stage II.

Liver metastasis was the second common site of recurrence after curative resection with stage II gallbladder carcinoma. Therefore, postoperative adjuvant chemotherapy for prevention of liver metastasis should be evaluated using multiinstitutional prospective randomized study.

The rate of port site recurrence in laparoscopic cholecystectomy for gallbladder carcinoma is unexpectedly high.[8–10] In our data, we experienced one case with port site recurrence among 12 patients who underwent initial laparoscopic cholecystectomy. Thus, we suggest that port sites should be excised when radical reoperation is performed.

It is important to understand the timing and influencing factors of recurrence after curative resection for stage II gallbladder carcinoma; these factors are important in making the decision for therapeutic modalities. In this study, although the precise of timing and influencing factors of recurrence were available in a limited number of patients, it gives some important suggestions. The results of this study confirm that recurrence after curative resection for gallbladder carcinoma occurred mostly within the first 2 years after operation in patients with gallbladder carcinoma at stage II. This study also showed that young age group (<60 years) and poorly differentiated tumor were common in the early recurrence group. Thus, we suggest that short-term follow-up evaluation with strictness is recommended in patients with young age group (<60 years) and poorly differentiated tumor.

According to our results, the size of tumor and tumor infiltration (T stage) were statistically insignificant. The lymph node metastasis seemed to have marginal significance with a P value of 0.0761, but is insignificant to be considered as a main factor. Although some studies have reported that lymph node metastasis was an important indicator on prognosis, we found that nodal metastasis was a minimal relationship to recurrence.[3,11–13] It may be explained by the fact that our study was only focused on same stage patients who underwent complete dissection of regional lymph node, so lymph node metastasis was not a significant factor on recurrence.

In this study, poor differentiation and infiltrating type were independent factors predicting tumor recurrence with stage II gallbladder carcinoma on multivariate analysis. These factors are important in predicting the possibility of recurrence in stage II gallbladder carcinoma. Therefore, high probability of recurrence in patients with infiltrating and poorly differentiated tumor support the notion that effective postoperative adjuvant modality should be developed for these patients.

Conclusions

Our study gives some important suggestions relevant to the management for stage II gall bladder carcinoma. First, aortocaval lymph node and liver were the most common site of recurrence after curative resection of stage II gall bladder carcinoma; this calls for further prospective study on the necessity of aortocaval lymph node dissection and postoperative adjuvant chemotherapy or chemoradiotherapy.

Second, young age group (<60 years) and poor differentiation tumor were common in the early recurrence group. So, young age group and poor differentiation tumor indicated the need for a close-up follow-up program.

Third, infiltrating type and poor differentiation tumors were independent factors predicting recurrence with stage II

gall bladder carcinoma. So, it suggests that large multicenter randomized control trials are necessary to clarify the role of adjuvant chemotherapy in infiltrating and poor differentiation tumors.

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48-Hour pH Monitoring Increases the Risk of False Positive Studies When the Capsule is Prematurely Passed

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Abstract Ambulatory wireless 48-h esophageal pH monitoring (Bravo Medtronic, Shoreview, MN, USA) has been shown to be more sensitive in detecting abnormal esophageal acid exposure compared with transnasal 24-h pH probes. However, accurate interpretation of the wireless monitoring data is paramount when contemplating surgical intervention for those with gastroesophageal reflux disease. The aim of this study is to evaluate the incidence of false-positive interpretations of this wireless monitoring data secondary to premature transit of the Bravo capsule into the stomach and subsequently into the duodenum prior to the completion of the 48-h study period. We reviewed 100 consecutive Bravo pH studies at our University Esophageal Motility Center. There were 58 women and 42 men included in our evaluation. Premature transit of the Bravo capsule into the stomach and subsequently into the small bowel was defined by a prolonged gastric pH phase with either evidence of alkalinization and no further reflux episodes or loss of communication with the Bravo capsule prior to the end of the 48-h data collection period. Of the 100 patients reviewed, 11% manifested evidence of early passage of the Bravo capsule resulting in a misinterpretation of the data as abnormal acid exposure. The mean time of inaccurate data after transit of the Bravo capsule was 18 h and 42 min. The mean length of time that the capsule was retained in the stomach prior to duodenal passage was 4 h. If the aforementioned data were included in the final interpretation of the study, it yielded a mean DeMeester score of 44.25 with a mean total time of pH <4 of 14.7% per case. Exclusion of the prolonged gastric phase from the final interpretation of each case resulted in a statistically significant reduction in the mean total time the pH <4 (4.33 vs. 14.7%, $p<0.05$) and the mean DeMeester score (12.81 vs. 44.25 $p<0.05$). The mean time from the initiation of esophageal pH data to the passage of the Bravo capsule into the stomach was 15 h and 22 min. The observation mandates meticulous inspection of the pH tracing by the interpreting physician throughout the entirety of a 48-h study to identify premature transit of the capsule. Tracings that show prolonged acid exposure or loss of communication with the Bravo capsule should be screened for the capsule's possible early dislodgement and premature advancement into the stomach.

Keywords Gastroesophageal reflux disease · Bravo pH monitoring · 24-h pH monitoring

Introduction

Esophageal pH recording provides quantitative data on both esophageal acid exposure and on the correlation between patient's symptoms and reflux events. Despite these strengths, the inherent weakness of the technique is its inability to prove causality between symptoms and acid reflux.[1,2,5] Alternatively, causality is reasonably assumed in clinical practice by the alleviation of suspected reflux during a therapeutic trial of proton pump inhibitor (PPI). In view of this alternative, the American Gastrointestinal Association (in 2001) concluded that the major indications for esophageal pH monitoring include the documentation

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Table 1 From the Initiation of Esophageal pH Data to the Passage of the Bravo Capsule into the Stomach

	Normal Tracing	Passed Probe	*p*
Patients	89	11	
Total time			0.001
pH <4	4.33	14.7%	
DeMeester score	12.81	44.25	0.008

of abnormal esophageal acid exposure in an endoscopy-normal patient who is being considered for antireflux surgery, evaluation of patients with refractory symptoms to PPI therapy and/or surgical therapies, evaluation of patients with noncardiac chest pain, and extraesophageal manifestation of GERD (laryngitis, asthma and chronic cough).[1,2,7] Ambulatory pH testing is especially useful for confirming the presence of GERD in the above-mentioned patient group. The conventional ambulatory 24-h pH monitoring requires the transnasal introduction of the catheter with the pH sensors into the esophagus. Frequently, this method produces discomfort and inconvenience and leads most patients to modify their daily activities.[2–5]

Bravo is a catheter-free pH monitoring system and is designed to minimize the discomfort associated with transnasal catheters. It also allows the patients to enjoy their regular diet and activities without the embarrassment and discomfort associated with traditional pH monitoring. The Bravo pH monitoring system (Medtronic, Minneapolis, MN, USA) uses a radiotelemetric capsule temporarily attached to the esophageal mucosa, which transmits pH data to a receiver carried on the patient's belt. Although effective in measuring esophageal exposure to acid, validation of the equipment by simultaneous measurement with the commonly used catheter-based system has been lacking.[1,2] Outcomes of the study results, however, depend on the accuracy of performance and interpretation of the data obtained. Standardization of the methodology, however, has enhanced the reliability and reproducibility of the technique, and esophageal pH monitoring is widely performed in both academic and community settings. Because the catheter is not externally tethered, accidental slippage is hard to determine clinically. Early passage may alter the data recovered from the Bravo device and complicate the final interpretation. Either false-positive or false-negative results may be encountered, depending on the acidity of the stomach and the duration of capsule stay in the gastric phase. The aim of our study is to determine the incidence of early Bravo capsule passage and the effects on the interpretation of esophageal acid exposure of such a study.

Methods and Materials

The records of consecutive patients with GERD symptoms and undergoing Bravo pH monitoring over 1-year period at our university setting were reviewed. Patients with past surgical history of the upper GI tract, bleeding diathesis or coagulopathy, esophageal strictures, severe gastrointestinal bleeding in the past 3 months, and advanced cirrhosis or significant comorbidities were excluded. Patients were instructed to stop taking PPI 7 days prior to the study.

All patients underwent upper endoscopy to measure the distance between the squamocolumnar junction and the incisors. The test results included the percentage acid exposure (total upright and supine) and the duration and number of reflux episodes. After the completion of endoscopy, the self-contained Bravo (Medtronic) delivery system was passed transorally into the patient's esophagus. The delivery system was positioned 6 cm above the squamocolumner junction. Suction was then applied with a vaccum pump through the suction channel to the catheter for 30 s (suction pressure >500 mm Hg), causing the adjacent mucosa to be drawn into the well of the capsule. Subsequently, the activation button of the delivery system was depressed, releasing an attachment pin that was driven through the well, thus attaching the capsule to esophageal mucosa. The activation button was twisted clockwise (90°) and re-extended, resulting in the release of the delivery system from the capsule. The endoscope was than reinserted in all cases to allow visual inspection of the attachment site. Care was taken not to dislodge the capsule with the endoscope.

The capsule was activated with a magnetic switch before application so that data collection began as soon as the capsule was in place. pH data were obtained at 6-s intervals and transmitted via radiotelemetry to a small, pager-sized receiver worn by the patient. Patients were encouraged to go about their usual activities, including work and exercise.

Table 2 Early Passage of the Bravo Capsule

Event	Time of pH Tracing (out of 48 h)
Start point of early detachment and initiation of incorrect recording	13 h and 40 min (range 0.40–35 h)
Period of probe located in the stomach (incorrect acid pH recording)	3 h and 11 min (range 2.10–5.50 h)
Period of probe located in duodenum and small bowel (incorrect alkaline pH recording)	15 h and 21 min (range 1.40–43.3 h)
Total time of incorrect recording	18 h and 32 min (range 2.5–47.4)

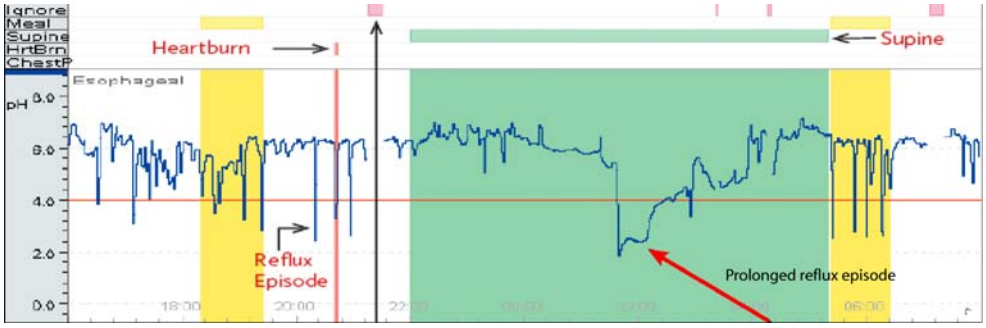

Figure 1 Intraesophageal normal Bravo capsule location in a patient with GERD.

They were also instructed to consume their usual diet without restrictions. While showering, the patients were instructed to place the receiver on the bathroom floor or lavatory so as to keep the receiver as close to them as possible. At the end of the recording period (48-h), the patients returned the receivers and the data were uploaded to a personal computer, analyzed using software provided by Medtronic, and then interpreted by a physician. pH data analyzed include the total time and percentage of time with pH <4.0, the total number of reflux episodes, the number of episodes during which pH was <4 for 5 min or more, the total duration of pH recording, total time and percentage of time upright, supine and postprandial reflux, DeMeester score, and symptom index. During this study, patients also kept a diary recording of food intake, symptoms, and activity, including position changes, and this information was used in the interpretation of the pH data, as during traditional pH testing. The capsule is designed to dislodge from the esophageal mucosa within 7–10 days and subsequently pass through the gastrointestinal tract to be expelled in the stool. Premature transit of the Bravo capsule into stomach and subsequently into the small bowel was defined by the prolonged gastric pH phase with evidence of alkalinization and no further reflux episodes prior to the end of the 48-h data collection period.

Results

One hundred consecutive patients underwent Bravo capsule placement over 12 months. There were 58 women and 42 men (women, mean age 40±14; men, mean age 42±16). Of the 100 patients reviewed, 11% manifested evidence of early passage of the Bravo capsule, resulting in misinterpreting the data as abnormal acid exposure (see Table 2). Exclusion of the prolonged gastric phase from the final interpretation of each case resulted in a statistically significant reduction in the mean total time the pH <4 (4.33 vs. 14.7%, $p<0.05$) and the mean DeMeester score (12.81 vs. 44.25 $p<0.05$). The mean time from the initiation of esophageal pH data to the passage of Bravo capsule into the stomach was 15 h and 22 min (Tables 1 and 2). No false-negative studies were demonstrated, as abnormal pH findings are indicative of premature passage of the capsule into the stomach.

Discussion

This study describes our experience with the Bravo catheter-free esophageal pH monitoring system in the first 100 consecutive patients studied over a 12-month period at our university. No major complications occurred, and the Bravo capsule was well tolerated by the patients.

Since the United States Food and Drug Administration approved the Bravo system as a class 1 pH monitoring system, very few studies have evaluated performance, tolerability, associated symptoms, and accuracy with this novel technology.[2,5,6] Successful placement of the pH capsule ranged between 92 and 100%.[1,2,3] The results after using this technology have failed to focus on the false positives secondary to the slippage of capsule, resulting in the recording of inaccurate data. Our study addresses this issue. One non-US study, by Tu et al.[7] in Taiwan, reported

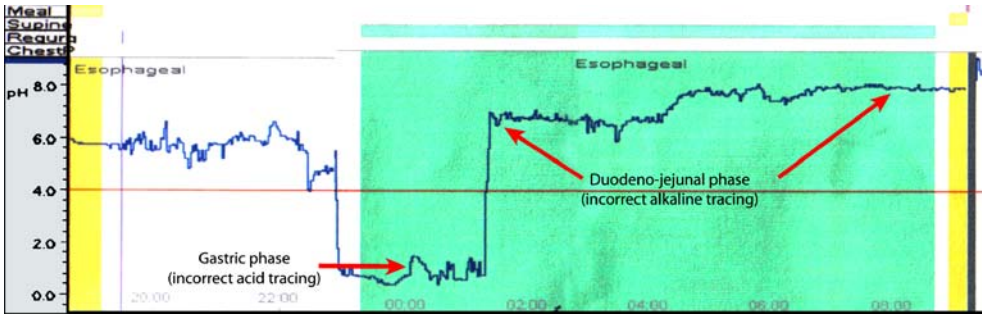

Figure 2 Bravo capsule into the stomach with short gastric phase. Gastric phase with incorrect acid tracing.

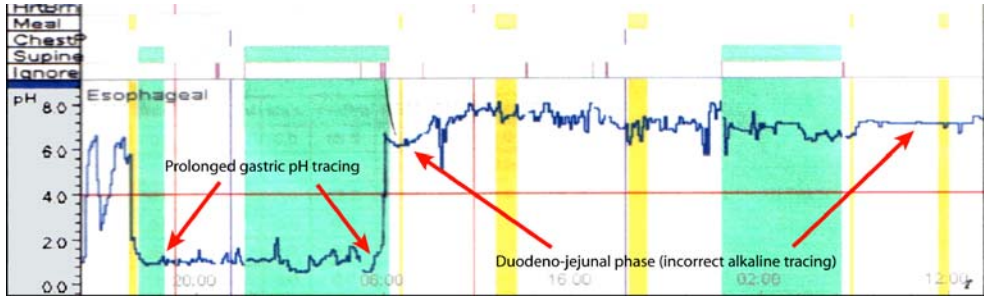

Figure 3 Prolong gastric pH tracing. Duodeno–jejunal phase with incorrect alkaline tracing.

that, compared to the western experience, the wireless pH monitoring system achieved a comparable rate and recording efficacy. We were able to retrieve data for 100% of our subjects, a percentage comparable to those of Ward et al.[1] (92%) and Pandolfino[8] (89%). The studies performed by Pandolfino[8] and Ward et al. concluded that wireless pH monitoring produces significantly less impairment of daily activities and oropharangeal symptoms compared to traditional pH monitoring. However, these studies did not focus on the accuracy of the data influenced by the early detachment of the Bravo capsule.

We did not experience any difficulty at the initial placement of the Bravo capsule. All placements were confirmed with repeat endoscopy. We obtained normal tracing in 89% of our patients, whereas 11% showed inaccurate data due to slippage of the capsule into the stomach and the small bowel prior to the completion of the 48-h study. Difficulty in attachment, however, has been reported as the most common problem, but it can be improved with more operator experience, adequate sedation, and appropriate material lubrication.

Previous studies have reported that a 48-h study detects more abnormalities than a 24-h study alone. Lin et al.[9] reported that 48 h identified 10% additional abnormal studies that were not detected on 24-h analysis. Portale et al.[5] demonstrated that the Bravo system detects a significantly greater percentage of total time, pH <4, and a higher number of reflux episodes in normal subjects when compared to the conventional. These studies do not mention early passage of the Bravo capsule that could contribute to the 10% increase in an abnormal result as per Lin et al.[9]

In one study, Bruely des Varannes et al.[10] found that the Bravo capsule underrecorded acid exposure by nearly 30% compared with the catheter pH system. The difference was explained by the failure of the Bravo capsule to detect short reflux events. However, the concordance between the Bravo capsule and the catheter-based pH was 88% for the diagnosis of GERD.

The catheter-free pH monitoring system (Bravo) has been proposed as an alternative and promising method for 24-h pH; however, accurate results mandate meticulous inspection of the pH tracing by the interpreting physician throughout the entirety of a 48-h study to identify premature transit of the capsule. Falsely elevated esophageal acid exposure can be recorded by the computer as the result of early passage of the Bravo probe into the stomach (Figs. 1, 2, and 3). Tracings that show prolonged acid exposure should be manually reviewed for signs of possible early dislodgement and premature advancement into the stomach.

Conclusion

This study demonstrates that the meticulous inspection of the pH tracing by the interpreting physician is vital to gain accurate results. This interpretation should be done throughout the entirety of a 48-h study to identify the premature transit of the capsule.

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Recurrent Heartburn after Laparoscopic Fundoplication is Not Always Recurrent Reflux

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Abstract
Introduction A small cohort of patients present after antireflux surgery complaining of recurrent heartburn. Many of these patients have been empirically recommenced on proton pump inhibitors.
Objective The aim of this study was to determine whether patients with symptoms that suggest recurrent reflux had objective evidence of reflux, and to determine predictors of recurrent reflux.
Methods We identified all patients from an existing database who had undergone pH monitoring for "recurrent heartburn" after fundoplication. These patients were then cross-referenced to another database, which recorded the outcomes for patients who had undergone a laparoscopic fundoplication. Patients complaining of dysphagia or other problems without heartburn were excluded from analysis.
Results Seventy-six patients were identified who met the inclusion criteria. Fifty-six (74%) of these had a normal 24-h pH study. Thirty-five patients (63%) with a normal pH study were on medication for heartburn at the time of referral. Three factors were found to be associated with an abnormal 24-h pH study: a partial fundoplication (P=0.039), onset of symptoms 6 months or more after surgery (P<0.001), and a good symptom response when antireflux medication was recommenced (P=0.015).
Conclusions Not all patients complaining of recurrent heartburn after fundoplication have evidence of abnormal reflux. Objective evidence of abnormal esophageal acid exposure should be confirmed before recommencing antireflux medication.

Keywords Laparoscopic fundoplication · Recurrent heartburn · Recurrent reflux · 24-h pH study

Introduction

Since the first laparoscopic fundoplication was performed in 1991, the popularity of fundoplication for the treatment of gastroesophageal reflux has escalated.[1,2] In 1999, fundoplications accounted for 87 of every 100,000 hospital discharges in the United States. This represented an eighfold increase in the rate of surgery over the previous decade.[3] Reports looking at 5-year follow-up results after laparoscopic fundoplication suggest that 86 to 96% of patients are satisfied with the outcome of surgery. However, there are also data that imply "surgical failure" rates of up to 30%.[3,4]

What determines a "surgical failure" in patients who have undergone a fundoplication? Symptom control is often used as a marker of surgical outcome. In 2001, Spechler et al.[5] reported "...62% of surgical patients were taking antireflux medication on a regular basis" with the inference of

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apparent failure of surgical treatment. However, three recent studies have shown that recurrence of symptoms after fundoplication might be a poor indicator of reflux status. These investigators found that only 23 to 39% of patients who had symptoms suggestive of recurrent reflux had abnormal esophageal acid exposure confirmed when they underwent 24-h pH monitoring.[6–8] Hence, it is possible that many of the patients who use antisecretory medications after fundoplication might not actually have recurrent reflux. These patients might be taking medication unnecessarily.

We undertook this study to determine: 1) whether patients who complained of "recurrent heartburn" after fundoplication had an abnormal 24-h pH study and, 2) whether specific symptoms and/or other patient factors can be identified to predict which patients will have abnormal reflux when investigated by 24-h pH monitoring.

Materials and Methods

Patient Selection and Clinical Follow-up

All patients who underwent pH monitoring in the Department of Surgery at the Royal Adelaide Hospital after a laparoscopic fundoplication for "recurrent heartburn" were identified by comparing a database that is used to store pH study reports, with a clinical database that records the outcome for all laparoscopic fundoplications performed by surgeons associated with the Departments of Surgery at the University of Adelaide, and Flinders University, in Adelaide, South Australia. Patients were included in this study if they had undergone a laparoscopic fundoplication (Nissen or partial) for reflux disease that had been diagnosed before surgery by either an abnormal 24-h pH study (esophageal pH<4 for more than 4% of the study) and/or endoscopy with evidence of esophagitis (minimum Savary–Miller grade I[9]). Patients were excluded if they had undergone an initial open fundoplication, or if they had undergone postoperative pH monitoring to investigate nonreflux (symptoms without heartburn). Patients with other symptoms as well as heartburn (e. g., acid regurgitation, cough, nasal symptoms, or dysphagia) were not excluded from analysis. In addition to the information that was obtained from the databases, some medical records were reviewed when needed, to review clinic correspondence, endoscopy reports, and operation reports.

The patients identified were divided into two subgroups according to the outcome of the pH study—normal 24-h pH study (pH <4 for <4% of the study duration), abnormal 24-h pH study (pH <4 for> 4%). Clinical follow-up data for these patients were collected prospectively by a research nurse. This was achieved by using a combination of postal questionnaires and telephone interviews at 3 months, 12 months, and yearly after fundoplication.

A range of clinical variables was used to compare the two groups of patients: age, gender, body mass index (greater vs less than 30 kg/m^2), preoperative pH study (pH <4 for more vs less than 4% of the study), type of fundoplication (partial vs total), postoperative heartburn score, esophageal motility (decreased motility ≤50% primary peristalsis, increased motility = contraction amplitudes >100 mmHg throughout esophagus and >180 mmHg in distal esophagus), onset of symptoms more than vs less than 6 months after fundoplication, and response to antireflux medication postoperatively. Heartburn was assessed using a 0 to 10 analog scale (0 = no heartburn, 10 = severe heartburn). In addition, a series of yes/no questions were asked to determine the patient's ability to relieve symptoms of bloating by belching, their ability to belch normally, and whether or not they experienced dysphagia. Patients were also asked about the postoperative use of antireflux medication, including the type, dose, and frequency of use. Patients were asked to rate their improvement after treatment with antireflux medication: no response, mild–moderate response (incomplete eradication of heartburn symptoms), good response (no heartburn symptoms on medication).[10,11]

Esophageal Manometry

Esophageal manometry was performed using an eight-channel catheter with a 6-cm sleeve (Dentsleeve Pty Ltd, Adelaide, Australia). Data were recorded using an eight-channel Grass polygraph (Model 7D, Grass Instrument Co., Quincy, MA, USA). Esophageal contractility and lower esophageal sphincter (LOS) relaxation were determined by analysis of 10 wet swallows (5 mL each, 30 s apart).[12] The basal end-expiratory LOS pressure (mmHg) was measured at 10-s intervals during the rest period (excluding swallow activity).

24-hour pH Study

Acid-suppressing medications were discontinued for 2 days (H_2 blockers and prokinetics) to 5 days (proton pump inhibitors) before the study. A single sensor Zinetics antimony pH probe was positioned 5 cm proximal to the LES and pH data were collected for a period of 24 h using an ambulatory pH Digitrapper Mk III (Medtronic Functional Diagnostics, Denmark). Data were analyzed using EsopHogram ver2.01 (Polygram for Windows ver 2.04, Synectics Medical © 1996). A reflux event occurred if the pH dropped below pH 4.0 for longer than 5 s.[13] In this study, where all patients had undergone a fundoplication, a cut-off value of 4% was used to define "abnormal reflux".

Endoscopy

Preoperative upper gastrointestinal endoscopy data was available for 60 of 76 (79%) patients. The degree of esophagitis was graded according to the Savary–Miller classification (Grade 0 = no mucosal ulceration, Grade I = single linear ulcer in distal esophageal mucosa, Grade II = multiple noncircumferential ulcerations, Grade III = circumferential ulceration, Grade IV = chronic complicated lesions [deep ulcers, strictures, Barrett's esophagus]).[9] Patients were identified as having Barrett's esophagus by the presence of visible columnar mucosa in the tubular esophagus.

Statistical Analyses

SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL) was used to perform data analysis. Data were expressed as mean ± range or number (percentage) as appropriate. Pearson's chi-square tests and Mann–Whitney *U* tests were used where applicable to compare variables between the two groups of patients. A stepwise forward binary logistic regression analysis was also performed to confirm significant predictors for the presence or absence of abnormal esophageal acid exposure. Differences were considered significant at $P<0.05$.

This study was approved by the Clinical Research Ethics Committee of the Royal Adelaide Hospital.

Results

Out of 3,763 pH studies in the Royal Adelaide Hospital pH/manometry database, and 1,717 individual patients who had undergone a laparoscopic fundoplication in our institution, 76 patients were identified who met the inclusion criteria for this study. Some of the characteristics of these patients are summarized in Table 1. Female patients constituted 33 (43%) of the 76 patients, and the mean age at time of study was 57 years (range 28–80). Preoperative body mass index (BMI) values could be calculated for 72 of 76 patients. Twenty-seven (38%) were obese (BMI ≥30 kg/m^2). Fifty-three patients (63%) had an abnormal preoperative 24-h pH study, and 55 patients (65%) had a minimum of Savary–Miller grade I[9] esophagitis on endoscopy. Twenty-four patients (32%) had undergone a partial fundoplication (anterior 90° [15 patients], 180° [8], or 270° [1]), and the other 52 patients (68%) had undergone a total fundoplication (360°). The time interval between fundoplication and subsequent postoperative 24-h pH testing ranged from 2 months to 13 years, with a mean time interval of 3.7 years.

Table 1 Demographic Data on 76 Patients with Recurrent Symptoms After Fundoplication

	Normal pH Study (n=56)	Abnormal pH Study (n=20)	P Value
Age, mean ± SD, years	57±12.1	57±13.9	0.77
Gender, M/F	21/35	12/8	0.08
BMI ≥30[a], no. (%)	24 (43)	3 (15)	0.03
pH study >4%[b], no. (%)	36 (64)	14 (70)	0.72

[a] BMI = body mass index (kg/m^2) calculated on 72/76 patients
[b] Preoperative pH monitoring

Table 2 Symptom Profile of Patients with Recurrent Symptoms After Laparoscopic Fundoplication

	Patients (n=76) no. (%)
Heartburn	76 (100)
Dysphagia[a]	52 (68)
Acid regurgitation[a]	44 (58)
Cough[a]	44 (58)
Chest/abdominal pain	38 (50)
Sore throat[a]	2 (3)

[a] Not sole symptom for any patient included in this study

Recurrent symptoms experienced by the study group are listed in Table 2. Heartburn, dysphagia, and acid regurgitation were the most common complaints, followed by cough, nasal symptoms, and chest or abdominal pain. As stated in the "Materials and Methods" section, any patient suffering solely from such symptoms without heartburn was excluded from the study.

Postoperative 24-h pH studies were normal in 56 patients (74%) and abnormal in the remaining 20 patients (26%). These formed the two study groups. There was no difference between the study groups with regard to age, gender, or preoperative 24-h pH monitoring outcomes. Patients with a preoperative body mass index ≥30 kg/m^2 were significantly more likely to have a normal postoperative 24-h pH study (P=0.03).

We found that 46 of 56 patients (82%) who had a normal pH study, had a pH <4 for 1% or less of the study duration, and 7 of 56 (13%) had a pH <4 for between 1.1 and 2% of the study duration. The remaining three patients had pH <4 between 2.1 and 3% of the study, with no patients having a pH <4 for 3.1 to 4% of the study. Patients with an abnormal 24-h pH study were more likely to have a strong correlation between symptoms and reflux events identified at pH monitoring ($P<0.0001$).[14] The converse was true for patients with a normal postoperative pH study ($P<0.0001$; Table 3).

Thirty-five of the 56 (63%) patients with a normal postoperative pH study, and 17 of 20 patients (85%) with an abnormal study, were taking antireflux medication at the time of their clinical review. Table 4 summarizes the use of

Table 3 Symptom-Reflux Event Correlation Between a Positive 24-h pH Study, Symptom Index (SI), and Symptom Sensitivity Index (SSI)

	Normal pH Study (n=56)	Abnormal pH Study (n=20)	P Value
SI ≥50%[a], no. (%)	4 (7)	9 (45)	0.0001
SSI ≥ 10%[b], no. (%)	11 (20)	13 (65)	0.0001

[a] SI =% of reflux associated symptom episodes
[b] SSI =% of symptom associated reflux episodes

antireflux medication in each group. The majority of patients were taking proton pump inhibitors.

Eleven of 20 patients (55%) with an abnormal pH study went on to have revisional surgery. The following diagnoses were made at surgery: slipped wrap (3), disrupted wrap (3), herniated wrap (2), etiology for failure unclear (3). Five of 56 patients (9%) with normal 24-h pH monitoring eventually went on to have revisional antireflux surgery. These were all patients who had troublesome dysphagia and heartburn.

Postoperative variables that were not associated with the 24-h pH study outcome are listed in Table 5. No difference was seen between the two groups for postoperative heartburn score, abnormal esophageal motility, and ability to relieve symptoms of bloating. Three variables were found to be significantly associated with an abnormal postoperative 24-h pH study (Table 6). These were: a partial fundoplication (P=0.039), onset of symptoms more than 6 months after surgery (P<0.001), and a good response to antireflux medication (P=0.015). These postoperative variables were entered into a binary logistic regression model (with the exception of a good response to antireflux medication as a result of incomplete data on all patients) and the same factors (onset of symptoms more than 6 months postoperatively, partial wrap) remained highly significant (P<0.02).

Eighteen of the 56 patients who had a normal pH study had an endoscopy performed near the time of the pH study. Four of these patients had an equivalent or improved grade of esophagitis compared to the preoperative endoscopy findings. The other 14 patients had no evidence of esophagitis nor disruption/herniation of their fundoplication.

Table 4 Use of Antireflux Medication After Fundoplication

	Normal pH Study (n=56)	Abnormal pH Study (n=20)
Antireflux medication, no. (%)	35 (63)	17 (85)
H_2 blockers, no. (%)	6 (17)	3 (18)
Proton pump inhibitors, no. (%)	29 (83)	14 (82)

Table 5 Postoperative Variables Not Significantly Associated with a Positive 24-hr pH Study

	Normal pH Study (n=56)	Abnormal pH Study (n=20)	P Value
Heartburn score, mean ± SD	5.5±3.5	6.3±3.9	0.29
Esophageal motility, no. (%)			
Normal[a]	44 (79)	16 (80)	0.46
Increased[b]	2 (4)	0 (0)	0.46
Decreased[c]	6 (11)	4 (20)	0.46
Bloat relief, no. (%)	29 (52)	8 (40)	0.81

[a] >50% primary peristalsis on manometry
[b] Hyperdynamic esophagus on manometry ≥100 mmHg proximal esophageal contraction amplitudes, and distal esophageal contraction amplitudes >180 mmHg
[c] ≤50% primary peristalsis on manometry

Discussion

Only 20 of 76 patients in this study who were assessed for recurrence of "heartburn" after fundoplication had an abnormal 24-h pH study. The remaining 56 patients had no objective evidence of abnormal esophageal acid exposure. In this latter group, 95% had acid in the esophagus (pH <4) for 2% of the study duration or less, suggesting that the negative results were unequivocal findings.

Our results are similar to those reported from three other centers where only 23 to 39% of patients investigated with pH monitoring for recurrent reflux symptoms had an abnormal pH study.[6–8] It seems that many patients are taking antireflux medication unnecessarily because they are prescribed these medications after a fundoplication without objective evidence of reflux.[15,16] In our study, 63% of patients with "reflux" symptoms, but a normal pH study, were taking antireflux medications at the time of their assessment. This appears to be inappropriate treatment.

Table 6 Intraoperative and Postoperative Variables Significantly Associated with a Positive Postoperative 24-h pH Study

	Normal pH Study (n=56)	Abnormal pH Study (n=20)	P Value
Partial wrap[a], no. (%)	14 (25)	10 (50)	0.039
Onset of symptoms > 6 mo postoperatively[b], no. (%)	23 (41)	17 (85)	0.001
Good response to anti-reflux medication[c], no. (%)	15 (27)	12 (60)	0.015

[a] Partial wrap includes 90° (15), 180° (8), 270° (1)
[b] Data available on 75/76 patients
[c] Data available on 41/76 patients

A reasonable question to ask is: are there any factors that can alert the physician that a patient's symptoms are more likely to be caused by true recurrent reflux? A number of studies have shown that postoperative heartburn, dysphagia, and chest pain can all occur in the absence of abnormal esophageal acid exposure.[4,6–8,17,18] Galvani et al.[6] found the symptom of acid regurgitation a reliable indicator of pathologic reflux. However, in our experience, less than 60% of patients complained of regurgitation postoperatively and there was no significant association of a positive 24-h pH study with this symptom.

We assessed nonsymptom-related variables (except postoperative heartburn score, and the ability to relieve bloat symptoms) to determine whether anything can be identified that could guide the physician toward the correct diagnosis. We did not find any significant association between pH outcomes, and age, gender, or esophageal motility. Obesity was significantly associated with a normal pH study, rather than an abnormal study. The lack of association between obesity and pathologic reflux is strongly supported by recent publications.[19–22] Anvari et al.[21] documented no difference in outcome between 70 morbidly obese patients and 70 non-obese patients as measured by postoperative 24-h pH results. Obese patients had a low 1.4% recurrence rate, requiring reoperation during a follow-up period of almost 4 years.[21] We have no ready explanation for our finding other than a possible type I statistical error.

Three variables were found to be significantly associated with pathologic reflux: a partial fundoplication, onset of recurrent symptoms 6 months or more after surgery, and a good response to antireflux medication. In particular, 9 of 20 patients with an abnormal pH study had undergone an anterior partial fundoplication. In two randomized trials, we have previously shown that an anterior 90° partial fundoplication is followed by a high rate of patient satisfaction and few adverse effects (dysphagia and wind-related complaints) compared to total fundoplication. However, there is a trade-off with less effective long-term control of reflux.[23,24]

In contrast to other publications on this topic[6–8], most patients in our study had a long time interval between fundoplication and recurrent symptoms and postoperative pH testing (mean of 3.7 years). Therefore, we were able to differentiate between patients whose recurrent symptoms began in the early period after surgery, and those whose symptoms developed years later. Anecdotally, it has been our experience that patients who develop recurrent heartburn, and who do not have abnormal esophageal acid exposure, tend to report symptoms at the first or second postoperative visit. Our results support this observation. In addition, patients who have a normal pH study often state that antireflux medication only controlled their symptoms "somewhat" or "not at all." Again, this is supported by the current study. In contrast to this, patients who report a good response to antireflux medication probably have recurrent reflux, and it is likely that this will be confirmed by 24-h pH monitoring.

The more complex issue is how to manage the patient who has a normal pH study. The sparse literature on this topic suggests that if reflux and other pathology is excluded, then a functional diagnosis is likely.[3,25] We are now studying these patients in greater detail to rule out other causes of "heartburn": biliary disease, peptic ulcer disease, gastritis, irritable bowel syndrome, and functional dyspepsia.

Based on the Rome Consensus, functional dyspepsia is defined as persistent or recurrent pain in the upper abdomen in the absence of other pathology for at least 12 weeks in the previous year.[26] However, many of the patients who had a normal pH study seem to have symptoms arising from the esophagus itself. This raises the question of whether a condition of "irritable esophagus" exists in a similar fashion to "irritable bowel." In other words, one could hypothesize that this small subgroup of patients might suffer from an overly sensitive esophagus, and this could be caused by altered sensory receptors on esophageal mucosa or abnormal processing of neurotransmitters.[27–29] This is a question that requires further study. As none of these patients had moderate to severe dysphagia, it seems unlikely that obstruction caused by a "tight" fundoplication causing intermittent esophageal distension, or spasm, was a cause of the heartburn.

The present study has limitations. First, it does not address recurrence of heartburn in fundoplication patients who have not undergone pH monitoring at our institution, but whose symptoms have been successfully treated by reinstitution of antireflux medication. Therefore, this report could be biased toward patients in whom antireflux medication has not been successful. Second, other studies have established that the reproducibility of 24-h pH monitoring is only of the order of 70 to 80%.[14] We defined our cut-off value for abnormal reflux at 4% as we felt that any value above this number is likely to be highly relevant in patients with recurrent symptoms who have previously undergone a fundoplication. Also, we found excellent symptom-reflux event correlation within our 24-h pH recordings, which supports accurate reporting of our results. Nevertheless, had we repeated the test in our negative group, it is possible that a small proportion may have had a positive test.

Third, it is possible that symptoms experienced by patients with a normal pH study could have been caused by nonacid reflux. However, studies have shown that nonacid reflux is very uncommon.[30,31] It is therefore unlikely that nonacid reflux was a cause of our patients' recurrent symptoms.

Conclusion

Fifty-six patients (74%) complaining of recurrent heartburn after laparoscopic fundoplication, who were referred for 24-

h pH monitoring, had no evidence of abnormal esophageal acid exposure. Although "62% of surgical patients were taking antireflux medication on a regular basis" in the widely cited JAMA article of Spechler et al.[5], it is likely that many of their patients were started on medical therapy without objective testing, and some may not have had abnormal reflux. Furthermore, it appears there is a small group of patients who are proven to have abnormal reflux before surgery, who have an intact fundoplication, and yet continue to have reflux symptoms for reasons that are not clear. Further investigation of this perplexing group of patients is needed.

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Epidural Administration of Morphine Facilitates Time of Appearance of First Gastric Interdigestive Migrating Complex in Dogs with Paralytic Ileus After Open Abdominal Surgery

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Abstract Morphine is known to delay gastric emptying and intestinal transit, although epidural morphine is believed to decrease postoperative complications. However, these findings are still controversial and based only on clinical observations. We investigated the effects of epidural morphine administration on gut motility by measuring interdigestive migrating complex after open surgery in dogs. Twenty-eight beagles were divided into four groups (n=7 each) to receive epidural saline (control group), epidural morphine, epidural ropivacaine, or low-dose continuous intravenous morphine. Strain gauge force transducers were sutured under open operation to the serosal surface of the stomach, duodenum, jejunum, and ileum to monitor gut motility. Time of appearance of first interdigestive migrating complex from the stomach propagated to the distal intestine was significantly shorter in the group that received epidural morphine compared with the other three groups. These results suggest that epidural administration of morphine may facilitate recovery from paralytic ileus after open abdominal surgery, perhaps through its effects on the central nervous system.

Keywords Anesthesia · Animal model · Ileus · Motility · Pain · Surgery

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Introduction

Facilitating postoperative recovery is an old but constantly evolving challenge for the surgical team.[1] In particular, temporary disturbance of gastrointestinal motility is inevitable after abdominal surgery and may lead to bacterial growth and increased postoperative morbidity and mortality.[2] Epidural anesthesia/analgesia has recently been found to decrease postoperative complications, including paralytic ileus.[3–6] However, these findings are still controversial.[7] Moreover, the evidence of the usefulness of epidural anesthesia/analgesia is based on clinical observations such as first flatus, first bowel movement, duration to first oral intake, and frequency of nausea and abdominal symptoms.[8–10] Thus, the clinical effects of epidural anesthesia/analgesia on the time of appearance of the first interdigestive migrating complex (IMC) originating in the stomach propagated to distal intestine has yet to be fully confirmed in an animal model.

Morphine is known to induce contractions in the intestine, leading to disturbances in the rhythm of intestinal motility and subsequent constipation.[11] In contrast to the well-established effects of morphine on the gastrointestinal tract, epidural administration of morphine plus bupivacaine has been reported to facilitate recovery from paralytic ileus after surgery,[12] suggesting the existence of differential effects of epidural morphine from those of intravenous morphine on the recovery process of gut motility from paralytic ileus after open intraabdominal operations. We hypothesized that this effect on gut motility may take place via the indirect analgesic effects of epidural morphine on the central nervous system. Moreover, local anesthesia is often used together with narcotics for epidural analgesia to provide an effective blockade of the sympathetic nerves, which are also considered to play a role in the recovery of gut motility.[13] Because local anesthesia is usually not separated from epidural analgesia, the role of blocking sympathetic nerves in early recovery of gut motility remains uncertain. We included ropivacaine, a commonly used local anesthetic, in this study to investigate the role of inhibition of sympathetic nerves in the recovery of postoperative ileus.

The IMC of the digestive tract is known to have a cyclic and propagating action under normal conditions,[14] and consists of four phases. These phases occur almost simultaneously in the stomach and duodenum and then migrate distally in sequence over the entire small bowel. Phase I is a period of quiescence; phase II is a period of intermittent, low-amplitude contractions; phase III is a brief burst of regular, high-amplitude contractions; and phase IV is a brief transition back to the quiescence of phase I.[15] The interdigestive contractile activity disappears during abnormal conditions such as open intraabdominal surgical procedures, reflecting intestinal paralysis. Using strain gauge force transducer (SGTs) is a well-known method of investigating gut motility including IMC in animal models.[16–18] In particular, a previous report proved that the appearance of IMCs in the stomach (gastric IMCs or GIMCs) was correlated with both gastric emptying and small intestinal transit time.[19] The present study examined the effects of epidural analgesia on gut motility in an SGT dog model using epidural administration of saline, morphine, or ropivacaine, and compared these results with continuous intravenous injection of low-dose morphine.

Materials and Methods

Ethical Issues

All experimental protocols were approved by the Ethical Committee for Animal Use at Jikei University. All surgical procedures were performed under general anesthesia. For the 7 days during which gut motility was monitored, animals were administered 400 kcal/day via the central vein. Finally, animals were sacrificed using an overdose of thiopental.

Operation

A total of 28 beagles (body weight, 10–12 kg) were randomly assigned into four groups (n=7 each) to receive epidural saline (control: 1 mL/h); epidural ropivacaine (1 mL [2 μg]/h); epidural morphine (1 mL: 0.08 mg/h); or intravenous morphine (1 mL: 0.08 mg/h). All beagles were prepared for operation with Niflek (Ajinomoto Pharmaceutics, Kawasaki, Japan) to clear the intestinal lumen of solid stool, and had been fasted for 20 h before abdominal surgery.

General anesthesia was induced using thiopental; after intubation and maintenance with isoflurane, the operation was performed. An SGT (Star Medical, Nishi-nippori, Tokyo, Japan) was sutured to the serosal surface of each of the gastric bodies, including the duodenum, jejunum (10 cm distal to the ligament of Treiz), and ileum (10 cm proximal to the ileocecal junction). SGTs were pulled through the abdominal wall subcutaneously to the bottom of the scapula, then connected to the transmitter. A catheter was inserted into the right cervical vein for parental nutrition. Animals were fasted throughout the investigation.

Drug Infusion

Preoperatively, an 18-gauge epidural catheter was inserted into the epidural space at the L1-2 spinal level, and then pushed three spinal segments upwards to the level of the lower thoracic vertebrae in all dogs. Catheter tip placement was confirmed under fluoroscopy.

Continuous epidural infusion of saline (0.9% NaCl) with or without drugs was started at 1 mL/h through the epidural catheter before the abdominal operation after general anesthesia and was maintained for 7 days. Monitoring of gut motility began immediately after the operation and was also maintained for 7 days. Continuous intravenous infusion of morphine was applied concomitant with continuous epidural infusion of saline at 1 mL/h.

Drug injections and the continuous gastrointestinal recording were performed until the end of day 7. Intolerable pain after operation is thought to affect recovery for the first 24–48 h. Thus, analgesia was maintained during this period. Continuous administration of analgesia after recovery from intolerable pain was maintained to investigate the effects of narcotics on the recovery of gastrointestinal motility in a nonsevere pain condition.

Monitoring of Gut Motility

The phase-III-like contractions were defined as a concerted grouping of high-amplitude contractions lasting >5 min. Phase-III-like contractions in the stomach propagated routinely to the distal small intestine. Therefore, GIMC was separated from other phase-III-like contractions observed in the small intestine and considered a primary endpoint.

Gastrointestinal Motility

Normal gastrointestinal motility is classified into two states: fasting state and postprandial state (Fig. 1). The fasting state comprises four phases: I, silent state; II, random irregular contractions; III, frequent high-amplitude contractions; and IV, rapid decrease in frequency and intensity of contractions after phase III. In normal dogs, phase III shows a cyclic appearance at intervals of 90–120 min in the stomach, and propagates to the distal intestine. Based on previous studies,[20,21] the appearance of GIMCs after the open intraabdominal operation was considered to indicate normalization of the digestive tract in this study.

Statistical Analysis

To adjust the significance level to account for multiple testing in the data sets, permutation tests were applied to detect significant differences in duration until first GIMC or phase-III-like contraction at each of the four sites in the gastrointestinal tract (stomach, duodenum, jejunum, and ileum) for each condition (saline control, epidural morphine, epidural ropivacaine, and intravenous morphine). The distribution of maximum t statistics based on 10,000 random permutations was compared with observed values to determine P values for each situation. $P<0.05$ was considered statistically significant. All tests were performed using STATA 8.0 software (STATA Corporation, College Station, TX, USA).

Results

On the day of operation (day 0; Fig. 2a), the saline and ropivacaine groups showed no signs of contractions. In contrast, a bundle of high-amplitude contractions was evident in epidural morphine group and some contractions

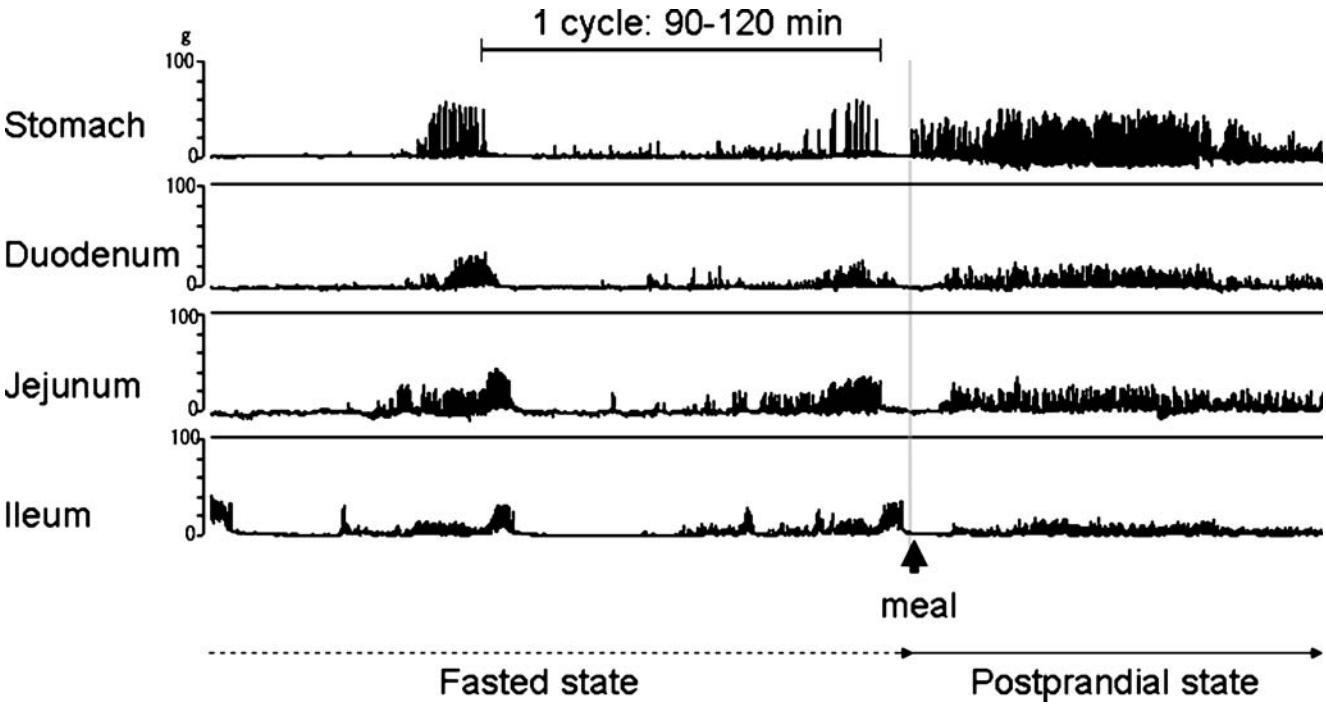

Figure 1 Gastrointestinal motility in the physiologic state monitored using strain gauge force transducers (SGTs). Normal gastrointestinal motility is classified into two states: fasting and postprandial. The fasting state comprises fou phases: I, silent state; II, random irregular contractions; III, frequent high-amplitude contractions; and IV, rapid decrease in frequency and intensity of contractions compared with phase III. In normal dogs, phase III shows a cyclic appearance at intervals of 90–120 min in the stomach and propagates to the distal intestine. Appearance of these interdigestive migrating contractions after open surgery reflects normalization of digestive tract function. With food intake, this cyclic activity disappears and continuous irregular contractions appear, representing the postprandial state.

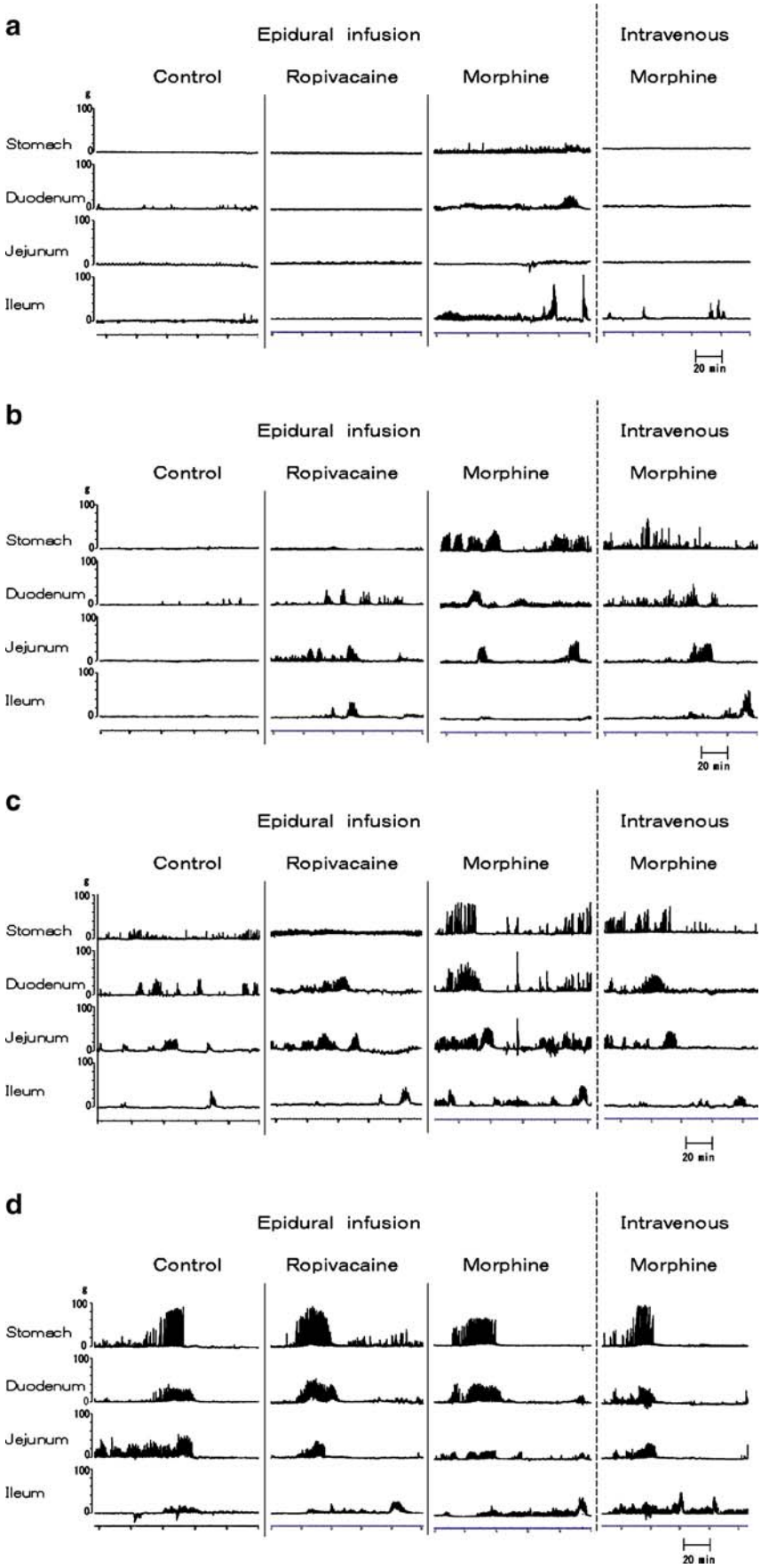

Figure 2 Postoperative gastrointestinal motility. Gastrointestinal motility was monitored using strain gauge force transducers (SGTs) at the gastric body, duodenum, jejunum, and ileum for 24 h postoperatively. The panels of epidural infusion with saline, ropivacaine, morphine, and continuous intravenous infusion of morphine were fused from left to right, respectively. (a) The day of surgery (day 0); (b) day 1; (c) day 2; (d) day 7.

were evident in the intravenous morphine group from the beginning of recording. Overall, the first phase-III-like contraction appeared at a median of 2.7 h (range, 1.8–14.8 h) after starting monitoring in the ileum. On day 1 (Fig. 2b), the saline group was still in a total paralytic state. In the ropivacaine group, low-amplitude contractions started to appear and some were recognized as phase-III-like contractions. In the epidural and intravenous morphine groups, higher amplitude contractions clearly propagating to the distal intestine were observed and extended from the duodenum. On day 2 (Fig. 2c), clear phase-III-like contractions were recorded in the jejunum, ileum, and sometimes in the duodenum for the saline and ropivacaine groups, but clear, rhythmic, phase-III-like contractions were not yet evident in the stomach. Conversely, phase-III-like contractions were seen in the stomach in both the epidural and intravenous morphine groups. By day 7 (Fig. 2d), clear GIMCs propagating to distal intestine were apparent in the stomach in all four groups.

Duration to first appearance of phase-III-like contractions and GIMC at each site were compared among groups. The first GIMC appeared earlier in the epidural morphine group than in the control (P=0.014), intravenous morphine (P=0.027), or ropivacaine groups (P=0.003; Fig. 3). Moreover, there were significant differences in time to first appearance of phase-III-like contractions between groups, with the epidural morphine group showing an earlier appearance of phase-III-like contractions than control in all parts of the small intestine ($P \leq 0.003$ in duodenum, jejunum, and ileum). The intravenous morphine group showed an earlier appearance of phase III contractions than the control group ($P \leq 0.02$ in all regions) but a later appearance than the epidural morphine group in the small intestine ($P \leq 0.05$ in jejunum and ileum; duodenum: NS). Ropivacaine did not alter duration to first phase-III-like contractions compared with the control group in any area except the jejunum (P=0.0062).

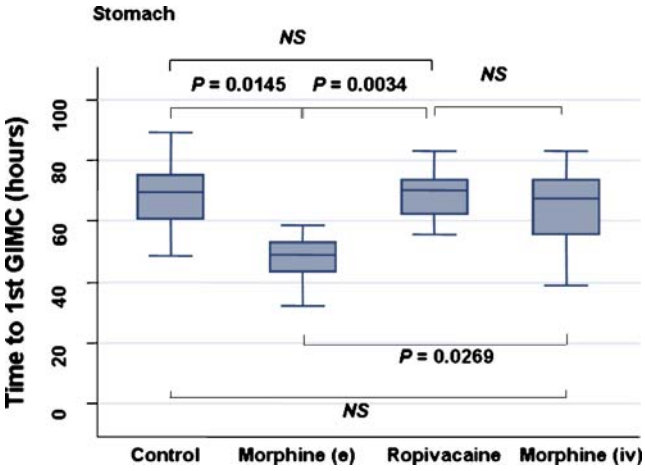

Figure 3 Duration until first appearance of gastric interdigestive migrating complex by group in the gastrointestinal tract. To adjust the level of significance to account for multiple testing of data sets, permutation tests were applied to detect significant differences in duration until the first interdigestive migrating complex at each site in the gastrointestinal tract (stomach, duodenum, jejunum, ileum) under each condition (saline control, epidural morphine, epidural ropivacaine, intravenous morphine). The distribution of maximum t statistics based on 10,000 random permutations was compared with observed values to determine P values for each situation.

Discussion

We demonstrated that only epidural administration of morphine significantly facilitated recovery of GIMC, which has been reported to correlate with the recovery of gastric emptying and intestinal transport from the paralytic state after an open intraabdominal operation.[19] Phase-III-like contractions first appeared in the ileum, followed by the jejunum, duodenum, and finally in the stomach; this pattern of recovery of interdigestive motility matched that of a previous report.[19] Moreover, phase-III-like activity in all regions of the small intestine and stomach recovered earlier postoperatively after epidural morphine. However, the postoperative IMC recorded by means of a multipressure sensor probe placed intraoperatively into the jejunum did not correlate with the first passage of flatus and stool.[22] Thus, although differences in phase-III-like activity in the small intestine between groups were statistically significant, we cannot say that they were clinically significant.

Morphine is known to delay gastric emptying and intestinal transit, suppress intestinal secretion of water and electrolytes, and suppress transport of bile into the duodenum[23]; however, epidural morphine can shorten postoperative ileus.[6,24] This clinical knowledge is consistent with our results using an animal model. Epidural morphine and intravenous morphine showed differential effects on the recovery pattern of gut motility after open intraabdominal operations. Low doses of morphine equivalent to the dose used in our study have been reported to induce premature phase III activity in dogs[25–27] and humans,[28] whereas a supramaximal dose of morphine did not affect migrating myoelectric complexes by initiating premature phase III contractions.[29] In this study, intravenous morphine facilitated the appearance of phase-III-like contractions, which has been reported previously.[30] Intravenous morphine, however, did not significantly facilitate the first appearance of GIMC, which is consistent with the finding that morphine administration has no effect on gastric motility, whereas it markedly increases duodenal contractility.[31] To our knowledge, this is the first study in which differential effects of epidural and intravenous morphine were demonstrated by measuring GIMC and phase-III-like contractions. Moreover, a previous review

noted that the primary sites of action for morphine with respect to inhibition of gastrointestinal function are in the peripheral nervous system, whereas analgesic activity resides primarily in the central nervous system.[32] Thus, the additive effect of epidural morphine to intravenous low-dose morphine may occur indirectly through analgesic or other actions on the central nervous system. By targeting sympathetic nerves around the spinal cord and the sensory fields of the central nervous system, epidural administration of ropivacaine allows analgesia at very low dosages compared with intravenous infusion.[33] In this study, epidural infusion of ropivacaine was expected to exert a strong blockade on the sympathetic nerves and affect motility, but showed no significant effects compared with control. This finding may mean that suppression of the sympathetic nerves may not represent a major pathway of recovery from postoperative ileus.

It is not the return of interdigestive activity but rather gastric emptying and/or bowel movements that keeps a patient from leaving the hospital. Thus, a limitation of this study was that we did not evaluate the time to recovery of gastric emptying and intestinal transport. Furthermore, the acetaminophen absorption technique generally correlates well to scintigraphy of liquid phase gastric emptying.[34] However, because the dogs were kept in a fasting condition as a result of the nature of the experimental design, acetaminophen infusion to monitor gastric emptying was not done. Instead, we used GIMC that migrates down the bowel as the primary endpoint and phase-III-like contractions as the secondary endpoint.

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Laparoscopic Repair of Colonoscopic Perforations: Indications and Guidelines

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Abstract Iatrogenic colonic perforation is one of the most serious potential complications of colonoscopy. Standard management is surgical repair. No prospective data exist to clearly define the indications for laparoscopic repair. We report the largest case series to date of laparoscopic repair of colonoscopic perforations. A retrospective review was performed of all patients undergoing either exploratory laparoscopy with conversion to open repair, or laparoscopic repair of colonoscopic perforation. Exploratory laparoscopy for the attempted repair of colonoscopic perforations was performed in 11 patients at our institution. The mean colonic perforation size was 2.7 cm. Three cases were converted immediately to open laparotomy. A fourth patient that underwent primary laparoscopic repair of a 4-cm tear developed a leak at the repair site, necessitating reoperation. A fifth patient in whom exploratory laparoscopy was unrevealing underwent separate laparotomy for continued sepsis. Six patients underwent successful laparoscopic repair. Most perforations secondary to colonoscopy warrant rapid exploratory laparoscopy. Extensive inflammation or fecal soilage may require colonic diversion. Inability to laparoscopically localize the area of perforation or doubt regarding the security of the repair should prompt conversion to laparotomy. Laparoscopic repair of colonic perforations in experienced hands is a viable alternative to the open approach.

Keywords Colonoscopy · Colonic perforation · Laparoscopy · Laparoscopic surgery

Introduction

Colonoscopy is an effective tool for both diagnosis and therapy of colonic lesions but carries a small risk of complications, the most serious and feared of which is colonic perforation. Perforation can rapidly progress to peritonitis and sepsis, carrying significant morbidity and mortality.

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Perforation of the colon during colonoscopy may occur due to mechanical or thermal injury. Excessive mechanical pressure may be exerted along the shaft of the colonoscope during advancement or rotation, or at the instrument tip. In addition, pneumatic pressure from excessive insufflation can lead to tearing and perforation. Thermal injury occurs during "hot" biopsy or polypectomy. In contrast to perforations from mechanical forces, these injuries are often smaller with less peritoneal contamination.[1,2]

Controversy exists over the ideal management of colonoscopic perforation. Treatment strategies range from nonoperative management to open colonic diversion. In order to avoid further patient trauma, minimally invasive methods, such as laparoscopic repair, have been developed. However, colonic perforation secondary to colonoscopy is so infrequent that no single institution has been able to gather sufficient data to definitively state the circumstances under which minimally invasive treatment is appropriate. No prospective studies exist, and retrospective studies range from single-case reports to a handful of patients treated with attempted laparoscopic repair.[2–12] We initially reported our

results with three patients in 1994,[10] and we now provide our updated experience with laparoscopic repair of colonoscopic perforations. This represents the largest case series reported to date.

Material and Methods

After approval of the Mayo Clinic Scottsdale Institutional Board Review, a retrospective review of all patients undergoing colonic endoscopy at Mayo Clinic Scottsdale from the facility's inception in June 1987 through July 2004 was performed. A comprehensive computerized search was performed using diagnostic codes to identify patients who had both a colonic endoscopy and any type of bowel perforation within a defined period of 30 days. From these results, charts were reviewed to select the patients that had either exploratory laparoscopy with conversion to open repair or laparoscopic repair of iatrogenic colonic perforation caused by colonoscopy.

Results

From January 1993 through July 2004, 11 patients underwent exploratory laparoscopy for attempted repair of colonoscopic perforations at our institution. Detailed data are summarized in Table 1. The mean patient age was 72 (range 64–85). Seven (64%) were female and four (36%) were male. All but two (82%) patients had undergone at least one prior abdominal or pelvic surgery. All four diagnostic colonic endoscopies resulted in forceful injuries that were described after surgical exploration as linear colonic tears or lacerations. All seven therapeutic colonoscopies resulted in small, focal injuries from polypectomy. The mean defect size was 2.7 cm (range 1–4 cm).

Operative and outcome data are summarized in Table 2. Seven (64%) of the 11 patients underwent laparoscopic repair. Six of the seven were repaired by oversewing the perforation with either one or two layers of sutures. One was repaired with a laparoscopic linear stapler.

Six of the patients recovered uneventfully. One patient developed an abscess caused by a leak at the site of previous laparoscopic repair of a 4-cm perforation. Exploratory laparotomy was performed on this patient on postoperative day number seven, with sigmoid colectomy and end colostomy formation. The mean hospital stay following perforation and laparoscopic repair was 7.6 days (range 4–16 days). The only additional postoperative complication was new-onset atrial fibrillation in one patient that spontaneously resolved.

Three (27%) cases were converted from exploratory laparoscopy to open laparotomy. One was converted because the perforation appeared to be into the lesser omental bursa and was difficult to access laparoscopically. The second case was converted because the perforation was deep in the pelvis and the patient was unable to tolerate elevated airway pressures caused by steep Trendelenburg

Table 1 Patient Demographics, Colonoscopic Procedure, and Mechanism of Perforation

Patient	Age	Sex	Abdominopelvic Surgical History	Procedure	Aim	Mechanism
1	72	F	Hemorrhoidectomy, hysterectomy, oophorectomy, open appendectomy	Colonoscopy	Diagnostic	Polypectomy/ cautery
2	66	F	Open cholecystectomy	Colonoscopy	Diagnostic	Mechanical injury
3	79	M	TURP	Colonoscopy	Therapeutic	Polypectomy/ cautery
4	68	M	Right adrenalectomy	Colonoscopy	Diagnostic	Perforated diverticulum
5	72	F	Hysterectomy, appendectomy	Flexible sigmoidoscopy	Diagnostic	Mechanical injury
6	72	F	Total abdominal hysterectomy	Colonoscopy	Diagnostic	Mechanical injury
7	78	M	Open cholecystectomy, drainage intra-abdominal abscess	Colonoscopy	Diagnostic	Mechanical injury
8	85	F	Total vaginal hysterectomy	Colonoscopy	Therapeutic	Polypectomy/ cautery
9	70	M	None	Colonoscopy	Therapeutic	Polypectomy/ cautery
10	64	F	None	Colonoscopy	Therapeutic	Polypectomy/ cautery
11	71	F	Hysterectomy	Colonoscopy	Diagnostic	Mechanical injury

TURP = transurethral resection of the prostate

Table 2 Operation, Perforation Description, and Outcome

Patient	Operation	Perforation Location	Perforation Size	Hospital Days Postcolonoscopy	Complications
1	Laparoscopic primary suture repair	Cecum	1 cm	6	None
2	Laparoscopic primary suture repair	Sigmoid	1 cm	4	None
3	Laparoscopic primary suture repair	Transverse	1.5 cm	5	None
4	Laparoscopic primary suture repair	Sigmoid	2 cm	6	None
5	Laparoscopic stapled repair	Sigmoid	4 cm	7	None
6	Laparoscopic primary suture repair; subsequent laparotomy with sigmoidectomy and end colostomy	Sigmoid	4 cm	16	Bladder injury during laparoscopy requiring repair; re-exploration laparotomy 7 days later for abscess from repaired perforation
7	Laparoscopic primary suture repair	Sigmoid	No mention	9	Atrial flutter
8	Exploratory laparoscopy, then laparotomy later same day	Not found	Not found	10	Reoperation, sepsis, respiratory failure, death
9	Exploratory laparoscopy, converted to open primary suture repair	Transverse	0.2 cm	8	None
10	Exploratory laparoscopy, converted to open primary suture repair	Transverse	2 cm	8	None
11	Exploratory laparoscopy converted to open primary suture repair due to difficulty maintaining proper airway pressures while in Trendelenburg	Sigmoid	1 cm	9	Anemia, new onset atrial fibrillation

positioning. The third case was converted to appropriately manage a large segment of small bowel that appeared hyperemic and inflamed from fecal soilage. Of this group, the mean perforation size was 1.1 cm (range 0.2–2 cm) and the mean hospital stay was 8.3 days (range 8–9 days).

The final case was a patient with severe obstructive pulmonary disease admitted to the hospital 10 days previously for hematochezia. Following therapeutic colonoscopy, the diagnosis of colonic perforation was made, based upon radiographic demonstration of free intra-abdominal air. Exploratory laparoscopy performed by a highly experienced colorectal surgeon was unrevealing. Postoperatively, the patient developed sepsis and was returned to the operating room later the same day for open laparotomy. Again, no perforation was found and no specific repair was performed. The patient continued to decline postoperatively and life support was eventually withdrawn on postoperative day 10 in accordance with the family's wishes. The diagnosis of colonic perforation remains in doubt.

Discussion

One of the most devastating complications of colonoscopy is perforation of the colon, which may result in significant morbidity and even mortality. Suggested treatments of colonic perforations range from observation to segmental resection and diversion of the fecal stream to the exterior. Most surgeons prefer exploration, while some authors[13,14] prefer nonoperative management of select cases. Visualization of the peritoneal cavity by the endoscopist and the development of signs of peritoneal irritation[14] are absolute indications for surgery. The timely application of exploratory laparoscopy may prevent the development of inflammation and further injury that would make more invasive measures, such as open laparotomy or colonic diversion, necessary.

A total of 21 cases of laparoscopically attempted repair, not including our previous data, were found in the literature. Detailed data are summarized in Table 3. In 17 patients (81%), the repair was accomplished laparoscopically without conversion to open laparotomy. The largest series, published by Wullstein et al.,[11] included seven patients in whom exploratory laparoscopy was performed. Four of their seven cases were performed completely laparoscopically. Two others had extensive injuries, necessitating open conversion. Their first patient had an intraoperative technical complication that also led to open conversion after laparoscopic repair. Allam et al.[4] described a single case in which they used a laparoscopically assisted approach to perform a minilaparotomy for repair of a perforation.

Perforation size was described in 17 of the reported cases. Only three patients in whom perforation size was greater than 2.5 cm underwent full laparoscopic repair (largest 5 cm). Thirteen (62%) perforations were in the sigmoid colon, two (10%) in the rectum, three (14%) in the cecum, and three (14%) in the transverse colon or left

Table 3 Reported Minimally Invasive Repairs of Colonoscopic Perforations

Investigator	Number of Patients	Injury Location	Size	Procedure	Results	Postop Day
Agresta, F	2	(1) Diverticular perforation subperitoneal rectum (2) Sigmoid	(1) Not described (2) Microperforation	(1) Irrigation/ drainage (2) Single suture repair	Unremarkable recovery	Not described
Regan, MC	1	Pelvic colon	Not described	Serosal pursestring suture	Unremarkable recovery	3
Goh, PM	1	Upper sigmoid	2.5 cm	Tangential transverse resection with laparoscopic linear stapler	Unremarkable recovery	5
Allam, M	1	Rectosigmoid	7.6 cm	Laparoscopically assisted minilaparotomy/end colostomy	Unremarkable recovery/colostomy closure 4 weeks later	5
Hayashi, K	1	Middle sigmoid	1.5 cm	Tangential transverse resection with laparoscopic linear stapler/drainage	Unremarkable recovery	Not described
Velez, MA	1	Distal sigmoid	5 cm	Primary suture repair	Unremarkable recovery	3
Wullstein, C	1	Cecum	<1 cm	Primary suture repair	Unremarkable recovery	3
	3	2 Sigmoid, 1 left flexure	<2.5 cm	Tangential transverse resection with laparoscopic linear stapler	1 intraoperative rupture of staple l ine/conversion to open. Others unremarkable recovery	11, 5, 9
	1	Cecum	>2.5 cm	Ileocecal resection	Unremarkable recovery	8
	2	Transverse colon, deep rectum	>2.5 cm	Laparoscopy/ conversion to open procedure	Not described	Not described
Yamamoto, A	5	4 Sigmoid, 1 cecum	1, 1, 1.5, 1.5, 5 cm	Tangential transverse resection with laparoscopic linear stapler	3 patients vunremarkable recovery. 1 prolonged due to Parkinson's disease. 1 prolonged recovery because developed dissecting aortic aneurysm	13, 13, 16, 29, 101
Miyahara, M	1	Transverse colon	Not described	Primary suture repair	Unremarkable recovery	15
Mehdi, A	1	Sigmoid	Not described	Primary suture repair	Septic shock postop day 2 requiring vasopressors, then uneventful recovery	11

colonic flexure. Outcome is briefly described in all but one patient. Only one patient developed a related complication of septic shock on postoperative day 2 after laparoscopic repair, which resolved.[7] Two other patients had unrelated complications requiring prolonged hospitalization lengths of 29 and 101 days.[12] If these two patients are excluded, the mean number of postoperative days until discharge of the remaining 13 patients that underwent totally laparoscopic repair, in whom data are available, was 8.8 days.

The surgeon should communicate closely with the endoscopist when deciding upon appropriate patient management. The endoscopist can provide important information about the quality of the patient's bowel preparation and often a description of the endoscopically visualized injury.

Such factors may influence the surgeon's choice of laparoscopy vs. laparotomy.

If a colonoscopic perforation is to be repaired laparoscopically instead of diverted, the same conditions should be met as during open surgery. The elapsed time between the injury and intervention should be as short as possible. The abdomen should be relatively clean and free of fecal soilage and inflammation, and there should also be no residual pathology. The operating surgeon and team should be comfortable with laparoscopic techniques, such as mobilization of the colon and intracorporeal suturing.

Wullstein et al. proposed an algorithm based on size, including perforation and area of necrosis, that may be used as a guide to choosing type of repair. They felt that the upper size limit for sutured repair is 1 cm. Between 1 and 2.5 cm, they suggested a transverse tangential stapled resection, and above 2.5 cm, a segmental resection.[11] Valez[2] has shown that sutured repair is possible for a 5-cm tear.

In our experience, the defect size is less important than the condition of the bowel to be repaired and the level of contamination and inflammation present. We have used both sutured and stapled repair techniques with good results to repair defects of up to 4 cm. Following trocar placement and appropriate laparoscopic mobilization of the affected portion of colon, the defect is located and inspected. The defect should be free of significant inflammation and the colon mobilized well enough to perform a tension-free repair. The method of closure is based on surgeon preference but should be comparable to open techniques. We prefer a two-layer closure, beginning with a running 3-0 braided, absorbable suture. We use a semicircular SH needle via a 10-mm trocar, although a ski needle through a 5-mm trocar or an automated endoscopic suturing device through a 10-mm trocar may be used alternatively. Next, an outer layer of interrupted seromuscular 3-0 silk sutures should be placed. If the injury is in the sigmoid colon, the bowel may be occluded with a clamp and air insufflated into the rectum with the repair underwater to ensure the absence of a leak. For a stapled repair of a longitudinal tear, we place full-thickness stay sutures on both sides of the middle of the tear. While suspending each suture up and away from the other, an endoscopic stapler with a bowel load is fired across the lips of the defect transversely, avoiding luminal narrowing. Should the injury be so extensive as to preclude primary repair, a colostomy may be performed laparoscopically.

Some perforations may be difficult to locate. Communication with the endoscopist again can be helpful in targeting the search. Perforations may at times be visualized by insufflating the colon or rectum while it is underwater. If a perforation cannot be found, we favor immediate conversion to laparotomy.

Conclusion

Except in unusual circumstances, colonic perforation secondary to colonic endoscopy warrants rapid evaluation by exploratory laparoscopy if an experienced laparoscopic surgeon is available. Colonic wall defect size should not be the determining factor in choosing between laparoscopic or open repair but may influence the surgeon to choose one laparoscopic technique over another. Extensive peritoneal inflammation, fecal soilage, or complex colonic injury may require colonic diversion. Inability to laparoscopically localize the area of perforation should prompt conversion to laparotomy. Conversion to an open procedure should also be performed if there is any doubt regarding the security of the repair. Laparoscopic repair of colonic perforations in experienced hands is a viable alternative to the open approach.

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The Prognostic Significance of Total Lymph Node Harvest in Patients with $T_{2–4}N_0M_0$ Colorectal Cancer

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Abstract In patients with radically resected colorectal carcinoma, lymph node involvement is particularly important for a good prognosis and adjuvant therapy. The number of such lymph node recoveries is still controversial, with recommendations ranging from 6 to 17 nodes. The aim of this study is to determine if a specified minimum number of lymph nodes examined per surgical specimen can have any effect on the prognosis of patients who have undergone curative resection for $T_{2–4}N_0M_0$ colorectal carcinoma. Between September 1999 and January 2005, a total of 366 patients who underwent radical resection for $T_{2–4}N_0M_0$ colorectal carcinoma were retrospectively analyzed in a single institution. All specimen segments were fixed, with node identification performed by sight and palpation. We excluded 186 patients who received postoperative adjuvant chemotherapy via oral or intravenous transmission to prevent possible chemotherapeutic effects on patients' prognosis; therefore, a total of 180 patients with $T_{2–4}N_0M_0$ colorectal carcinoma were enrolled into this study. After the pathological examination, a mean of 12 lymph nodes (range 0–66) was harvested per tumor specimen. No postoperative relapse was found in this group, where the number of examined lymph nodes was 18 or more. Univariate analysis identified the size of the tumor, depth of invasion, grade of tumor, and number of examined lymph nodes, which were significantly correlated with postoperative relapse (all $P<0.05$). Meanwhile, both the depth of tumor invasion and the number of harvested lymph nodes were independent predictors for postoperative relapse ($P<0.05$). The 5-year overall survival rate of $T_{2–4}N_0M_0$ colorectal carcinoma patients who had 18 or more lymph nodes examined was significantly higher than those who had less than 18 nodes examined ($P=0.015$). Nodal harvest in patients undergoing radical resection for colorectal carcinoma was highly significant in the current investigation. Our results suggest that harvesting and examining a minimum of 18 lymph nodes per surgical specimen might be taken into consideration for more reliable staging of lymph node-negative colorectal carcinoma.

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Keywords Colorectal cancer · Lymph node number · Postoperative relapse · Survival

Introduction

The presence or absence of lymph node metastasis is pivotal in predicting the clinical outcome of patients who have undergone radical surgery for colorectal carcinoma (CRC). The presence of lymph node metastases often determines the use of adjuvant therapy; such adjuvant therapies have been shown unequivocally to provide a disease-free and overall survival benefit in patients with node-positive disease.[1] Furthermore, the presence of nodal metastases provides important prognostic information.[2] There is a consistent risk of substaging tumors and understaging patients when no sufficient lymph nodes are retrieved. Therefore, an accurate assessment of the pathologic status of the tumor lymph nodes in the resected specimen is essential for reducing the risk of understaging.

The number of lymph nodes required for accurate staging of patients is controversial, with recommendations in the literature ranging from 6 to 17 nodes.[3–7] Current guidelines from the American Joint Committee on Cancer recommend the assessment of 12 nodes or more for accurate staging.[8] Many factors that can lead to difficulty in establishing the minimum number have been identified. Among them is the lack of a constant number of lymph nodes in the various parts of the large bowel, the extent of surgical lymphadenectomy, and the skill and energy of the pathologist.[9]

Many of the previous recommendations have been based on studies regarding the number of nodes examined in relation to node-positive rates rather than to postoperative relapse and survival data. Only recently have data from large clinical trials demonstrated a correlation between nodal harvest and long-term survival in patients with node-negative disease.[9,10] The aims of this study are to evaluate the clinical outcome of patients who have undergone radical surgery for $T_{2–4}N_0M_0$ CRC and to determine if a specified minimum number of examined lymph nodes per surgical specimen can have any effect on postoperative relapse and long-term survival.

Material and Methods

Between September 1999 and January 2005, a total 925 patients underwent radical resection or palliative procedures for CRC at the Department of Surgery, Kaohsiung Medical University Hospital. Altogether, 366 patients (39.5%) had tumors classified as $T_{2–4}N_0M_0$ CRC, that is, tumors that have invaded between the muscularis propria and the pericolic–perirectal tissue of the large bowel wall without lymph node metastases, and all these patients received radical resection. Radical resection is defined as any gross residual tumor that does not remain in the surgical bed, and the surgical resection margin is pathologically negative for tumor invasion. One hundred and eighty-six $T_{2–4}N_0M_0$ CRC patients who had received postoperative adjuvant chemotherapy were excluded to prevent the possible chemotherapeutic effects on patients' prognosis, and the remaining 180 patients were enrolled into this study. For proximal colon tumors, lymphadenectomy was extended to the origin of the ileocolic, right colic, and middle colic arteries. For distal colon lesions and rectal tumors, it was extended to the origin of the inferior mesenteric artery along the preaortocaval space. Total mesorectal excision was performed in all patients with tumors of the middle and lower rectum and a distal clearance of at least 2 cm from the edge of the tumor. Clinical stage and pathological features of the primary tumors were defined according to the sixth edition of the TNM staging system of the International Union Against Cancer.[11]

All the surgical specimens were fixed in 10% formalin solution and routinely processed for paraffin embedding. The number of examined lymph nodes was ascertained by reference to the histopathologic report of each patient. Lymph nodes were identified in the surgical specimens by sight and palpation. Routine histological examination was performed using hematoxylin and eosin staining. Histological processing of the specimens was the same for all patients. No special fat clearance or staining techniques were employed. The mesenteric and adventitial fat was carefully displaced by manual pressure, visually inspected for lymph nodes, and palpated for the presence of firm tissue that was indicative of a lymph node. Representative sections were examined in all grossly involved lymph nodes; grossly uninvolved lymph nodes smaller than 3 mm were submitted whole, and those 3 mm or larger were bivalved and submitted for routine hematoxylin and eosin examination. The following histopathologic features were assessed for each tumor specimen including tumor type (classified as adenocarcinoma or mucinous carcinoma), invasive depth (classified as T2, T3, and T4), and tumor grade (classified as well, moderately, and poorly differentiated).

The median follow-up period was 36 months (range 18–68 months). All 180 patients were routinely followed up on until their deaths. The serum carcinoembryonic antigen level was measured every 3 months for the first 2 years, and every 6 months for the following 3 years. Abdominal ultrasonography was performed every 6 months. Annual computed tomography for the chest and abdomen was also carried out. The development of new recurrent or metastatic lesions after operation was defined as a postoperative relapse. The median time to recurrence of these patients was 18.3±4.5 months. The number of lymph nodes examined per specimen was recorded to determine if a

specific cutoff could affect clinical outcome. The cutoff we considered to be the best indicator for separating patients with regard to survival was that which showed the clearest rise in statistical significance. Patients were further divided into two groups based on the threshold of the adequate number of examined lymph nodes.

Statistical Analysis

All data were analyzed by the Statistical Package for the Social Sciences, version 11.5 (SPSS Inc., Chicago, IL, USA). The univariate analysis of clinicopathologic features between the two groups was compared using the chi-square test. The multivariate analysis of independent prognostic factors for postoperative relapse was determined using logistic regression analysis. The cumulative survival rates were calculated by the Kaplan–Meier method, and the differences in survival rates were analyzed by the log-rank test. A P value of less than 0.05 was considered to be statistically significant.

Results

The clinical and pathologic data regarding the 180 $T_{2–4}N_0M_0$ CRC patients are summarized in Table 1. There were 84 men (46.7%) and 96 women (53.3%). The average age was 69.0 years (range 32–93 years). The number of sites where the tumor was at the colon was 132 (73.3%) and 48 (26.7%) at rectum. One hundred and sixty-eight patients (93.3%) were classified as adenocarcinoma in histology, and 12 patients (6.7%) as mucinous carcinoma. With regard to the histological types of these tumors, 25 were well-differentiated carcinoma, 135 were moderately differentiated carcinoma, and 20 were poorly differentiated carcinoma. A mean number of 12 lymph nodes (range 0–66) were examined per tumor specimen. The average value of identified lymph nodes in tumors of the right colon, left colon, and rectum was 14.84, 11.28, and 10.12, respectively. From the relationship between postoperative relapse and the number of examined lymph nodes, we found that those with 18 or more lymph nodes had no postoperative relapse. Therefore, the adequate number of lymph nodes to separate the $T_{2–4}N_0M_0$ CRC patients into subgroups was set at 18 in our study. The incidence of postoperative relapse among the subgroups with lymph nodes <18 was not prominently different, with a range of 25.0 to 30.8%. On the basis of this finding, 180 $T_{2–4}N_0M_0$ CRC patients were divided into two groups (group 1: examined lymph nodes fewer than 18; group 2: examined lymph nodes equal to or more than 18). By univariate analysis, there were no significant differences regarding the age, gender, tumor site, invasive depth, tumor grade, and tumor type between the two groups, except for the tumor size between the two groups (Table 2; $P<0.001$).

Moreover, the presence of postoperative relapse significantly correlated with the tumor size ($P=0.040$), invasive depth ($P=0.031$), histology ($P=0.047$), and the number of examined lymph nodes (Table 3; $P<0.001$). Figure 1 details the overall survival rate of CRC patients according to the number of examined lymph nodes (≧ 18 or <18). The survival rate of the number of examined lymph nodes <18 group was significantly lower than that of the examined lymph nodes ≧ 18 group using a log-rank test ($P=0.015$). Using multivariate logistic regression analysis, both the number of examined lymph nodes ($P=0.005$) and depth of tumor invasion ($P=0.028$) were demonstrated to be independent predictors for postoperative relapse (Table 4). Regarding the sites of postoperative relapse, 17, 9, 8, 7, 6, and 6 were attributed to the liver, local recurrence, peritoneal carcinomatosis, the bones, retroperitoneal causes, and the lungs, respectively.

Discussion

Lymph node involvement is one of the most important prognostic factors after radical surgery for CRC.[12–14] The

Table 1 Clinicopathologic Characteristics of 180 $T_{2–4}N_0M_0$ Colorectal Cancer Patients

Variables	Number of Patients (%)
Age (years)	
<60	148 (82.2)
≧60	32 (17.8)
Gender	
Male	84 (46.7)
Female	96 (53.3)
Maximum tumor size (cm)	
≧ 5	62 (34.4)
<5	118 (65.6)
Location	
Colon	132 (73.3)
Rectum	48 (26.7)
Differentiation	
Well	25 (13.9)
Moderately	135 (75.0)
Poorly	20 (11.1)
Depth of tumor invasion	
T2	54 (30.0)
T3 and T4	126 (70.0)
Type of tumor	
Adenocarcinoma	168 (93.3)
Mucinous	12 (6.7)
Number of examined lymph nodes	
≧ 18	35 (19.5)
<18	145 (80.5)

Table 2 Univariate Analysis of Clinicopathologic Features of $T_{2–4}N_0M_0$ Colorectal Cancer Patients Between the Number of Examined Lymph Nodes <18 and ≧ 18

	Lymph Nodes <18 (*N*=145)	Lymph Nodes ≧ 18 (*N*=35)	*P* value
Age (years)			
<60	26 (17.9)	6 (17.1)	0.913
≧ 60	119 (82.1)	29 (82.9)	
Gender			
Male	72 (49.6)	12 (34.3)	0.102
Female	73 (50.4)	23 (65.7)	
Maximum size			
<5 cm	106 (73.1)	12 (34.3)	<0.001
≧ 5 cm	39 (26.9)	23 (65.7)	
Location			
Colon	104 (71.7)	28 (80.0)	0.320
Rectum	41 (28.3)	7 (20.0)	
Histology			
WD	18 (12.4)	7 (20)	0.357
MD	112 (77.2)	23 (65.7)	
PD	15 (10.4)	5 (14.3)	
Depth of tumor			
T2	48 (33.1)	6 (17.1)	0.064
T3 and T4	97 (66.9)	29 (82.9)	
Type of tumor			
A	135 (93.1)	34 (97.1)	0.371
M	10 (6.9)	1 (2.9)	

WD = well-differentiated, MD = moderately differentiated, PD = poorly differentiated, A = adenocarcinoma, M = mucinous carcinoma

prognostic value of lymph node involvement in patients who undergo resection for CRC has been well established.[14–17] In addition to the prognostic significance of nodal metastases, the presence of tumor cells in the regional node basin is an important criterion for a recommendation of adjuvant systemic therapy.[18] An accurate examination of the surgical specimen is mandatory to assess the lymph node status of the tumor correctly.[6] Theoretically, all the lymph nodes should be harvested from the surgical specimens and examined to confirm a tumor is negative of lymph node involvement.

The actual number of lymph nodes must be harvested and examined in the resected bowel because CRC has not yet been determined definitively. Despite recent interest in this subject, the number of nodes required to accurately stage patients is controversial, and considerable variation exists among studies. The variability in the number of lymph nodes in the various regions of the large bowel, the extent of surgical lymphadenectomy, the searching ability for lymph nodes by pathologists, and the different statistical methods employed in different studies are major impediments. These variables most probably explain the lack of agreement in determining a universally valid minimum number of lymph nodes. Scott and Grace found that when at least 13 lymph nodes are examined histologically, more than 90% of the specimens containing nodal metastases can be identified.[18] In 1990, the Working Party Report to the World Congress of Gastroenterology in Sydney recommended that a minimum of 12 lymph nodes be recovered.[5] Goldstein et al.[6] reported the probability of correctly classifying a colorectal tumor as node-positive when 17 lymph nodes are examined. Similarly, the high-risk groups for recurrence/metastasis were identified in Dukes' A and B CRC patients with harvested lymph nodes ≦ 14.[19] Hernanz et al.[20] and Caplin et al.[21] demonstrated that a minimum of six lymph nodes examined per specimen is necessary for correct Dukes' B staging, and six or fewer lymph nodes examined in Dukes' stage B CRC patients correlated with poorer survival when compared with examining seven or more. Moreover, it has been suggested that the examination of at least 14 nodes after resection of T2 or T3 carcinoma of the colon and rectum will accurately stage the lymphatic basin.[22]

As a rule, three variables must be considered when examining the issue of nodal harvest: patient factors, surgical factors, and pathologic factors. Patient factors

Table 3 Univariate Analysis of Clinicopathologic Features between $T_{2–4}N_0M_0$ Colorectal Cancer Patients with and Without Postoperative Relapse

	Postoperative relapse (+) (*N*=42) (%)	Postoperative relapse (-) (*N*=138) (%)	*P* value
Age (years)			
<60	7(16.7)	25(18.1)	0.830
≧ 60	35(83.3)	113(81.9)	
Gender			
Male	20(47.6)	63(45.7)	0.823
Female	22(52.4)	75(54.3)	
Maximum size			
<5 cm	22(52.4)	96(69.5)	0.040
≧ 5 cm	20(47.6)	42(30.5)	
Location			
Colon	31(73.8)	101(73.2)	0.936
Rectum	11(26.2)	37(26.8)	
Histology			
WD	2(4.8)	22(15.9)	0.047
MD	32(76.2)	104(75.4)	
PD	8(19)	12(8.7)	
Depth of tumor			
T2	7(16.7)	47(34.1)	0.031
T3 and T4	35(83.3)	91(65.9)	
Type of tumor			
A	37(88.1)	131(94.9)	0.120
M	5(11.9)	7(5.1)	
Number of examined lymph nodes			
<18	42(100)	103(74.6)	<0.001
≧ 18	0(0)	35(25.4)	

WD = well-differentiated, MD = moderately differentiated, PD = poorly differentiated, A = adenocarcinoma, M = mucinous carcinoma

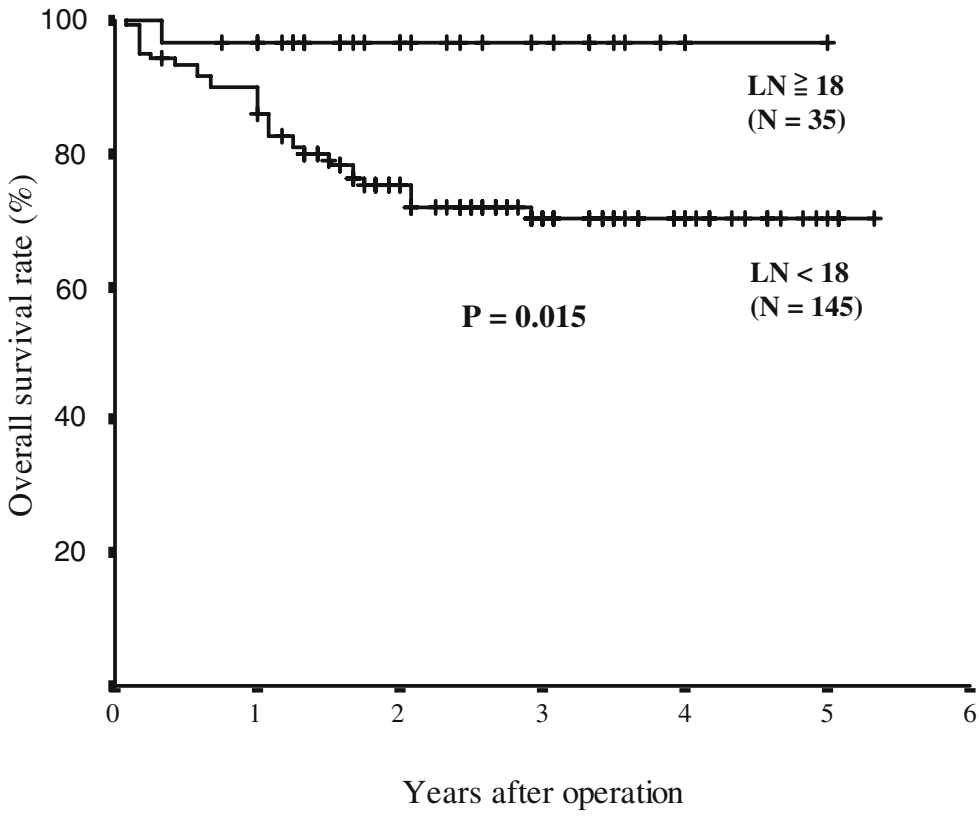

Figure 1 Cumulative overall 5-year survival rates of $T_{2–4}N_0M_0$ colorectal cancer patients were analyzed by the Kaplan–Meier method with the differences compared by a log-rank test. Patients who had 18 or more lymph nodes examined had a significantly higher survival rate than those who had less than 18 nodes examined (P=0.015).

may reflect anatomic or individual variability in nodal harvest.[23] For example, some studies have demonstrated that right-sided resections are associated with a greater nodal harvest than left-sided resections.[20] Likewise, the mean number of examined lymph nodes for right-sided resections is more than left-sided resections in our investigations. Leopoldo et al.[24] have also reported that lymph node retrieval in TNM stage II CRC patients is affected by the patient's age, gender, tumor grade, and tumor site. Conversely, we have identified that a significant number of retrieved lymph nodes ≧ 18 is influenced by tumor size in our analysis. The incidence of ≧ 18 lymph node harvest increases with larger-sized ≧ 5 cm tumors.

Nevertheless, the impact of the pathology and surgery as it pertains to colorectal lymph node harvest is largely unreported. Given that this is a single institution, fairly uniform pathological and surgical techniques would be expected. It is possible that some of the variability is patient related, i.e., that the number of lymph nodes in a given patient varies, and that this may have an independent effect on survival. In the present study, no postoperative relapse was observed in $T_{2–4}N_0M_0$ CRC patients with a harvest of 18 or more lymph nodes. On the other hand, our findings reveal that the number of lymph node retrievals of at least 18 was significantly related to the postoperative relapse for $T_{2–4}N_0M_0$ CRC patients, in addition to the conventional depth of tumor invasion in TNM staging. Indeed, the poorer overall survival rate is also observed in $T_{2–4}N_0M_0$ CRC patients with 17 or fewer lymph nodes examined. Because the number of examined lymph nodes has been proven to be crucial in the prediction of postoperative relapse for $T_{2–4}N_0M_0$ CRC patients, the examination of 17 or fewer lymph nodes

Table 4 Correlation between Postoperative Relapse and Clinicopathologic Features of $T_{2–4}N_0M_0$ CRC Patients Using Multivariate Logistic Regression Analysis

Variables	β	SE	P value	Hazard Ratio	95% Confidence Interval
Tumor size (≧ 5 cm/<5 cm)	0.713	0.500	0.154	2.040	0.766–5.437
Histology (PD/WD+MD)	0.125	0.759	0.869	1.133	0.256–5.018
Depth (T3+T4/T2)	1.039	0.473	0.028	2.826	1.117–7.147
Lymph node retrieval (<18/≧ 18)	3.019	1.081	0.005	20.481	2.462–170.399

WD = well-differentiated, MD = moderately differentiated, PD = poorly differentiated, β = coefficient, SE = standard error

in $T_{2–4}N_0M_0$ CRC patients should be considered during follow-up meticulously for postoperative surveillance. Perhaps patients with $T_{2–4}N_0M_0$ CRC with 17 or fewer nodes examined might be potential candidates for post-operative adjuvant chemotherapy. Despite the numbers of lymph node harvest or the extent of lymph node dissection not greatly improving the accurate tumor staging, the increase of examined numbers of tumor-free lymph nodes would probably decrease the incidence of understaging or alter further therapies for these patients. However, additional work in larger patient populations by means of long-term follow-up studies is mandatory for confirming this hypothesis.

Conclusion

In summary, the recovery and examination of at least 18 lymph nodes per surgical specimen may be essential in reaching a more strict level of accuracy when defining operations for $T_{2–4}N_0M_0$ CRC as a curative resection. An increase in the number of tumor-free lymph nodes has been suggested as clinically important, and this parameter should be taken into consideration in CRC patients without metastatic lymph nodes. Further investigation regarding surgical and pathologic standardization is needed with the goal of reducing variability, thus permitting more consistent staging of patients with CRC.

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Successful Duct-to-duct Biliary Reconstruction after Right Hemihepatectomy. Operative Planning Using Virtual 3D Reconstructed Images

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Abstract Accurate knowledge of partial anatomy is essential in hepatic surgery but is difficult to acquire. We describe the potential impact of a new technique for constructing three-dimensional virtual images of the portal vein, hepatic artery, and bile ducts and present a representative case. An 80-year-old man was suspected of having papillary cholangiocarcinoma arising in S8 of the liver and extending to the hepatic hilum intraluminaly. Right hemihepatectomy with bile duct resection was planned. However, it was uncertain whether duct-to-duct biliary reconstruction would be possible based on the appearance of the confluence of the right and left hepatic ducts on cholangiogram and conventional computed tomograph. Virtual three-dimensional images of the liver were constructed and revealed vascular and biliary anatomy. They showed that the upper margin of bile duct excision would be 19 mm from the umbilical point of the left portal vein, and that the site of the left branch of the caudate lobe bile duct could be preserved. Based on this information, we performed a sphincter-preserving biliary operation safely without complications. Planning complex biliary surgery may be improved by the use of virtual three-dimensional images of the liver. This approach is especially useful in candidates for postoperative regional chemotherapy.

Keywords Liver resection · 3D · Duct-to-duct biliary reconstruction

Introduction

Evolution in surgical techniques has made hepatectomy much safer.[1,2] However, it is still sometimes difficult to manage tumors encroaching on the hepatic hilum. Most of these cases are treated by hepatectomy with bile duct resection and Roux-en-Y bilioenteric anastomosis. Although duct-to-duct biliary reconstruction can preserve the sphincter of Oddi and has potential clinical advantages, few case reports of duct-to-duct biliary reconstruction after hepatectomy with bile duct resection have been published.[3] One reason is that it is difficult to obtain a clear tumor margin with limited bile duct resection. Additionally, the risk of postoperative complications, such as anastomotic leak or stricture, is probably greater than with bilioenteric anastomosis.

Until recently, standard liver resection has been planned based on two-dimensional (2D) computed tomography and ultrasonography. However, the superiority of using 3D virtual images in hepatobiliary surgery has been documented.[4] 3D images provide useful information concerning the relative positions of anatomic structures in the liver. Because the biliary branching pattern around the hilum shows great variability,[5–7] 3D images can be used to define the individual anatomy and enhance the safety of surgical procedures.

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We describe a case of duct-to-duct biliary reconstruction after right hemihepatectomy that shows the advantages of using 3D images in operative planning.

Case Presentation

An 80-year-old man with a history of sigmoid colon cancer 6 years previously was referred with a hepatic mass. A recent computed tomography (CT) scan showed a hypodense mass in the right anterior segment of the liver, with dilatation of the peripheral intrahepatic bile ducts (Fig. 1a). This tumor extended to the hilus within the right hepatic duct (Fig. 1b). Endoscopic retrograde cholangiopancreatography (ERCP) revealed obstruction of the right anterior segmental bile duct with a polypoid shadow defect and possible encasement of the root of the posterior bile duct (Fig. 2). These findings suggested that the tumor extended to the confluence of the hepatic ducts. The posterior branch was seen entering the left hepatic duct. The patient underwent upper GI fiberscopy and colonoscopy, neither of which demonstrated any mucosal lesions. The differential diagnosis was primary intrahepatic cholangiocarcinoma, intraductal papillary type, versus metastatic liver cancer from the sigmoid colon. Based on the finding of 2D CT and cholangiograpy, right hemihepatectomy with bile duct resection was planned. The estimated percentage of liver resection was 62% of the total liver volume. Portal vein embolization through the ileocolic vein was performed.

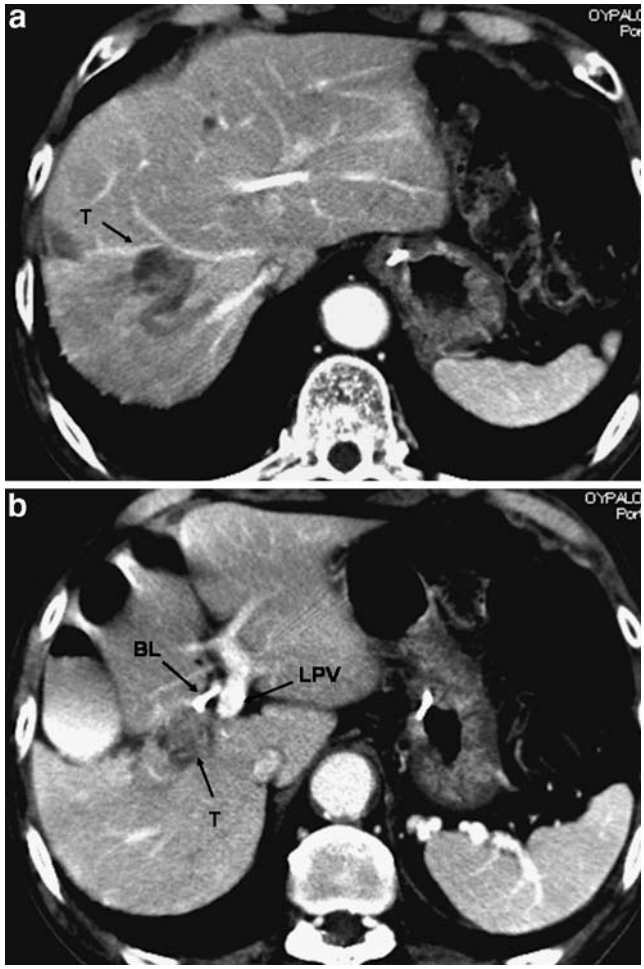

Figure 1 (a) Computed tomography scan shows a hypodense mass in the anterior segment of the right lobe of the liver with dilatation of peripheral intrahepatic bile ducts. T = tumor. (b) This tumor extends to the hilus within the right hepatic duct. T = tumor; BL = the left hepatic duct; LPV = the left portal vein.

The method of biliary reconstruction was the topic of some discussion. Because the patient was a candidate for postoperative regional chemotherapy, preservation of the sphincter of Oddi by duct-to-duct biliary reconstruction was desirable. Based on the cholangiogram, the left hepatic duct needed to be cut just peripheral to the confluence with the posterior bile duct to obtain a clear margin, and the common hepatic duct needed to be divided 15 mm to the hilar side from of the origin of the cystic duct. However, we felt it would be difficult to define the anatomy intraoperatively because biliary bifurcation within the hilar plate would not be visible. Moreover, it was unclear whether caudate lobectomy was necessary because the branching pattern of the caudate lobe bile duct and portal branches was not depicted by either 2D CT or cholangiogram.

Operative Planning

3D images of the liver were created from multidetector row computed tomography data sets using HepaVision and InterventionalPlanner, software tools specifically developed for 3D visualization and virtual resection of the liver. These tools were developed using the prototype platform at the Center for Medical Diagnostic Systems and Visualization at the University of Bremen (MeVis, Bremen, Germany). To start, based on the cholangiogram, markers identifying the upper and lower margins of transsection were placed on the 3D image. The upper marker was placed just peripheral to the root of the posterior bile duct, and the lower marker was placed 15 mm from the origin of the cystic duct. 3D images indicated that the distance between the incision in left hepatic duct and the umbilical portion of the left portal vein was 19 mm (Fig. 3a), and the distance between the upper and lower margins of transsection was 20 mm. The images clearly showed that the biliary branches of the left caudate lobe joined the left hepatic duct and that the portal branches of the left caudate lobe were not involved by tumor. On the other hand, the right half of the caudate lobe needed to be resected because the biliary branches of the right caudate lobe joined the posterior hepatic duct. The line of trans-

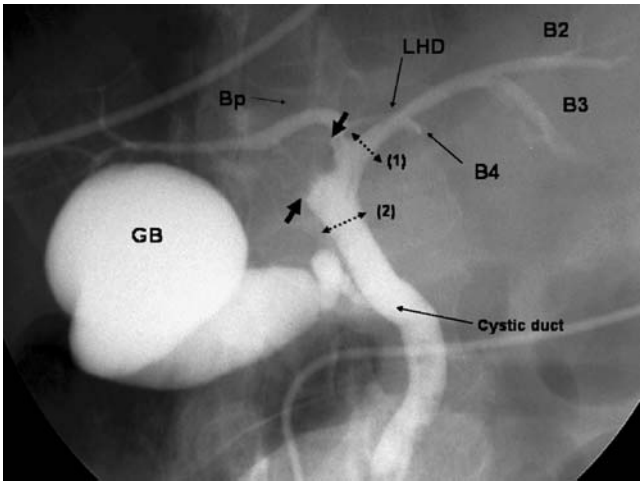

Figure 2 Endoscopic retrograde cholangiography reveals obstruction of the right anterior segmental bile duct with a polypoid shadow defect (thick arrow) and possible encasement of the root of the posterior bile duct. The posterior branch enters the left hepatic duct. The left hepatic duct needs to be cut just peripheral to the bifurcation of the posterior bile duct to obtain a clear margin (1), and the common hepatic duct needs to be cut 15 mm proximal to the confluence with the cystic duct (2). LHD = the left hepatic duct; Bp = bile duct of the posterior segment; GB = gall bladder; B2 = bile duct of segment 2; B3 = bile duct of segment 3; B4 = bile duct of segment 4.

section was simulated (Fig. 3b), and right hemihepatectomy including the right half of the caudate lobe and duct-to-duct biliary reconstruction was planned.

At operation, there was no evidence of disseminated disease. The liver was examined carefully using intraoperative ultrasonography and palpation. A large tumor was appreciated within segment 8 that extended to the hilum. There was no evidence of other intrahepatic disease. After intraoperative ultrasound, the right lobe of the liver was mobilized. The hepatic ligament was divided, and the falciform ligament was taken back to just above the vena cava. Small branches of the short hepatic vein were divided to expose the right half of the vena cava, and the right hepatic vein was encircled. The head of the pancreas was mobilized to allow subsequent duct-to-duct biliary reconstruction, and lymphadenectomy in the hepatic hilum was performed. The gallbladder was removed, and the common bile duct was identified and encircled by tape. A 4-0 Prolene stitch was placed in the left hepatic duct 19 mm from the right border of the umbilical point as a marker for the planned site of transsection. The right hepatic artery was ligated and divided, the right portal vein was encircled and divided, and parenchymal transsection was begun along the demarcation line using the Cavitron ultrasonic surgical aspirator (CUSA) and bipolar irrigation electric cautery. The Pringle maneuver was not performed. Next, the bifurcation of the hepatic duct was excised. The left hepatic duct was divided at the previously marked point, and the lower bile duct was divided 20 mm from the upper margin of the transsection. Finally, the right hepatic vein was cross-clamped, divided, and oversewn. The specimen was removed and inspected. Grossly, the surgical margin was clear by about 5 mm. Frozen section examination of the cut ends of the upper and lower bile ducts showed no cancer. The common hepatic duct was brought up to the cut end of the left hepatic duct, and a duct-to-duct anastomosis was

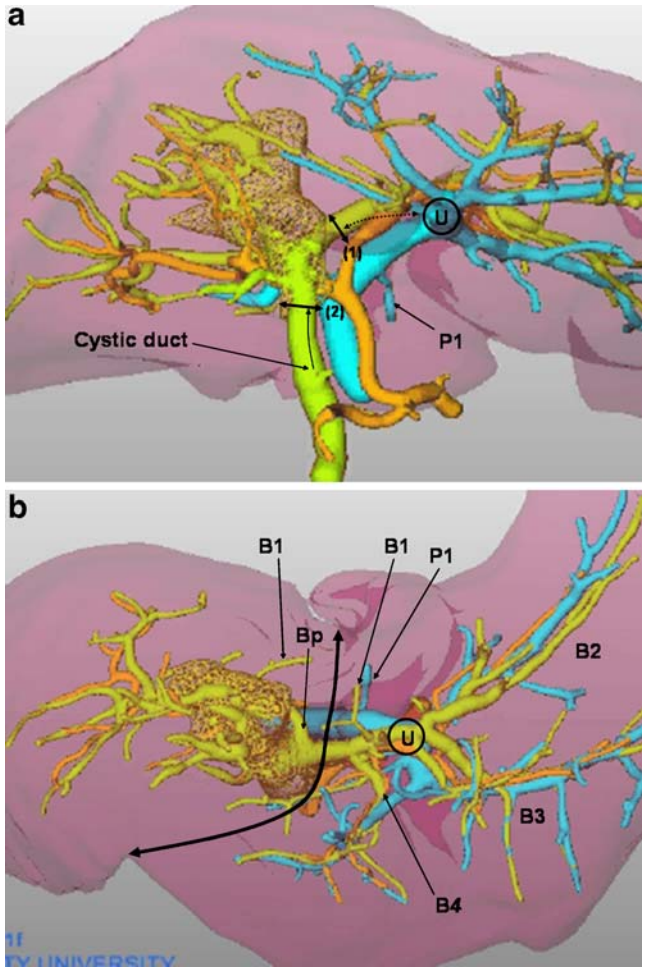

Figure 3 (a) Virtual three-dimensional (3D) reconstruction of the hepatic hilum. 3D image shows the portal vein, hepatic artery, and bile duct simultaneously. The upper marker was placed just peripheral to the root of the posterior bile duct (1). The lower marker was placed 15 mm proximal to the origin of the cystic duct (2). The distance between the point of left hepatic duct transsection and the umbilical portion (U) of the left portal vein is 19 mm (interrupted line). The distance between the upper and lower markers is 20 mm. The bile duct from the left caudate lobe joins with the left hepatic duct and the portal branches of the left caudate lobe are not involved by tumor. However, the right half of the caudate lobe needs to be resected because its bile duct branches joined the posterior hepatic duct. P1 = portal branch of segment 1. (b) Right hemihepatectomy including the right half of the caudate lobe was planned. Bp = bile duct of the posterior segment; B1 = bile duct of segment 1; B2 = bile duct of segment 2; B3 = bile duct of segment 3; B4 = bile duct of segment 4; P1 = portal branch of segment 1.

performed using interrupted 5-0 polydioxanone (PDS) sutures (Fig. 4). The anastomosis was splinted with a 4-Fr. retrograde trans-hepatic biliary drainage tube (RTBD). The time of operation was 7 h and the estimated blood loss was 400 ml.

The postoperative course was uneventful. Postoperative tube cholangiography showed neither anastomotic leakage nor biliary stricture (Fig. 5), and the patient was discharged 20 days after operation. The final pathologic diagnosis was metastatic sigmoid colon cancer. Surgical margins were all clear. The patient is alive without recurrence or biliary stricture 1 year after surgery.

Discussion

Although considerable progress has been made in liver surgery, reports of duct-to-duct biliary reconstruction for tumors encroaching on the hepatic hilum are rare.[3] One reason is the difficulty in obtaining a clear margin and satisfactory anastomosis with limited bile duct resection. Thus, hepaticojejunostomy is generally used for reconstruction in this kind of procedure. However, duct-to-duct biliary reconstruction offers some advantages.

First, hepatic arterial infusion chemotherapy (HAI) is associated with an increased risk of liver abscess,[8] and biliary stasis consequent to bilioenteric anastomosis further increases the risk of liver abscess.[9] Second, the risk of acute cholangitis is increased, and can be life-threatening. Preservation of sphincter function should decrease the risk of biliary infection.

Duct-to-duct biliary reconstruction is common in living-related liver transplantation. Kasahara et al.[10] reported that the incidence of biliary leakage and stricture after duct-to-duct biliary reconstruction is 4.7% and 26.6%, respectively, whereas, the incidence of biliary leakage after Roux-en-Y choledochojejunostomy is 12.4%. Although the incidence of biliary stricture with duct-to-duct anastomosis is higher than with bilioenteric anastomosis, three fourths of strictures could be managed endoscopically. We should expect further reductions in complication rates as surgical techniques evolve.

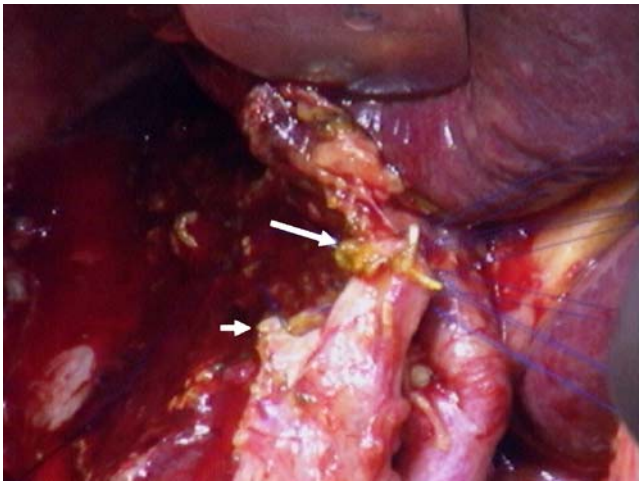

Figure 4 Duct-to-duct anastomosis was performed using interrupted 5-0 polydioxanone (PDS) sutures. Long arrow: anastomotic site; short arrow: cut end of the cystic duct.

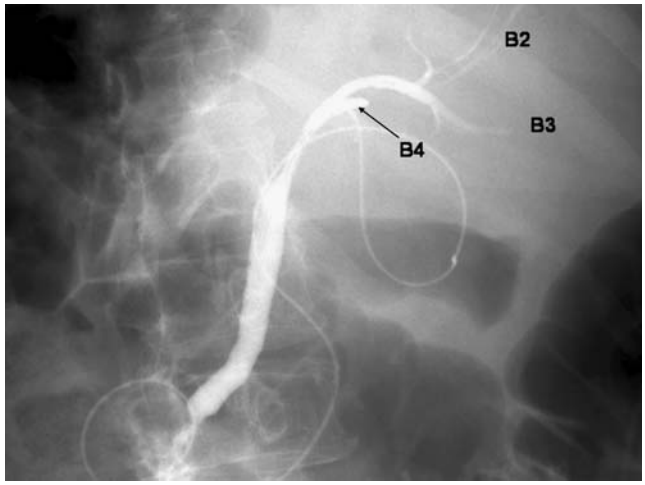

Figure 5 Postoperative tube cholangiography shows neither anastomotic leakage nor biliary stricture. B2 = bile duct of segment 2; B3 = bile duct of segment 3; B4 = bile duct of segment 4.

The bifurcation pattern of hilar bile duct cannot be observed directly intraoperatively, because it is covered by the hilar plate. Thus, the point of hepatic duct transsection must be planned preoperatively. However, it is difficult to plan bile duct resection by cholangiography alone, as the point of transsection must be established relative to the position of the umbilical portion of the left portal vein. Several authors have recommended 3D imaging for operative planning in hepatobiliary surgery to facilitate venous reconstruction in living-related liver transplantation.[11–13] However, 3D imaging to plan biliary reconstruction has not been reported. We found that preoperative 3D images of the portal triad is helpful in assessing the distance between the planned site of left hepatic duct transsection and the umbilical portion of the left portal vein. In this case, we anticipated at least a 2-cm defect, which we felt was technically manageable by choledochocholedochostomy.

Additionally, knowing the anatomy of the caudate lobe branches of both the portal vein and bile duct helps to prevent iatrogenic injury, which may result in persistent biliary fistula.[14] This region is difficult to define using conventional cholangiography. Furukawa et al.[15] reported that 3D cholangiography eliminates overlap of different biliary branches. In the present case, 3D images showed the distribution of caudate lobe branches so that the left caudate lobe could be preserved.

In this patient, extraluminal bile duct invasion was minimal. This allowed us to resect a relatively short segment of the common hepatic duct. Some authors have reported that macroscopic intrabiliary extension indicates a less aggressive behavior. This characteristic has been investigated for both metastatic tumors from colon cancer and primary intrahepatic cholangiocarcinoma.[16,17] If this patient had had diffuse sclerosing infiltration extending to the bifurcartion of the hepatic duct, Roux-en-Y hepaticojejunostomy would have been necessary.[18]

In conclusion, duct-to-duct biliary reconstruction was performed successfully in a patient with a hepatic metastasis encroaching on the hepatic hilum. Preoperative 3D imaging to visualize the anatomic structures in the hepatic hilum is a new tool that will help surgeons individualize treatment and reduce morbidity and mortality of hepatic surgery.

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Diagnosis and Surgical Management of Gallbladder Cancer: A Review

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Abstract Gallbladder cancer is one of the most lethal carcinomas and continues to pose many challenges for surgeons. Identifiable risk factors for carcinoma of the gallbladder include cholelithiasis, an anomalous pancreaticobiliary junction, and focal mucosal microcalcifications. Adenocarcinoma is the primary histologic type in most patients and the tumor is frequently associated with Kras and p53 mutations. Radiologic and endoscopic advances in endoscopic ultrasonography and magnetic resonance cholangiopancreatogram, plus helical computed tomography, have enhanced preoperative staging. Surgical options include cholecystectomy for disease limited to the mucosa (Tis/T1) or a radical cholecystectomy (subsegmental resection of segments IVB and V plus a hepatoduodenal ligament lymphadenectomy) for advanced disease without signs of distant metastasis (T2-4/N0-N2). Some surgeons have advocated more radical hepatic resection including extended right hepatectomy or central bisegmentectomy plus caudate lobectomy. Japanese surgeons have reported studies that included patients having a pancreaticoduodenectomy to improve distal ductal margins and lymphadenectomy for T3 and T4 cancers. These patients have a lower rate of local recurrence but no survival advantage. Options for adjuvant therapy remain limited. Radiation therapy with fluorouracil radiosensitization is the most commonly used postoperative treatments. Current trials are investigating the role of capecitabine, oxaliplatin, and bevacizumab in the management of gallbladder carcinoma.

Keywords Gallbladder cancer · Biliary tract · Gastrointestinal cancer

Introduction

Gallbladder cancer (GBC), a rare and highly lethal disease first described in 1777 by deStoll,[1] is the most common malignant neoplasm of the biliary tract and the seventh most common gastrointestinal cancer.[2] During 2005, it was estimated that 7,480 cases of cancer of the biliary tract, the majority arising from the gallbladder, were diagnosed in the United States, and 3,340 patients were expected to die from this disease.[3] In most patients, GBC presents at an advanced stage, often at the time of cholecystectomy for presumed chronic cholecystitis. GBC poses a challenge for both the clinician and the surgeon to improve outcomes.

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Epidemiology

GBC affects women more commonly than men in all populations, with some series reporting prevalences three to five times higher for females.[4] The highest frequency occurs among women over 65 years of age with a long history of gallstones. In our experience at Mayo Clinic Rochester, the female-to-male ratio was 2.5:1.[5]

GBC has a worldwide geographic distribution that correlates with the prevalence of gallstone disease. The world's highest prevalence of GBC is in Bolivia (15.5/100,000), but GBC is also common in Chile, Northern India, and Central European countries. In the United States,

the prevalence varies among ethnic groups, with Native Americans and Mexican Americans having the highest rate of GBC. African Americans have the lowest prevalence of GBC at 1/100,000.[4,6]

Risk Factors and Pathogenesis

Knowledge of the pathogenesis of GBC is limited, and currently, this disease is considered to have a multifactorial etiology. In general, factors associated with gallstone disease, such as obesity, a high fat and carbohydrate diet, multiple pregnancies, and the use of estrogens, correlate with an increased risk of GBC.[7,8]

Cholelithiasis is the best known risk factor for GBC. Chile, which has the world's highest mortality from GBC, has one of the highest prevalences of cholelithiasis.[9,10] The vast majority of patients with gallbladder neoplasms also have gallstones. The risk of GBC is four to five times higher in patients with gallstones than in acalculous individuals.[11] A study of 2,583 residents of Rochester, MN, with cholelithiasis found a threefold increase in the risk of GBC in men who had stones. Interestingly, the risk was not increased for women.[12]

The mechanism by which cholelithiasis predisposes to GBC has yet to be established. A large stone size and the duration of gallstone symptoms are factors that have been associated with the development of gallbladder neoplasia.[13] The risk of GBC has been proportional to the size of the stones in some studies. Because gallstone size can be related to the age of the calculus, the time the stone has been present is probably the more relevant factor in the pathogenesis of GBC.[14] Chronic inflammation of the gallbladder mucosa by gallstones may predispose to malignant transformation via a sequence evolving from atypia to dysplasia to carcinoma in situ and, finally, to invasive carcinoma.[15] Mutations of the p53 gene may have an important role in this sequence of events.[16]

Despite the association with cholelithiasis, only 1–3% of patients with gallstones develop GBC and some patients with GBC do not have stones.[17] Ransohoff and Gracie estimated the incidence of GBC for symptomatic gallstone patients to be 0.00078 per year after analyzing 4,781 patients comprising 11 cohorts and approximately 32,134 person-years of follow-up.[18]

Porcelain gallbladder has traditionally been regarded as a risk factor for GBC. The association was first reported in the 1960s, when an Argentinean study reported 16 GBC cases in 26 patients with calcified gallbladders.[19] This observation prompted the recommendation of cholecystectomy in all patients with a calcified gallbladder wall because of the high associated risk of GBC. Recent observations, however, report an overall lower incidence of GBC, 5% (2/44), in patients with calcifications within the gallbladder wall. When the pattern of calcification is further examined, the incidence of GBC is increased in the presence of focal mucosal calcification to 7% (2/27) when compared to those with intramural calcifications.[20] It seems that the pattern of calcification is more important than the mere presence of calcifications, with focal mucosal calcifications posing the greatest risk over diffuse intramural calcifications.

An anomalous junction of the pancreaticobiliary duct (AJPBD) also increases the risk of GBC. This anatomic variant allows pancreatic secretions to reflux into the biliary tree and induce chronic inflammation and metaplastic epithelial changes. It is found more frequently in Asia. GBC occurs in approximately 10–18% of Asian patients with this anatomic variant.[21,22,23]

Most small gallbladder polyps are asymptomatic, benign lesions that do not progress to cancer. Neoplastic polyps can harbor foci of carcinoma and are a predisposition for GBC. Polyp characteristics associated with an increased risk of malignancy include polyp diameter greater than 10 mm, patient age greater than 50 years, presence of gallstones, solitary polyps, and symptomatic polyps.[24] Polyps larger than 10 mm should be treated with cholecystectomy, whereas polyps smaller than 10 mm in patients without other risk factors can be followed with serial ultrasonography. Other conditions associated with an increased risk of GBC are xanthogranulomatous cholecystitis,[25] chronic typhoid infection,[26] adenomyomatosis of the gallbladder, and inflammatory bowel disease.[27,28]

Pathology

The dysplasia–carcinoma sequence for GBC has been proposed in the literature. Black demonstrated areas of carcinoma in situ in most specimens with invasive GBC.[29] The time to progress from dysplasia to carcinoma is estimated to require 10 to 15 years.[30] GBC typically does not present with adenomatous polyps but with a background of chronic mucosal inflammation. The validation for the frequent occurrence of an adenoma–carcinoma sequence, as seen in colon cancer, remains unproven in GBC.

The majority (60%) of GBCs arise in the fundus of the gallbladder, whereas 30% occur in the body and 10% in the neck.[18] The vast majority of GBCs (85–90%) are adenocarcinomas. Squamous-cell and adenosquamous carcinomas (2–10%), undifferentiated carcinomas (2–7%), and rare primary gallbladder neoplasms (<5%) (small-cell carcinoma, clear-cell carcinoma, neuroendocrine carcinoma, sarcoma, melanoma, and lymphoma) comprise the other histologic types of GBC (Table 1). Adenocarcinomas are subdivided into papillary, tubular, and nodular variants.

Table 1 Cellular Types of Gallbladder Cancer Based on AJCC Cancer Manual 6th Edition

Type
Carcinoma in situ
Adenocarcinoma, NOS
Papillary carcinoma
Adenocarcinoma, intestinal type
Mucinous carcinoma
Clear cell adenocarcinoma
Signet-ring cell carcinoma
Adenosquamous carcinoma
Squamous cell carcinoma
Small cell (oat cell) carcinoma[a]
Undifferentiated carcinoma[a]
Carcinoma, NOS
Carcinosarcoma

NOS=not otherwise specified
[a] Grade 4 by definition

Papillary carcinomas grow into the lumen of the gallbladder and behave less aggressively. They are less likely to invade the liver and have a lower incidence of lymph node metastasis. The gallbladder can be the site of distant metastasis from other primary cancer sites, with lung and melanoma being the most common metastatic tumors.[31,32]

Studies examining the molecular changes in GBC have noted frequent mutations of p53 and K-ras. The reported prevalences of GBC harboring p53 mutations range from 35–92%.[33,34,35] K-ras and p53 mutations have been associated with GBC in patients having AJPBD, suggesting that the reflux of pancreatic juice might contribute to a carcinogenic environment.[36] The detection of a K-ras mutation might serve as a useful tool in screening early GBC in patients with AJPBD.

Clinical Presentation

A significant barrier to improving the outcomes of GBC is the delayed clinical presentation in most patients, primarily due to a lack of specific symptoms and low clinical suspicion. Because the symptoms of GBC are usually nonspecific, at least 20% of patients are diagnosed at the time of cholecystectomy for biliary colic and cholelithiasis. Abdominal pain is the most common symptom of GBC (73%), followed by nausea and vomiting (43%), jaundice (37%), anorexia (35%), and weight loss (35%).[5] Constitutional symptoms, ascites, and a palpable mass are all indicative of advanced disease and poor prognosis.[37] Other less common presentations include duodenal obstruction, gastrointestinal bleeding, or hematobilia due to the invasion of adjacent bowel or vessels.[38,39]

Staging

The staging of GBC is a critical component of the comprehensive management and reporting of this neoplasm because the depth of invasion through the gallbladder wall and extent of lymph node metastasis dictate the operative management and correlate with prognosis. GBC is also classified by grade according to the level of differentiation, from well differentiated, grade 1, to poorly differentiated, grade 4. Grade-3 cancers are the most common.[40] The grading of GBC has no prognostic impact.

Multiple staging classifications have been described for GBC. The Nevin–Moran classification system,[41] originally described in 1976, and frequently used in the past, has been replaced by newer systems. The modified Nevin–Moran classification system,[42] the TNM system developed by the International Union Against Cancer and the American Joint Committee on Cancer (AJCC),[43] and the Japanese Biliary Surgical Society staging system[44] differ primarily in the value given to nodal metastasis. While controversy persists regarding which system is superior in predicting survival, the TNM system is used most commonly.

The modified Nevin staging system divides GBC into five stages according to the depth of penetration, the status of the hepatoduodenal ligament lymph nodes, and the presence of metastasis. Stage I has disease limited to the mucosa, stage II disease has tumor invasion into the muscularis layer, stage III includes neoplasms with transmural direct liver invasion, stage IV denotes the presence of lymph node metastasis, and stage V denotes distant metastasis. Fong and colleagues found that the modified Nevin system was not only superior to the 5th edition AJCC staging system in predicting prognosis, but also was useful in selecting patients for adjuvant therapy and stratification in clinical trials.[45]

The TNM classification system is the most accepted system worldwide (Table 2). This classification is based on depth of primary tumor invasion, local tumor extension, presence of metastasis to lymph nodes, and distant metastasis. The 5-year survival rates, stratified according to TNM stage (based on AJCC 5th edition staging criteria), derived from the National Cancer Database, were 60% for stage 0, 39% for stage I, 15% for stage II, 5% for stage III, and 1% for stage IV (Fig. 1).[2]

Single institution experiences with aggressive surgical management of GBC have shown improved 5-year survival rates by AJCC stage (5th edition) when compared with data pooled from multiple institutions.[5,46,47] T stage is a critical prognostic factor in GBC. The 5-year survival rate for patients with T1 neoplasms is greater than 85%; Yamaguchi reported a 100% 5-year survival in patients with T1 neoplasms in his analysis of 70 patients.[48] There are reports of 5-year survivals as high as 75% after radical resection for

Table 2 AJCC Staging, 6th Edition

Stage	T	N	M
0	Tis	N0	M0
IA	T1	N0	M0
IB	T2	N0	M0
IIA	T3	N0	M0
IIB	T1–T3	N1	M0
III	T4	Any N	M0
IV	Any T	Any N	M1

TNM classification of gallbladder cancer, 6th edition. For primary tumors, TX denotes the primary tumor cannot be assessed; T0 denotes no evidence of primary tumor; Tis denotes carcinoma in situ; T1 denotes that tumor invades lamina propria or muscle layer, T1a denotes tumor invades lamina propria, and T1b denotes tumor invades the muscle layer; T2 denotes tumor invades the perimuscular connective tissue; no extension beyond the serosa or into the liver; T3 denotes tumor perforates the serosa (visceral peritoneum) and/or directly invades the liver and/or one other adjacent organ or structure, such as the stomach, duodenum, colon, or pancreas, omentum or extrahepatic bile ducts; and T4 denotes tumor invades main portal vein or hepatic artery or invades multiple extrahepatic organs or structures. For regional lymph nodes, NX denotes the regional lymph nodes cannot be assessed, N0 denotes no regional lymph node metastasis, and N1 denotes regional lymph node metastasis. For distant metastasis, MX denotes the distant metastasis cannot be assessed, M0 denotes no distant metastasis, and M1 denotes distant metastasis.
T=primary tumor, N=lymph nodes, M=metastasis

patients with stage II disease. Five-year survival rates for patients with stages III and IVA remain below 33% with aggressive treatment, but are superior to the dismal results of a nonaggressive approach (<10%).[49]

Diagnostic Imaging

Ultrasonography is usually the first imaging test used to evaluate symptoms of biliary tract disease. For many reasons, ultrasonography has been an imperfect test to diagnose GBC. Ultrasonographic images often do not distinguish between GBC and chronic cholecystitis, especially in the early stages of GBC. The sensitivity of ultrasonography to recognize GBC is on the order of 44% (Fig. 2).[50] If, however, there is tumor infiltration of the liver or lymph node metastasis, the yield from ultrasonography will be higher. With direct infiltration into the liver, loss of the normal plane between the hepatic parenchyma and the gallbladder can be appreciated readily by an experienced radiologist. Haribhakti et al.,[51] in evaluating the effect of ultrasonography on GBC staging, found an overall accuracy of 38%. The majority of patients in the Haribhakti's study were understaged because of missed distant metastases and local tumor infiltration.

With the advent of endoscopic ultrasonography (EUS), more centers have incorporated EUS as a preoperative staging modality. GBC appears on EUS as a hypoechoic mass with or without gallbladder wall calcifications.[52] EUS can be used to obtain samples of the primary tumor, enlarged lymph nodes, or liver masses for cytology via fine needle aspiration. This is a tool that improves the sensitivity of diagnosing from 74 to 90% when compared to diagnosis with transabdominal ultrasonography alone.[53] Another potential adjunct to EUS is endoscopic retrograde cholangiopancreatography (ERCP). ERCP not only allows the endoscopist to obtain tissue for pathologic evaluation from lesions that extend into the biliary tree, it also identifies AJPBD and extrinsic compression of the extrahepatic ducts by tumor.

A more reliable diagnostic modality is computed tomography (CT). CT can readily evaluate not only the T stage but also the extent of locoregional disease (Fig. 3). Findings on CT may suggest strongly the presence of lymph node metastases, local invasion of the liver, and vascular involvement. Kim et al.[54] reported an overall accuracy of 71% with preoperative CT imaging. The

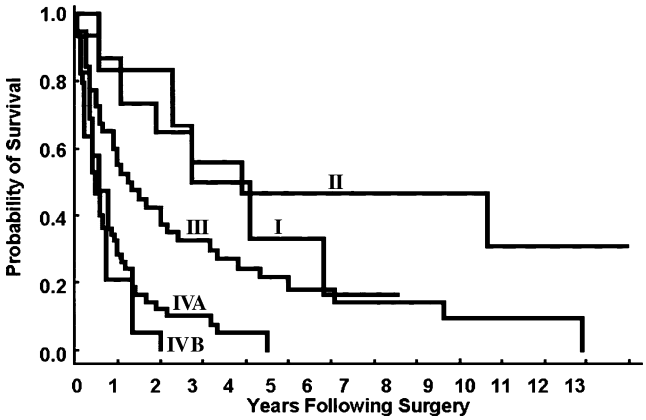

Figure 1 Survival according to TNM staging system. Modified from Taner et al.[5] (based on 5th edition).

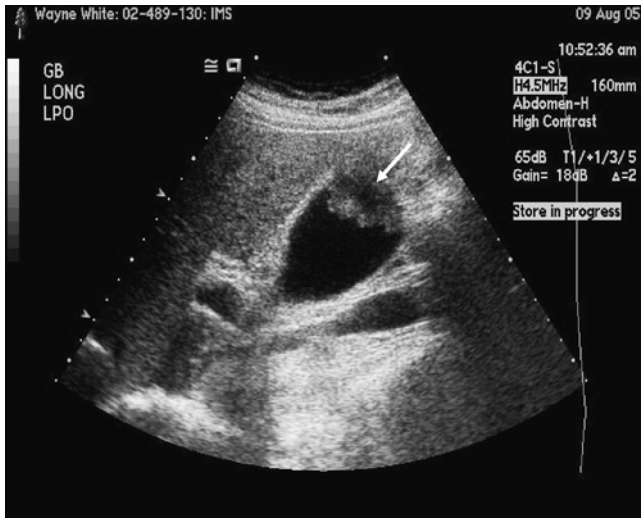

Figure 2 Ultrasonographic finding of gallbladder cancer (*white arrow*).

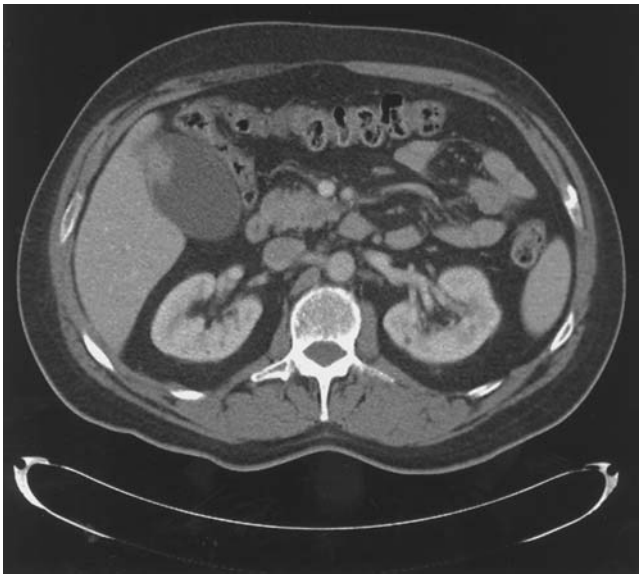

Figure 3 CT of mass in gallbladder later shown to be gallbladder cancer.

accuracy of CT is variable depending on the morphology of the neoplasm. For example, a T1 carcinoma with only a thickened wall is often missed on CT (sensitivity of 54% in these lesions). If, however, there is a large intraluminal mass, then a sensitivity of up to 89% has been reported. The ability of CT to accurately image gallbladder wall thickening improves with two-phase CT. This modality allows for enhanced visualization of wall thickening in the arterial and venous phases, which can help differentiate malignancy from chronic cholecystitis.[55]

As improvements in the technology of magnetic resonance imaging (MRI) have been introduced, the older diagnostic tools of selective celiac or hepatic angiography, ERCP, and transhepatic cholangiography have been replaced by magnetic resonance cholangiopancreatogram and magnetic resonance angiography (MRA) in the evaluation of GBC. When standard MR is combined with MR cholangiography and three-dimensional MRA, the sensitivity and specificity for vascular invasion can approach 100 and 87%, respectively. MRA is useful in diagnosing hepatic artery and portal vein invasion. When compared to CT, the sensitivity for MRI improved from 50% to 67–100% with a specificity of MRA of 89–100%. The detection rate of lymph node metastasis by MRI remains poor (57%).[56,57]

Surgical Treatment

The goal for treating GBC is an R0 resection. Because of advanced stage at presentation, only a third of patients are potential surgical candidates. GBC primarily invades the hepatic parenchyma, the hepatoduodenal ligament structures, and surrounding organs (duodenum, transverse colon, stomach, and small bowel). If preoperative imaging reveals hepatic metastases, encasement of the main portal vein or proper hepatic artery, or gross celiac or para-aortic lymphadenopathy, the patient is not eligible for surgical resection. Staging laparoscopy should be routinely performed prior to celiotomy because of the high rate of occult metastatic GBC. If metastatic disease is found at laparoscopy, tissue biopsy can avoid a nontherapeutic laparotomy.[58]

Contraindications for surgical resection include gross vascular invasion or encasement of major vessels (T4), ascites, diffuse hepatic involvement, distant metastasis, and poor functional status. Despite these grim figures, the only hope for cure remains an operative resection. Ito and colleagues reported that, of the GBC patients seen in their institution over the last 20 years, only 38% were eligible for resection. The cohort that underwent complete resection (all stages considered) rather than palliative surgery had an improved overall survival, 31 compared to 13%.[59] In the same report, patients who had no surgery had a 0% 5-year survival.

Incidental and Early-Stage GBC (Tis/T1)

GBC is discovered during a cholecystectomy about 1–2% of the time. A systematic approach to every cholecystectomy can improve the prevalence of early diagnosis. For example, if the gallbladder dissection is difficult or if there is evidence of regional lymph adenopathy, carcinoma should be suspected. Back table examination of the specimen will help identify a suspicious lesion. If the gallbladder specimen reveals only mucosal involvement on microscopic examination (Tis), or a pathologic report returns with identification of an incidental cancer with only submucosal or muscular invasion (T1), a simple cholecystectomy is adequate therapy. If, on initial exploration, regional lymphadenopathy is observed or a biopsy-proven GBC with deeper penetration (>T2) is diagnosed, then a radical cholecystectomy should be considered (see below). If a surgeon is not trained in this operation, the patient should be transferred to another institution because the patient has only one chance for a curative resection.

If an incidental finding of GBC is discovered at the time of a laparoscopic procedure, the operation should be converted from a laparoscopic to an open procedure and the port sites removed to prevent the potential of port site recurrences (Fig. 4a).[60] The expected 5-year survival using this technique for stage-1 cancers approaches 100%.[61,62]

Advanced GBC (≥T2/N0–N2)

Unfortunately, over 60% of GBC patients present at an advanced stage and are not candidates for surgical resection.[63] Patients with evidence of T2/T3 and/or N1 (peri-choledochal) or limited N2 (celiac, superior mesen-

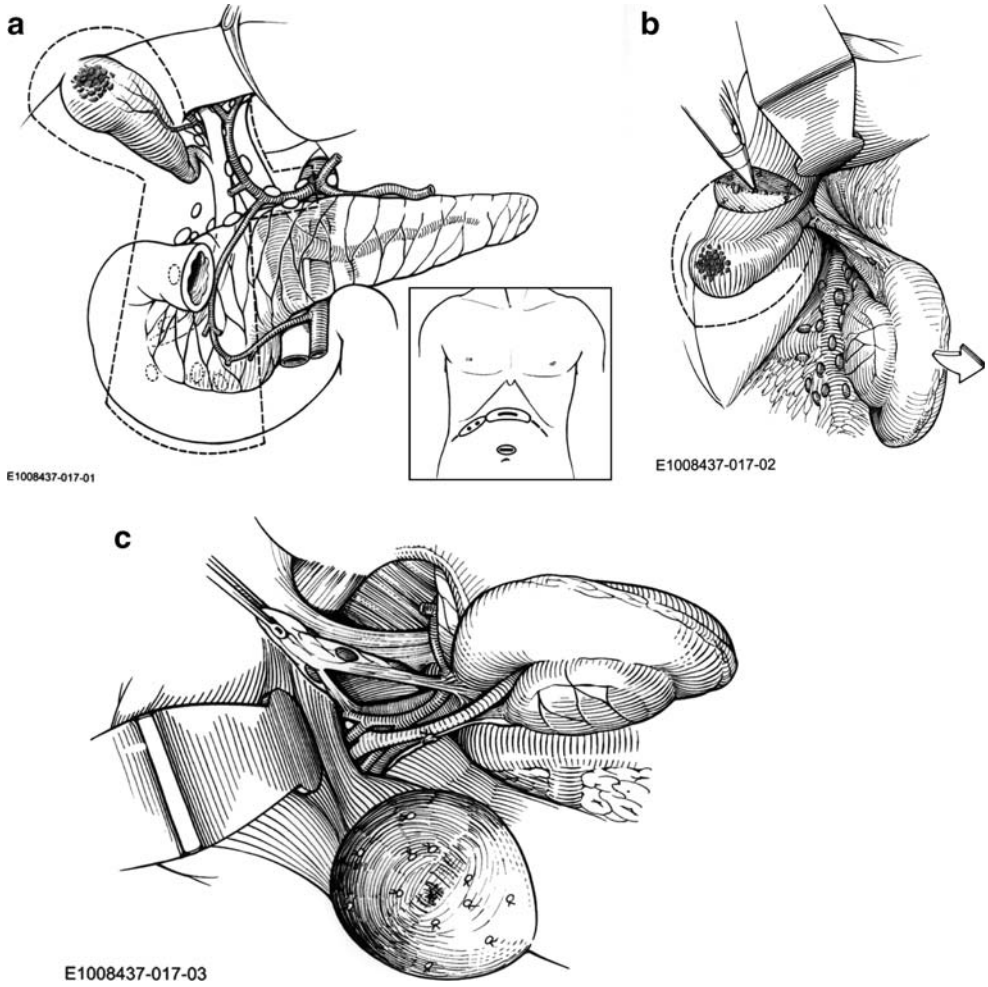

Figure 4 **a** Radical cholecystectomy. The *lower right inset* illustrates the typical right subcostal incision used for radical cholecystectomy, with inclusion of the port sites. The *main drawing* shows the borders of a radical cholecystectomy that includes resection of segment 4B and 5 of the gallbladder bed, along with the extent of the regional lymphadenectomy. **b** Radical cholecystectomy. Division of the hepatic parenchyma with an ultrasonic dissector. The duodenum has been mobilized (*arrow*) revealing the retroduodenal and retropancreatic lymph nodes posteriorly. The nodes are part of the N2 dissection that will be performed later. **c** Radical cholecystectomy. The gallbladder and liver surrounding the gallbladder have been resected, and the hepatoduodenal nodes have been freed from all surfaces but the anteromedial side of the portal triad.

teric, retro-portal, and posterior superior pancreaticoduodenal nodes) disease should be treated with radical cholecystectomy and lymphadenectomy combined with a hepatic resection to obtain an adequate margin (Figs. 4a, c and 5a). Most biliary surgeons believe that radical cholecystectomy should involve, at minimum, removal of the gallbladder with en-bloc subsegmental resection of the adjacent hepatic parenchyma of segments IVB and V and a regional lymphadenectomy that includes complete removal of the hepatoduodenal ligament lymph nodes, plus the common hepatic artery and retropancreatic lymph nodes (see Table 3). En-bloc resection of adherent adjacent organs such as the stomach, colon, duodenum, and pancreas should be performed as required.

Table 4 lists recent studies that have reported survival rates after "aggressive" surgical resections compared to palliative management.[54,64–66] Despite the fact that aggressive procedures can be performed safely by experienced surgeons, radical cholecystectomy does not seem to change the 5-year survival in this population.[37,42,67] Usually, the more radical resections, which include hepatectomy and/or pancreaticoduodenectomy (PD), are reserved for patients with resectable primary tumors and N0–2 disease (Fig. 5a).

The presence of para-aortic lymph nodes is treated as distant metastatic disease and radical surgery should not be employed. Kondo et al. examined 60 patients with nodal involvement at the time of operation; 38% had N3 (aortocaval nodes) disease (Fig. 5b). When patients with N3 disease were compared to patients with N1 or N2 involvement, survival was markedly worse for the N3 group.[68] The authors concluded that patients with N3

Table 3 Lymph Node Stations for Dissection

N1	N2	N3/M1
Cystic Pericholedochal	Superior mesenteric Posterior superior pancreaticoduodenal Retroportal Celiac axis	Interaortocaval nodes

N1/N2 dissections followed for resectable lesions. N3-positive nodes are considered as metastatic disease.

Table 4 Reports of Survival Among Advanced Cancer Patients

Study	Extensive surgery	Palliative surgery	Survival with palliative surgery at 1 and 5 years	Stage of disease	Survival with surgery at 1 and 5 years
Ishikawa et al. [64]	29	20	7%, 0%	IVA	71%, 13%
				IVB	17%, 0%
Wakabayashi et al. [65]	61	NM	NM	III	83%, 83%
				IVA	81%, 46%
				IVB	17%, 17%
Behari et al. [66]	42	139	NR	III	92%, 42%, 28%
				IV	88%, 0%
Ito et al. [59]	66	NM	12%, 0%	All	40%, 13%

NM=not measured, NR=not recorded

disease do not benefit from lymphadenectomy. It is prudent to biopsy any worrisome para-aortic lymph nodes at the beginning of an operation to exclude their involvement prior to proceeding with an aggressive resection.

Additional Options for Locally Advanced Cancers

Cystic Duct and Extrahepatic Biliary Resection

In patients who have had a prior cholecystectomy, there is the consideration of whether to resect the cystic duct stump to the level of the common bile duct. Spread of malignant cells to hepatoduodenal nodes from the gallbladder occurs via the lymphatics along the cystic duct. Resection of the cystic duct stump eliminates a hard mass that cannot easily be differentiated from cancer and helps obtain a more complete resection. There are no studies that directly address this issue, so an official recommendation cannot be made. It is our current practice to resect the cystic duct stump in patients with prior cholecystectomy, but analysis of our own data on GBC did not show any improvement in survival in this subpopulation.[5]

Extrahepatic biliary resection is not routinely performed for GBC. A growing group of surgeons recommend this technique for the management of T3 and T4 tumors. Shimizu et al. at Chiba University evaluated 50 consecutive patients who had extrahepatic biliary resections in conjunction with hepatic resection for GBC. They found the prevalence of hepatoduodenal ligament tumor involvement to be 60%.[69] Interestingly, most of these patients did not have evidence of macroscopic disease, making reliance on gross examination of the hepatoduodenal nodes inadequate as a guide regarding bile duct resection. These authors suggest that the threshold for extrahepatic biliary resection should be lowered in patients with penetration of GBC through the subserosa. A recent case report by Shikani et al. supports this practice. They reported a patient with GBC who survived for more than 7 years after extrahepatic biliary resection and inclusion of paraaortic nodes in the lymphadenectomy specimen.[70]

Hepatic Resections with Advanced Cancers

The role of hepatic resection is usually limited to subsegmental resection of segments IV and V 2 cm away from the gallbladder bed. This technique is adequate in GBC confined to the subserosal layer but may not be adequate in some T3 and T4 cancers. In animal experiments, it has been shown that the normal lymphatic drainage is from the cystic duct towards the hepatoduodenal

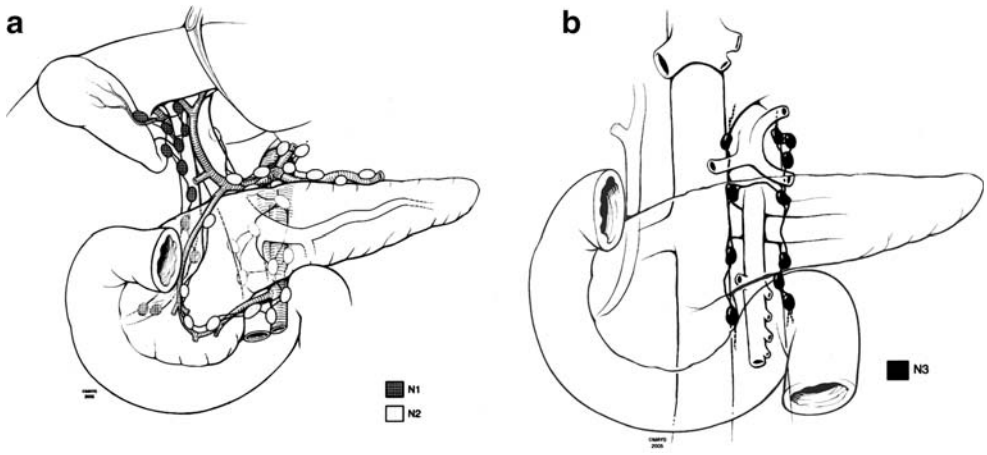

Figure 5 **a** N1 and N2 lymph node station. **b** N3 lymph node station (aortocaval nodes).

ligament. With lymphatic obstruction, the cystic duct lymphatic drainage is diverted into the adjacent hepatic parenchyma round the hilum and eventually back towards the hepatoduodenal ligament.[71] This supports the role of radical hepatic resections to include an extended right hepatectomy with or without a caudate lobe resection. Central bisegmentectomy plus caudate lobectomy is another option some surgeons have advocated for infiltrating GBC.[72]

PD and GBC

When patterns of surgical therapy for GBC are analyzed, western surgeons are customarily less aggressive than their Japanese counterparts. One such example is the use of PD for GBC. Several Japanese studies have reported on the feasibility of preforming a PD for some T3 and T4 GBCs.[73,74] The most common indications for PD have been infiltration of cancer into the pancreatic head and for metastasis to peri-pancreatic lymph nodes. Araida published the largest series of PD (n=93) for GBC.[75] All patients undergoing PD had T2–T4 lesions. When compared to patients who underwent extensive (N2, N3) lymphadenectomy alone, there was no survival benefit for PD if there was no hepatoduodenal ligament invasion or microscopic lymph node metastasis. There was, however, a lower recurrence rate for patients having PD if the patients had microscopic lymph node metastasis. Based on this study, there may be a small subset of patients that could benefit from PD, but for the majority of GBC patients, a thorough lymph node resection of the N1 and N2 nodes will be more helpful for long-term survival and reduced recurrence rates.

Palliative Treatment

If, at the time of surgical exploration, the patient's GBC is found to be unresectable, palliative procedures may be entertained. The rate of biliary obstruction in patients with GBC exceeds 60%. Management is individualized, but a Roux-en-Y hepaticojejunostomy may, in selected patients, be constructed at the proximal common hepatic duct or hilum. Gastric obstruction occurs in approximately 50% of the GBC patients that present with biliary obstruction. A gastrojejunostomy is often performed in this patient cohort to palliate or prevent this condition.

The nonoperative options of percutaneous or endoscopic endobiliary stents plus endoscopic enteric stenting and feeding tubes can be used in patients with poor functional status, limited life expectancy, or significant comorbidities. No controlled trials have compared the use of stents vs surgical bypass in this patient population. One small study published approximately 10 years ago showed fewer septic complications among patients who were palliated with a biliary-enteric bypass compared to those who were palliated with a biliary stent.[76] Given the high morbidity associated with stents, it is best for patients found to have locally advanced, unresectable disease at abdominal exploration to perform a biliary enteric bypass when possible. For patients with metastatic disease who have limited life expectancy, nonoperative relief of biliary and enteric obstruction usually provides a better means of palliaton.

Adjuvant Therapy

No drug therapy has proven efficacious for GBC. Traditional adjuvant chemotherapeutic regimens have generally included fluorouracil. External beam radiation is often used with fluorouracil chemosensitization, but there are few data to support its efficacy. At Mayo Clinic, adjuvant radiotherapy (54 Gy) with concurrent 5-fluorouracil (5-FU) was given to 21 consecutive patients over a 12-year period. The 5-year survival for the entire cohort was 33%, with a 65% 5-year survival for stages I–III and 0% survival for stage IV disease. The median survivals were 0.6, 1.4, and 5.1 years for patients with gross residual disease (R2), microscopic residual tumor (R1), and no residual disease (R0), respectively.[77] This study noted a superior survival rate than that seen with historic controls.

A prospective, randomized phase-III trial examined the role of adjuvant chemotherapy in 508 patients diagnosed with resectable pancreaticobiliary carcinomas, 140 of whom had GBC. Patients were randomized to receive surgical resection alone or operative therapy plus adjuvant chemotherapy with 5-FU and Mitomycin C. The latter group received Mitomycin C [6 mg/m^2 intravenously (IV)] during the operation and 5-FU (310 mg/m^2 IV) for five consecutive days during the first and third weeks postoperatively, followed by oral 5-FU (100 mg/m^2) starting the fifth postoperative week until tumor recurrence. The 5-year survival rate for patients in the adjuvant treatment group was 26%, compared with 14% in the control group (P= 0.04).[78]

Recently, gemcitabine has been compared to 5-FU and leucovorin in a phase-II trial in advanced biliary tract cancers including GBC. The results suggest that gemcitabine has equivalent activity compared to 5-FU and leucovorin.[79] Currently, two phase-II trials are evaluating new systemic treatments for GBC. In one study, 3-AP (Triapine) and gemcitabine are being evaluated. Capecitabine, oxaliplatin, bevacizumab, and radiation therapy are being tested in another study of patients with biliary tract cancer and GBC.

Conclusion

The best chance at cure for a GBC is with a Tis or T1 tumor incidentally discovered. Simple cholecystectomy is adequate therapy for these cancers. For more advanced GBC, the outcome is usually grim, but improved outcomes have been realized with aggressive radical operative therapy, most commonly, radical cholecystectomy and regional lymphadenectomy. With an increased level of suspicion and aggressive operative resection, survival outcomes should improve for patients who have limited GBC. Because most patients with GBC have apparent or occult metastases, they will not be cured until new systemic therapies improve the results seen with currently available treatments.

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The Use of Animal Models to Study Bacterial Translocation During Acute Pancreatitis

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Abstract Infection of pancreatic necrosis with intestinal flora is accepted to be a main predictor of outcome during severe acute pancreatitis. Bacterial translocation is the process whereby luminal bacteria migrate to extraintestinal sites. Animal models were proven indispensable in detecting three major aspects of bacterial translocation: small bowel bacterial overgrowth, mucosal barrier failure, and disturbed immune responses. Despite the progress made in the knowledge of bacterial translocation, the exact mechanism, origin and route of bacteria, and the optimal prophylactic and treatment strategies remain unclear. Methodological restrictions of animal models are likely to be the cause of this uncertainty. A literature review of animal models used to study bacterial translocation during acute pancreatitis demonstrates that many experimental techniques per se interfere with intestinal flora, mucosal barrier function, or immune response. Interference with these major aspects of bacterial translocation complicates interpretation of study results. This paper addresses these and other issues of animal models most frequently used to study bacterial translocation during acute pancreatitis.

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Introduction

Experimental models of acute pancreatitis exist for almost 150 years, with Claude Bernard first describing experimental pancreatitis by injection of bile and olive oil into the pancreatic duct of a rabbit.[1] Ever since, animal experiments were indispensable in providing insight in pathophysiology and treatment of acute pancreatitis. Experimental studies have major advantages over clinical studies, such as the availability of study subjects, standardization of disease severity, ability to perform invasive tests, extensive tissue sampling, and the possibility to test prophylactic treatment strategies.[2] Despite these advantages, some major aspects of the pathophysiology of acute pancreatitis remain unclear, mortality in severe acute pancreatitis is still as high as 5–28%, and optimal treatment strategies remain a topic of debate.[3,4]

In 1986, Beger et al. demonstrated a link between the intestinal flora, infection of pancreatic necrosis, and clinical outcome in patients with severe acute pancreatitis.[5] At the present time, infection of pancreatic necrosis is still regarded to be a main predictor of outcome during severe acute pancreatitis, and bacterial translocation of intestinal flora is considered to be the cause.[4]

Changes in intestinal motility and the associated shift of intestinal flora, mucosal barrier function, and the immune system were identified as pivotal aspects of bacterial translocation during acute pancreatitis.[6–11] This has greatly increased the understanding of bacterial translocation, but better insight into the exact mechanism of bacterial translocation and subsequent infection of pancreatic necrosis is needed to develop adequate prophylaxis and treatment strategies for patients with severe acute pancreatitis.

A multitude of animal models were used to study the mechanism of bacterial translocation, including radiolabeling, plasmid-labeled bacteria, or fluorescent beads.[12–15] Despite all these efforts, however, the exact origin, route, and mechanism of bacterial translocation causing infection of pancreatic necrosis are still unclear. The main reason for this uncertainty is the lack of an "ideal" animal model of acute pancreatitis to study pathophysiology of bacterial translocation and its treatment. The ideal model should be minimally invasive, standardized, reproducible, and resemble etiology, pathophysiology, disease course, and outcome of clinical acute pancreatitis, including response to treatment.[2] Experimental models used to study bacterial translocation in acute pancreatitis and its treatment all seem to have methodological restrictions that complicate the interpretation of study results. In 2000, Foitzik et al. reviewed the use of animal models of acute pancreatitis and their suitability for evaluating therapy and concluded that animal models should be designed to mimic etiology and clinical course of human pancreatitis to increase their value.[2] In addition, we would like to discuss the value animals studies and experimental models of acute pancreatitis have in face of their interference with one or more of the known aspects of bacterial translocation: intestinal motility and flora, mucosal barrier function, or the immune system.

The aim of this paper is to provide useful insights into the use of animal models to study bacterial translocation during acute pancreatitis, in the light of current knowledge of pathophysiology.

Animal Species and Housing Conditions

Before the late 1970s, larger laboratory animals such as dogs were predominantly used to study acute pancreatitis. But since the introduction of models of acute pancreatitis in small laboratory animals, mice or rats are generally favored for financial and ethical or practical reasons. Because of physiological and anatomical differences between species, choice of laboratory animal has important implications on the study results and extrapolation to the human situation.

Intestinal flora differs between animal species, largely depending on dietary demands and anatomical differences of the gastrointestinal tract and habits.[16–18] The protein-rich diet of dogs or cats results in lower counts of endogenous lactobacilli and higher counts of potential pathogens (e.g., clostridia species), compared to rats or mice with fiber-rich diets. Coprophagy, demonstrated by most rodents, also influences intestinal flora, resulting in higher counts of gram-negative bacteria in the proximal gastrointestinal tract.[19,20] Also, rats and mice are often bred and kept under specific pathogen-free conditions, introducing modifications of intestinal flora.

Intestinal barrier function also differs between species. In an experiment comparing small intestinal permeability between humans and rats, significant interspecies variation in urinary recovery of orally delivered mannitol was observed.[21]

Anatomical differences between species should also be considered. The relative size of the jejunum, ileum, cecum, and colon of different laboratory animals can influence origin and route of bacterial translocation during acute pancreatitis. In humans, retroperitoneal connections between the intestines and pancreas can greatly affect the clinical course of the disease.[22] Similar to humans, the dog pancreas is situated retroperitoneally. Rat and mouse pancreata, however, are almost fully enveloped by peritoneum, resembling a more intraperitoneal localization. Variation in retroperitoneal connections between intestines and the pancreas offers different routes for bacteria to translocate without being exposed to intraperitoneal immune cells.[23]

Experiments using small animals (e.g., mouse or rat) usually incorporate a larger number of animals compared to experiments with large laboratory animals (e.g., cat or dog). The use of a larger number of small laboratory animals improves statistical power of an experiment. On the other hand, the use of larger animals could resemble human pathophysiology better, but a smaller number of animals means lower statistical power and increased potential false negative or false positive results.

Models of Acute Pancreatitis

An abundance of animal models of acute pancreatitis is used to investigate bacterial translocation. Only models most frequently used for this purpose will be discussed. Baseline characteristics of the discussed models and their potential effects on intestinal flora, mucosal barrier, and immune function are summarized in Tables 1 and 2.

Table 1 Characteristics of Several Animal Models of Acute Pancreatitis

Model	Animal Species	Pancreatic Necrosis	Pancreatic Infection	Mortality	Invasiveness
Duodenal loop[24,25]	Rat	No	Considerable	High	Laparotomy
Choline-deficient diet[30–32]	Mouse	Yes	Little	High	Minimal
Duct ligation[34–37]	Rat/opossum	No/Yes	Little	Low	Laparotomy
Cerulein[44]	Mouse/rat	Yes/No	Little	Low	Minimal
Duct perfusion[48]	Rat/dog/pig	Yes	Considerable	Moderate to high	Laparotomy
Duct perfusion + cerulein[52]	Rat	Yes	Considerable	Moderate	Laparotomy

Duodenal Loop

Closing the duodenal lumen proximally and distally to the papilla of Vater results in reflux of the duodenal contents enclosed in the loop, including bile and pancreatic secretions, into the biliopancreatic duct.[24] In rats, this leads to acute pancreatitis of varying severity.[25] Discontinuation of the gastrointestinal tract leads to mucosal atrophy and functional changes to the mucosal barrier.[26] Furthermore, obstruction of bile flow into the intestine was shown to reduce intestinal motility, causing small bowel bacterial overgrowth and increased bacterial translocation.[27–29] Another major downside is the occurrence of reflux of duodenal contents, including bacteria, into the biliopancreatic duct. These obvious drawbacks of this model in experiments concerning bacterial translocation are the cause of its limited popularity.

Ethionine-supplemented Choline Deficiency

Lombardi et al.[30] described severe acute pancreatitis in young female mice after feeding a choline-deficient, ethionine-supplemented (CDE) diet.[31] Acute hemorrhagic pancreatitis ensues, as well as diffuse intraperitoneal fat necrosis and several systemic effects such as acidosis, hypoxia, and hypovolemia. In this model, mortality ranges from 0 to 100% after 4 days and can be controlled by varying the duration of the choline-deficient diet.[32] To ensure homogeneity and reproducibility, sex, age, and weight of the mice have to be closely matched, as well as food intake of all animals.[32]

Apart from these practical downsides of the model, systemic complications unrelated to pancreatitis (e.g., parotitis and fatty liver disease) render the model less useful for investigating systemic events (e.g., immune response) of acute pancreatitis.[31] Little is known of the effect of ethionine suppletion or choline deficiency on intestinal flora or mucosal barrier function. But the most important drawback of this model to study bacterial translocation is the low incidence of pancreatic infection (3–8%), even in severe necrotizing pancreatitis.[33]

Biliopancreatic Duct Ligation

In the duct ligation model, the common biliopancreatic duct is surgically clipped or tied at the sphincter of Oddi

Table 2 Aspects of Bacterial Translocation and Potential Confounding Factors of Animal Models

Aspect	Confounding Factor	Model
Intestinal motility and flora	Animal species	Potentially all models
	Housing conditions (SPF)	Potentially all models
	Diet	CDE diet
	Analgesics	Invasive models
	Laparotomy	Invasive models
	Bile flow	Duct ligation
	Cerulein	Cerulein models
	Intestinal manipulation	Invasive models
Mucosal barrier function	Stress	Potentially all models
	Diet	CDE diet
	Anesthetics	Invasive models
	Pancreatic proteases	Duct ligation
	Intestinal manipulation/puncture	Duct perfusion
Immune system	Stress	Potentially all models
	Diet	CDE diet
	Disease course/severity	Species-dependent
	Obstructive jaundice	Duct ligation, duodenal loop
	Intestinal manipulation	Invasive models

complex. The resulting obstruction of pancreatic secretions and potential biliary reflux into the pancreatic duct produce moderate pancreatitis, characterized by edema, moderate inflammation and hemorrhage, fat necrosis, and minimal acinar cell necrosis. Only in the American opossum does biliopancreatic duct ligation leads to severe acute pancreatitis with considerable necrosis.[34–37]

This model of acute pancreatitis greatly interferes with the pathophysiology of bacterial translocation. Obstruction of bile flow into the intestine causes small bowel bacterial overgrowth and bacterial translocation.[28] Also, exclusion of pancreatic proteases in the gut lumen alters intestinal permeability.[38,39] Apart from effects on the intestinal flora and mucosal barrier function, obstruction-induced jaundice also causes impairment of the immunesystem.[40–42] These effects complicate the interpretation of bacteriological results to study bacterial translocation.

Cerulein Infusion

Infusion of low doses of cerulein, a cholecystokinin analog, enhances production of pancreatic exocrine cell secretions without cell necrosis. In most species, infusion of supramaximal doses results in a decrease of secretion and acute pancreatitis with interstitial edema and inflammatory cell infiltration.[43] In mice, cerulein causes severe acute pancreatitis with necrosis of 40% of acinar cells.[44] In rats and other animals, however, cerulein-induced pancreatitis is usually mild and generally self-limiting. Moreover, pigs are reported to be insensitive to cerulein hyperstimulation.[45] It should be noted that cerulein is known to affect intestinal motility. Studies investigating the use of cerulein in man have shown absence of recognizable migrating motor complexes with decreased colonic transit time.[46] In general, experimental acute pancreatitis is associated with reduced small bowel motility, resulting in small bowel bacterial overgrowth and increased bacterial translocation to extraintestinal sites.[6,47] Thus, cerulein may interfere with intestinal flora by altering intestinal motility. Investigators should keep this in mind when designing a study and interpreting study results.

Biliopancreatic Duct Perfusion

Duct perfusion models are currently the most popular models of acute pancreatitis. Induction of acute pancreatitis involves infusion of bile, bile salts with or without bacteria, or activated pancreatic enzymes into the (bilio-)pancreatic duct. Early experiments mainly involved dogs, but currently, rats are used most frequently. Severity and reproducibility of acute pancreatitis and ensuing bacteriological results strongly depend on infusate, infusion pressure, volume, and time.[48]

The most commonly used infusates are solutions containing various concentrations of bile salts of varying hydrophobicity. Both chemical and pressure effects of infusion were suggested to play a major role in the pathogenesis of pancreatitis in perfusion models.[48,49] In both chemical- and pressure-induced pancreatitis, destruction of the pancreatic duct mucosal barrier is the key event. This is followed by pancreatic edema, autolysis, reduction of pancreatic blood flow, and, in severe cases, destruction of pancreatic parenchyma and formation of pancreatic necrosis.[50] Uncontrolled pressure-related damage causes variation in severity of the induced acute pancreatitis between study subjects, and thus should be avoided. Several experiments were performed to assess maximal pancreatic duct pressure before rupture of the duct epithelium causing increased and uncontrolled severity of acute pancreatitis. Data are conflicting, with rupture pressures varying from 15 to 82 mmHg.[48,49,51,52] A maximum infusion pressure of 30 to 50 mmHg is currently accepted for rat models.

Perfusion is usually performed by puncturing the duodenum and cannulating the papilla of Vater. The introduction of duodenal bacteria, through the papilla of Vater into the biliopancreatic duct could potentially be a confounding factor in transduodenal duct perfusion models. It was demonstrated, however, that significant bacterial infection of the pancreas ($>1\times10^2$ colony forming units per gram) because of the surgical procedure does not occur.[53]

Advantages of this model are the quick procedure of acute pancreatitis induction and the reproducibility of results. Other than duodenal puncturing and intestinal handling during surgery, both potentially affecting mucosal barrier function, no direct effects on intestinal flora or immune function are expected in this model.

Biliopancreatic Duct Injection and Cerulein Hyperstimulation

The combination of retrograde infusion of bile salts with superimposed cerulein hyperstimulation in rats was introduced by Schmidt et al. and was advocated as "a better model for evaluating therapy."[52] Although the disadvantages described for biliopancreatic duct injection and cerulein hyperstimulation all apply to this model, it was proven a very valuable model to examine bacterial translocation and treatment strategies. The major advantages are that histological and qualitative bacteriological results as well as reaction to treatment and disease course resemble human acute pancreatitis more closely than other models.[2,52] Although proven a very valuable model, potential model-related confounding factors as described above should always be kept in mind when interpreting results.

Disease Course

Especially in the severe form of acute pancreatitis, systemic events can be divided into two phases: early proinflammatory and late immunosuppressive.[54] In severe acute pancreatitis, the early phase is associated with a systemic inflammatory response syndrome (SIRS), potentially leading to multiple organ failure and early mortality. The late phase is characterized by immunosuppression, providing opportunity for infectious complications (e.g., infection of pancreatic necrosis) associated with sepsis and late mortality.[2,55] Laboratory animal species and experimental models, however, each show their own disease course of acute pancreatitis.

Animal models were mainly used to investigate the early phase of acute pancreatitis.[56] However, the model described by Schmidt et al. seems the most appropriate to investigate early and late systemic complications, considering that both phases can be discerned.[52,57] In this model, infection of pancreatic necrosis progresses at least until 96 h. When taking into account that disease course is more rapid in small rodents, timing could well correlate with data on the course of severe acute pancreatitis in humans, as described by Foitzik et al.[2], Beger et al.,[4] and Lankisch et al.[58]

Severity

Pancreatic necrosis is produced in several animal models of acute pancreatitis (Table 1). On the other hand, only duct perfusion, with or without superimposed cerulein hyperstimulation, and murine CDE models demonstrate mortality comparable to human necrotizing acute pancreatitis.[32,52,59] Models with high early mortality may be useful to investigate early phase systemic inflammatory response and organ failure, but are less adequate to investigate late infectious complications and associated (multiple) organ failure.

In most models, necrosis needs to be present for pancreatic infection to occur. It needs to be noted that this does not apply for the duodenal loop model in which reflux of duodenal contents into the biliopancreatic duct occurs.[60] In contrast, the murine CDE model produces elaborate necrosis, but is associated with very low rates of pancreatic infection.[33]

Culturing, Controls, and Route of Bacterial Translocation

In all animal models, factors such as analgesia, anesthesia, or surgical techniques can influence bacteriological results. Morphine-like analgesics have a significant effect on bowel motility and cause bacterial overgrowth and translocation to extraintestinal sites.[61] The anesthetic pentobarbital was suspected to be a factor in promoting bacterial translocation in a model of hemorrhagic shock.[62]

Also, stress causes mucosal barrier failure and bacterial translocation.[63] Surgical procedures are stressful events, but animal transport or handling alone could potentially cause stress-induced bacterial translocation. The influence of stress on adrenaline and corticosteroid levels could have its own effect on the function of the immune system, potentially influencing the systemic reaction to acute pancreatitis and bacterial translocation.

Proper sterile surgical techniques are very important when investigating bacterial translocation. If abdominal surgery is involved, control cultures of the peritoneal cavity to trace surgical contamination are of special importance. If peritoneal cultures are found to be positive, extra caution should be taken with interpretation of bacteriological analysis of abdominal organs. In case of surgical contamination or transperitoneal bacterial translocation, the peritoneal covering of the organ samples might be the cause of positive organ cultures, not the bacterial colonization in the organ itself (false positive culture).

Puncturing the duodenum in duct infusion models hypothetically causes spillage of duodenal contents onto the peritoneum, covering all abdominal organs. In rats, however, duodenal contents usually have low bacterial counts, mainly consisting of nonpathogenic lactobacilli only. On the other hand, a duct infusion study by Cicalese et al. reported positive peritoneal cultures at time of induction of pancreatitis of 16.6 to 33.3% of the studied rats.[15] Literature review of different animal models fairly frequently shows positive peritoneal cultures at the time of termination and organ sample collection of rats with acute pancreatitis. Positive peritoneal cultures are observed varying from 0–10% in minimally invasive models of acute pancreatitis (cerulein injection, CDE diet) to 8–100% in more invasive models (duct perfusion with or without cerulein hyperstimulation).[6,14,15,64–66]

Discussion

Changes in intestinal motility and flora, mucosal barrier function, and immune response were established as pivotal aspects in the process of bacterial translocation during acute pancreatitis. Early after the onset of acute pancreatitis, neurohormonal effects result in reduced small bowel motility.[6] This causes stasis of luminal contents and small bowel bacterial overgrowth with potential pathogens, including *Escherichia coli* and *Enterococcus* species. The abundant presence of luminal pathogens forms a challenge for the mucosal barrier. Furthermore, pancreatitis-associated

reduced intestinal blood flow results in mucosal ischemia and reperfusion damage.[67–69] Luminal bacteria, normally held at bay by the mucosal barrier, now have opportunity to penetrate into the intestinal epithelium. Local intestinal inflammation follows, further compromising mucosal barrier function. Pancreatitis and ensuing intestinal inflammation both contribute to a systemic proinflammatory response (SIRS), with damaging effects on distant organs.[70,71] If the systemic response is severe, multiple organ dysfunction syndrome (MODS) might follow.[72,73] If the patient survives the early phase, counterregulatory immunological pathways releasing anti-inflammatory cytokines result in a refractory state characterized by immunosuppression.[74,75] Persistent immunosuppression will render the patient liable for infection of pancreatic necrosis. Multiple organ dysfunction syndrome caused by infectious complications is considered accountable for so-called late mortality or "late septic death."[74,76]

Although animal models were proven indispensable in acute pancreatitis research, model-related problems are most likely the reason for important questions on pathophysiology and treatment strategies to remain unanswered. Current topics of debate include the route and origin of bacterial translocation and optimal prophylaxis and treatment strategies.

Several different routes of bacterial translocation were described and have directed efforts for many prophylactic and therapeutic strategies. Webster et al. showed bacteremia to occur early after induction of acute pancreatitis in CDE-induced acute pancreatitis, suggesting a hematogenous route.[77] Likewise, rapid passage of bacteria into the blood was found in other models of acute pancreatitis.[78] On the other hand, Runkel et al. found bacteria migrating to lymph nodes before their translocation to distant sites in a duct ligation model, suggesting a lymphogenous route.[79] Widdison et al. suggested transperitoneal translocation of bacteria originating from the colon in a feline model of severe necrotizing pancreatitis.[80] Other study groups, including our own, have provided proof of the role of the small bowel in the pathophysiology of bacterial translocation in acute pancreatitis or after morphine administration.[6,61,81]

The model of duct perfusion and cerulein hyperstimulation described by Schmidt et al. was proven very useful because it resembles human disease quite well, considering its biphasic disease course, pancreatic histology, "moderate" mortality, and the bacterial spectrum in pancreatic necrosis.[52] However, whether a confounder is introduced by puncturing the duodenum and cannulating the biliopancreatic duct is unknown. Therefore, to ensure quality of the presented study results, control cultures of the peritoneal cavity should be done when organ samples are analyzed bacteriologically. Peritoneal bacteria can potentially affect bacteriological analysis of all abdominal tissues. Widdison et al. washed abdominal samples before analysis, but this is not commonly performed.[80] A pilot study by Arendt et al. showed that washing removed 94–97% of intraperitoneally injected bacteria.[23] Immunohistologically localizing bacteria can help clarify if positive cultures of abdominal tissues are because of peritoneally located bacteria or actual bacterial colonization in the underlying organ tissue.

When experimentally evaluating therapy, treatment often starts before induction of acute pancreatitis. Obviously, this is an important reason why results cannot directly be translated to the clinical situation. On the other hand, these experimental studies provide proof of principle concerning the tested therapy. If prophylactically successful, the tested treatment strategy might be beneficial when started after the onset of acute pancreatitis and should therefore be further investigated. On the other hand, the faster course of acute pancreatitis in rodent models provides only a very short treatment window between the onset of the disease and early or late phase complications. This may lead to false negative effects of the therapy tested.

In conclusion, animal models of acute pancreatitis are indispensable tools, but model-related drawbacks often interfere with one or more pathophysiological aspects of bacterial translocation, complicating interpretation of results. When the ideal model of acute pancreatitis is not at hand, it is of major value that numerous alternatives are available. But with each experimental hypothesis, special care should be taken to select the most suitable model. Despite all the experimental work done, the route by which pancreatic infection occurs and gives rise to septic complications and mortality has not yet fully been elucidated. Optimal prophylactic and treatment strategies are also still widely debated. In the future, animal models will undoubtedly provide increasing understanding of these subjects, but model-related drawbacks should always be kept in mind when designing a study or when interpreting results.

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The Tethered Bezoar as a Delayed Complication of Laparoscopic Roux-en-Y Gastric Bypass: A Case Report

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Abstract Known complications of Roux-en-Y gastric bypass causing abdominal pain and obstructive symptoms include biliary colic, anastomotic ulcer, anastomotic stenosis, or internal hernia. This case report describes a new complication in a patient 15 months post-bypass: a bezoar at the gastrojejunal anastomosis, the nidus of which was a length of permanent suture material which had eroded through the gastric wall. We include endoscopic images of the bezoar, a review of the related gastric bypass literature, and describe the changes made in our practice as a result of this complication.

Keywords Bezoar · Gastric bypass · Postoperative complication

Introduction

Paralleling the increasing frequency of both open and laparoscopic Roux-en-Y gastric bypass (RYGB), the complications of these procedures have become more common. One of the most common presenting symptoms of a delayed postoperative complication of RYGB is epigastric, colicky abdominal pain. The differential of this symptomatology generally includes biliary colic, anastomotic ulcer, anastomotic stenosis, or internal hernia. This case demonstrates a new complication not reported before—the tethered bezoar.

The gastrojejunostomy in the laparoscopic RYGB can be constructed using a variety of techniques. The majority of these techniques use nonabsorbable suture or staples during the closure of the anastomosis. During postoperative endoscopy, this material is often noted to have necessitated into the lumen of the GI tract even when these originated as Lembert type, seromuscular sutures. These sutures have been implicated as a possible cause of ulcers after gastric bypass.[1]

Our patient is a 34-year-old woman who presented to the emergency room of our institution 15 months after a laparoscopic RYGB procedure for class III morbid obesity. She underwent an uneventful retrogastric, retrocolic laparoscopic RYGB with a linear stapled gastrojejunal anastomosis. The anastomosis was fashioned by suturing the Roux limb to the posterior wall of the gastric pouch using a running layer of 2.0 Surgidac, a nonabsorbable braided polyester material (Endostitch, US Surgical Corporation, Norwalk, Connecticut, USA). Enterotomies were made into the lumens of the gastric pouch and Roux limb, and an Endo-GIA (US Surgical Corporation) was then used to create a 2-cm anastomosis between the gastric pouch and Roux limb using 3.5 mm staples. The common gastroenterotomy was then closed with a running inner layer of 2.0 Polysorb, an absorbable suture (Endostitch, US Surgical Corporation), followed by an anterior running layer of 2.0 Surgidac.

In 3 months after her operation, the patient experienced epigastric pain which resolved on Zantac and Carafate. She was also diagnosed with cholelithiasis and managed non-operatively with Actigall. A follow-up ultrasound at 11 months demonstrated a normal gallbladder without cholelithiasis.

Approximately 15 months after her operation, the patient again began to complain of burning epigastric pain, exacerbated by eating, although thin liquids were

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less problematic. She was nauseated but did not vomit with her pain. She continued to pass flatus and have normal bowel movements. She was empirically restarted on 1 g of Carafate four times daily and a twice-a-day dosing of a proton pump inhibitor; however, her pain was refractory to these measures. Upon examination, she had a soft abdomen with mild tenderness localized to the epigastrium. Her stool guaiac was negative. Her white blood cell count and hematocrit were normal. Her liver function tests as well as blood chemistries were all within normal limits. Her presumptive diagnosis was an anastomotic ulcer with a possible anastomotic stricture. An upper endoscopy was planned for diagnosis and potential dilation.

The endoscopy revealed that the permanent sutures used to close the external layer of the gastrojejunal anastomosis had necessitated through the bowel wall such that the knotted end of the suture was hanging down the Roux limb with the other end tethering it to the anastomosis (Fig. 1).

The free end of the suture had developed a large bezoar, which was nearly obstructing the Roux limb. The suture was cut endoscopically, and the bezoar passed without difficulty (Fig. 2). The patient has been subsequently pain-free.

Review

The first case series of laparoscopic RYGB was published in 1994 by Wittgrove et al.[2] In the ensuing 11 years, there has been a large body of literature examining laparoscopic RYGB surgery for morbid obesity.

The laparoscopic RYGB operation has been shown to compare favorably with open RYGB in terms of operative blood loss, postoperative length of stay, return to activities of daily living, and return to work. The laparoscopic gastric bypass is equivalent to the open gastric bypass in terms of anastomotic leak rate, total costs, and percentage of excess body weight lost at 1 year.[3] The complication rates and types of complications differ between the two approaches. In general, the rates of wound infections (6.6% open vs 3.0% laparoscopic) and incisional hernias (8.6% open vs 0.5% laparoscopic) are higher in the open gastric bypass group. The rates of anastomotic stricture (0.6% open vs 4.7% laparoscopic) and bowel obstruction (2.1% open vs 3.2% laparoscopic) are higher in the laparoscopic group.[4]

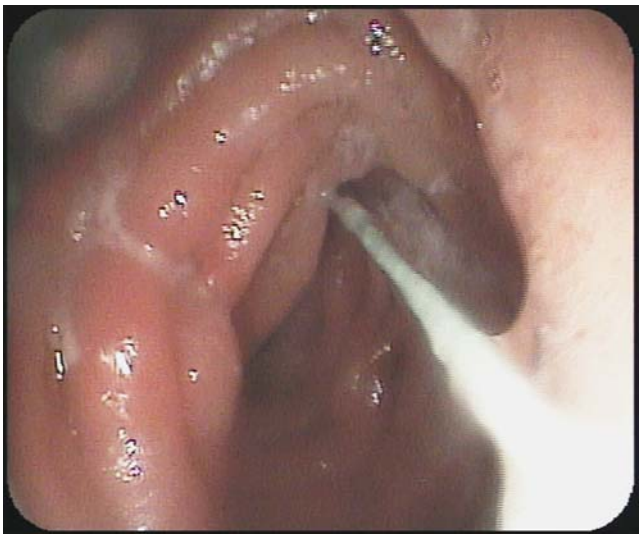

Figure 1 EGD image showing suture on tension being pulled by peristalsis on the bezoar into the distal Roux limb.

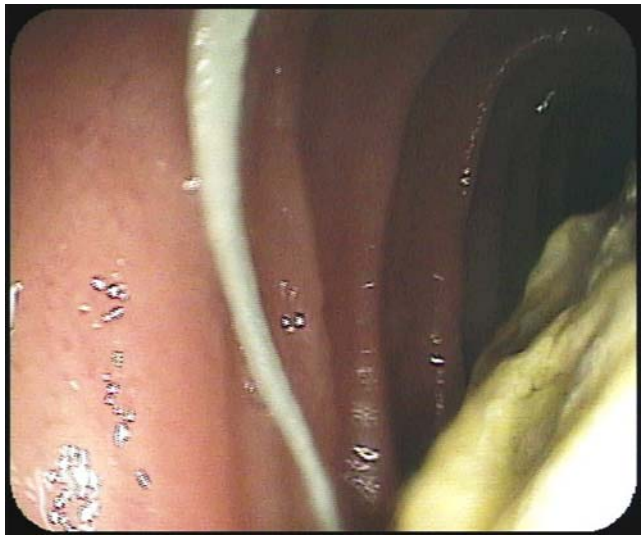

Figure 2 Small portion of bezoar visible in jejunum after cutting the suture.

A number of papers have described small bowel obstruction after laparoscopic RYGB both as early and late complications.[5–8] The vast majority of the described mechanisms for obstruction are narrowing of an anastomosis (kinking or stenosis) or internal herniation through one of the three known potential defects (between the mesenteries of the biliopancreatic limb and the Roux limb, through the transverse mesocolon defect, or posterior to the Roux limb—also called Peterson's defect).

In the case presented, the patient's symptoms were consistent with a proximal intermittent high-grade obstruction. Her ability to tolerate liquids, pass flatus, and have preserved bowel movements indicated that she did not have a complete obstruction of her upper gastrointestinal tract. The presumptive diagnoses were an ulcer and a possible stenosis of the gastrojejunostomy, and the planned intervention was esophagogastroscopy with dilation of the anastomosis. Upon entering the gastric pouch with the endoscope, the suture was visualized at the anastomosis under tension with a large bezoar attached. The retained suture also appeared to be causing a chronic abrasion injury

to the jejunal wall as it was under tension from the action of peristalsis on the bezoar.

The presumed etiology of the patient's recurrent symptoms is the intraluminal erosion of the knotted end of the outer, permanent suture line at the gastrojejunostomy which accumulated swallowed food particles resulting in a bezoar. This bezoar remained tethered to the gastrojejunostomy functioning as a ball valve causing her intermittent partial obstruction symptoms. The patient has remained symptom-free since the release of the bezoar.

The erosion of the nonabsorbable suture at the gastrojejunostomy is not an isolated phenomenon: there are several other patients in our series who have reported the spontaneous passage of suture material or who have visible suture on endoscopy of the gastrojejunostomy. Until now, none of these patients has been symptomatic with such suture erosions. However, we have begun to reevaluate the use of nonabsorbable suture material at the gastrojejunostomy in our practice. There are many bariatric surgeons whose practices include the use of nonabsorbable suture at the gastrojejunostomy, and it is for their consideration that we present this case.

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