2009 SSAT QUICK SHOT PRESENTATION

Should Elective Repair of Intrathoracic Stomach be Encouraged?

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Abstract

Background Given our aging population, patients with an intrathoracic stomach are an increasing clinical problem. The timing of repair remains controversial, and most reports do not delineate morbidity of emergent presentation. The aim of the study was to compare the morbidity and mortality of elective and emergent repair.

Methods Study population consisted of 127 patients retrospectively reviewed undergoing repair of intrathoracic stomach from 2000 to 2006. Repair was elective in 104 and emergent in 23 patients. Outcome measures included postoperative morbidity and mortality.

Results Patients presenting acutely were older (79 vs. 65 years, $p<0.0001$) and had higher prevalence of at least one cardiopulmonary comorbidity (57% vs. 21%, $p=0.0014$). They suffered greater mortality (22% vs. 1%, $p=0.0007$), major (30% vs. 3%, $p=0.0003$), and minor (43% vs. 19%, $p=0.0269$) complications compared to elective repair. On multivariate analysis, emergent repair was a predictor of in-hospital mortality, major complications, readmission to intensive care unit, return to operating room, and length of stay.

Conclusion Emergent surgical repair of intrathoracic stomach was associated with markedly higher mortality and morbidity than elective repair. Although patients undergoing urgent surgery were older and had more comorbidities than those having an elective procedure, these data suggest that elective repair should be considered in patients with suitable surgical risk.

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Introduction

The management of patients with an intrathoracic stomach has become a more common concern given the marked increase in the elderly population. There are a number of unresolved issues related to their clinical care, including the risk of developing acute symptoms, the morbidity and mortality associated with elective vs. emergent repair, and the optimal surgical approach once repair is undertaken. Early reports of the natural history of patients with an intrathoracic stomach suggested a risk of emergent presentation and/or death during observation of as high as 30%.[1] More recent studies have reported a smaller risk of developing acute symptoms requiring emergency intervention.[2] The latter one has led to acceptance of watchful waiting in otherwise asymptomatic or minimally symptomatic individuals.

In addition to the risk of developing acute symptoms, the morbidity and mortality of emergent repair as compared to elective repair is relevant to management decisions.

Mortality has been reported in up to 40% of patients requiring urgent or emergent surgical repair.[3,4] Most recent case series do not identify patients presenting emergently, nor do they delineate the prevalence of emergent vs. elective presentations or the outcomes following emergent repair. Recent large scale population analyses have suggested that mortality of emergent paraesophageal hernia repair is high relative to elective repair.[5,6] As such, the timing of repair remains controversial, particularly the relative benefit of watchful waiting vs. early elective repair and the risk of emergent repair. The aim of the study was to assess the perioperative clinical outcomes and compare the morbidity and mortality of elective and acute repair of patients with an intrathoracic stomach.

Patients and Methods

Study Population

The study population consisted of 127 consecutive patients seen between June 2000 and December 2006 who underwent primary repair of an intrathoracic stomach at the University of Rochester Medical Center. The primary presenting symptoms included chest pain in 26 patients (20%), heartburn in 24 (19%), abdominal pain in 22 (17%), nausea/vomiting in 16 (13%), respiratory symptoms in nine (7%), and dysphagia in eight (6%). All patients had preoperative endoscopic evaluation. Barium esophagram and motility evaluation were performed on each individual at the discretion of the surgeon. All patients had at least 25% of the stomach herniated into the chest as determined from endoscopic, radiographic, and intraoperative evaluation. Mean follow-up was 12.7 months (range 1–97). Patients presenting emergently were operated on in the same admission. Nine patients were transferred from an outside hospital, and one patient became acutely obstructed while in the hospital following orthopedic wrist surgery.

Outcome Assessment

Study variables included details of clinical presentation and demographics, operative repair, and perioperative hospital stay. Independent variables included age, gender, body mass index (BMI), chief presenting symptoms, acute vs. elective presentation, type III vs. type IV hiatal hernia (HH), operative repair, and presence of a significant comorbidity. Significant comorbidity was defined as having at least one of the following cardiopulmonary comorbidities: coronary artery disease, congestive heart failure, chronic obstructive pulmonary disease (COPD), or an arrhythmia. The primary outcome was in-hospital mortality. Secondary outcome measures included postoperative complications, readmission

to the intensive care unit (ICU), return to the operating room (OR), and length of stay (LOS). Postoperative complications were graded via the classification reported by Clavien into major (III, IV, and V) and minor (I and II).[7]

Surgical Technique

All patients presenting emergently were adequately resuscitated and medically optimized prior to surgery, and 18 of 23 had nasogastric tube decompression. Open transabdominal repair was conducted through an upper midline incision. While the precise operative details varied according to the individual surgeon, the operative steps included extensive mobilization and reduction of the intrathoracic stomach and any other abdominal organs from the thorax, hernia sac excision, assessment of adequate esophageal length, and diaphragmatic crural repair with interrupted sutures. A fundoplication, either full or partial, was subsequently performed over a bougie. If a fundoplication was not performed, gastric fixation with gastropexy or gastrostomy was performed. If needed, crural reinforcement with mesh was also performed. Laparoscopic repair consisted of standard five laparoscopy incisions. All patients underwent extensive mobilization and reduction of the intrathoracic stomach from the thorax into the abdomen, hernia sac excision, mobilization of anterior fat pad, assessment of adequate esophageal length, and diaphragmatic primary crural repair with interrupted sutures. A Collis gastroplasty was performed if warranted. A fundoplication was subsequently performed routinely over a bougie. Mesh reinforcement was employed if there was evidence of tension or crural compromise. Transthoracic repair consisted of standard left lateral thoracotomy incision with the patient in right lateral decubitus position. The operative steps included dissection, mobilization, and excision of the hernia sac, assessment of adequate esophageal length, reduction of the intrathoracic stomach into the abdominal cavity, and diaphragmatic crural repair with interrupted sutures. This was followed by fundoplication. Two patients underwent hernia reduction, crural closure, and gastric bypass; three hernia reduction and crural closure with gastropexy; and two hernia reduction only with gastropexy. Three patients had a Collis gastroplasty. Six patients had crural reinforcement with mesh, four in laparoscopic repair (all Surgisis; Cook Medical, AZ) and two in open repair (Surgisis and Gore-Tex; Gore Medical, IN). Choice of operation was determined by the individual surgeon.

Statistical Analysis

Nondisease-specific survival curves were plotted as determined by the Social Security Death Index on April 28, 2009, with survival analysis comparison conducted with

Mantel–Cox log-rank test. Chi-square was used to compare proportions of gender, comorbidity, acute vs. elective presentation, types of operative repair, intraoperative injury, major and minor complications, readmission to ICU, and return to OR. t test was used to compare age, BMI, and length of stay. Factors associated with acute presentation were assessed via logistic regression. Adjusting for age, gender, BMI, comorbidity, operative repair, and presentation, multivariate regression analysis with model selection was conducted to assess predictors for the six outcomes. Analyses were conducted using SAS 9.1 © Copyright 2007 SAS Institute Inc., Cary, NC 27513, USA on a Windows XP platform.

Results

The clinical characteristics of the 127 patients are shown in Table 1. There were 94 females and 33 males with a mean

Table 1 Clinical Characteristics of the Study Population

Variable	Value
Total patients	127
Age	
Mean±SD	68.0±13.5
Range	37.5–100.7
Gender	
Female	94 (74%)
Male	33 (26%)
BMI	
Mean±SD	29.6±6.5
Range	15.1–51.0
Type IV HH	8 (6%)
Comorbidity[a]	35 (28%)
Type of repair	
Laparoscopic	75 (59%)
Open transabdominal	39 (31%)
Transthoracic	13 (10%)
Presentation	
Emergent	23 (18%)
Elective	104 (82%)
In-hospital mortality	6 (5%)
Major complications	10 (8%)
Minor complications	30 (24%)
Readmission to ICU	7 (6%)
Return to OR	5 (4%)
Postoperative LOS	
Mean	8.6
Median	5

[a] Presence of either CAD, CHF, COPD, or arrhythmia

Table 2 Summary of the Surgical Repairs in the Study Population

Operative approach	Patients
Laparoscopic	
Nissen fundoplication	59
Toupet fundoplication	16
Open transabdominal	
Nissen fundoplication	20
Toupet fundoplication	12
Gastric bypass	2
Reduction and closure only	3
Reduction only	2
Transthoracic	
Nissen fundoplication	12
Belsey fundoplication	1
Additional	
Collis gastroplasty	3
Mesh	6

age of 68 years ranging from 38 to 101. Ninety-four percent of the patients had a type III hiatal hernia. The surgical approach was laparoscopic in 75 (four conversions), open transabdominal in 39, and transthoracic in 13 patients (Table 2). A fundoplication was added in 120 of the 127 patients and consisted of a Nissen in 91, Toupet in 28, and Belsey fundoplication in one. The overall in-hospital mortality was 5% (six of 127). Major postoperative complications occurred in 8% (ten of 127) and minor in 24% (30 of 127). Five patients (4%) required reoperation and seven required readmission to the ICU.

Table 3 compares the clinical characteristics and procedures in patients admitted for elective ($n=104$) and emergent/urgent ($n=23$) repair. The primary presenting symptoms in patients admitted emergently were nausea/vomiting in eight, abdominal pain in seven, chest pain in four, failure to thrive in two, and acute dysphagia and shortness of breath in one each. Elective patients on the other hand presented primarily with a symptom of gastroesophageal reflux disease. Heartburn was present in 23 patients, with chest pain in 22, epigastric pain in 15, respiratory symptoms in nine, nausea/vomiting in eight, dysphagia in seven, regurgitation in six, anemia in two, and 12 unknown. All emergent patients presented with acute high-grade obstruction, and two had endoscopic evidence of strangulation and mucosal compromise. Patients presenting emergently were older (79 vs. 65 years, $p<0.0001$) and had a higher prevalence of at least one comorbidity (57% vs. 21%, $p=0.0014$) than those admitted electively. The majority of patients presenting acutely (74%, 17 of 23) were approached via an open transabdominal repair, while

Table 3 Clinical Characteristics of Patients Undergoing Emergent vs. Elective Repair

Value	Emergent repair (N = 23)	Elective repair (N = 104)	p value
Age			
Mean±SD	79.3±11.8	65.4±12.6	<0.0001
Gender			
Female	16 (70%)	78 (75%)	0.80
Male	7 (30%)	26 (25%)	
BMI			
Mean±SD	28.7±10.8	29.8±5.6	0.55
Comorbidity[a]	13 (57%)	22 (21%)	0.0014
Type of repair			
Laparoscopic	6 (26%)	69 (66%)	0.0007
Open transabdominal	17 (74%)	22 (21%)	
Transthoracic	0 (0%)	13 (13%)	

[a] Presence of either CAD, CHF, COPD, or arrhythmia

the majority of elective repairs were laparoscopic (66%, 69 of 104).

Emergent repair was associated with significantly greater mortality (22%, five of 23, vs. 1%, one of 104, p=0.0007) than elective repair (Table 4). Sepsis was the cause of death in all patients presenting emergently. Urosepsis was present in two, pneumonia in one, *Clostridium difficile* colitis in one, and fungal sepsis in one patient. The single death following elective repair was secondary to fulminant *C. difficile* colitis. Both major (30% vs. 3%, p=0.0003) and minor (43% vs. 19%, p=0.0269) complications were higher in emergent repair than those with elective repair. Admission to the ICU was more common following emergent repair (22%, five of 23 vs. 2%, two of 104, p=0.0021) as was reoperation (13%, three of 23 vs. 3%, three of 104, p= 0.072) and median length of hospital stay (9 vs. 4 days, p< 0.0001). The size of the intrathoracic stomach was not significantly related to any presentation or clinical outcome variables. On multivariate analysis with model selection, adjusting for age, gender, BMI, comorbidity, and type of repair, acute presentation was an independent predictor of in-hospital mortality (OR=28.6, p=0.0029), major complication (OR=14.7, p=0.0003), readmission to the ICU (OR= 14.2, p=0.0024), and return to the operating room (OR=7.7, p=0.0313; Table 5). Emergent presentation (p=0.0106) and

open transabdominal (p=0.0266) repair were independent predictors of postoperative LOS.

At a median of 50.6 months postoperatively, 5-year survival for the entire study population was 85% and 9-year survival was 69% (Fig. 1). The 5-year survival for patients with emergent repair was 41% compared to 94% in patients repaired electively (p<0.0001; Fig. 2). The median survival following emergent repair was 48.6 months.

Discussion

The data show that emergent surgical repair of an intrathoracic stomach is associated with a greater than 20-fold increase in mortality (22% vs. 1%) than elective repair. Perioperative complications and postoperative LOS were also significantly greater. Further, emergent presentation was an independent predictor of in-hospital mortality, major complications, readmission to the ICU, return to the OR, and postoperative LOS. Likely partly responsible for these observations, patients undergoing urgent surgery were older (79 vs. 65 years) and had more comorbidities than those having an elective procedure. Mortality was largely due to sepsis, each of which initially presented with acute high-grade obstruction and developed subsequent postoperative sepsis.

Table 4 Outcomes of Patients Undergoing Emergent vs. Elective Repair

| Outcome	Emergent repair (N = 23)	Elective repair (N = 104)	p value
In-hospital Mortality	5 (22%)	1 (1%)	0.0007
Major Complications	7 (30%)	3 (3%)	0.0003
Minor Complications	10 (43%)	20 (19%)	0.0269
Readmission to ICU	5 (22%)	2 (2%)	0.0021
Return to OR	3 (13%)	3 (3%)	0.072
Postoperative LOS			
Mean	21.5	5.7	<0.0001
Median	9	4	

Table 5 Emergent Presentation as an Independent Predictor for the Various Outcomes on Multivariate Analysis with Model Selection

Outcome[a]	OR	95% CI	p value
In-hospital mortality	28.6	3.2, 259.4	0.0029
Major complications	14.7	3.4, 62.9	0.0003
Readmission to ICU	14.2	2.6, 78.7	0.0024
Return to OR	7.7	1.2, 48.8	0.0313

[a] Adjusting for age, gender, BMI, comorbidity, operative repair

With the advent of the laparoscopic approach, there have been numerous reports of surgical outcomes following repair of the intrathoracic stomach over the past 20 years. Most studies have focused on the technical details of repair, morbidity and mortality in the elective setting, and the prevalence of hernia recurrence. As such, there is paucity of recent outcome data in patients following emergent presentation. Modern case series generally fail to specify which patients present emergently, and if reported, the proportion of patients operated upon urgently is small, commonly less than 10% of the cohort (Table 6). Outcome data published in the 1960s–1980s in contrast reported an average prevalence of over 40% emergent repairs. Our cohort of 23 patients represents 18% of the total study population, outlining the prevalence of the problem in present day demographics.[8]

Patients of advanced age with multiple comorbidities are at an increased operative risk, and surgical repair is often discouraged even if they present with mild obstructive symptoms. Historical data suggest that up to one third of these patients may develop acute or worsening symptoms requiring emergent repair.[1] This high incidence of developing acute symptoms while being observed has recently been challenged. Calculated from a pooled analysis of five case series where the interval of known hernia prior to repair was documented, Stylopoulus and colleagues reported the annual probability of an asymptomatic patient developing acute symptoms requiring emergency surgery to be 1.16% per year, with a lifetime risk of 18% in patients older than
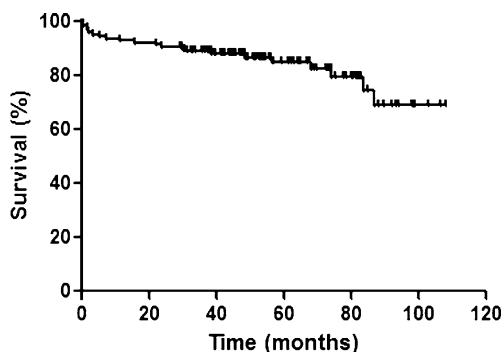
Figure 1 Overall nondisease-specific survival of patients following operative repair of an intrathoracic stomach.
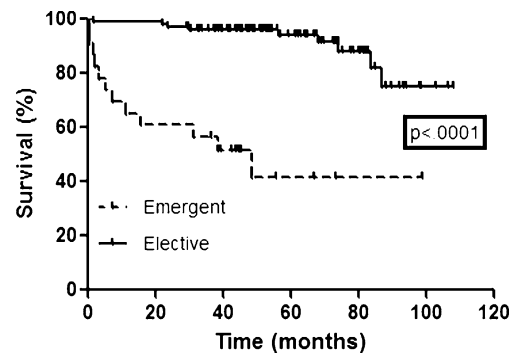
Figure 2 Nondisease-specific survival of patients with emergent (dashed line) vs. elective (solid line) repair of an intrathoracic stomach.

65 years.[2] Several caveats are important in interpreting this and similar data. First, the data may not be translatable to patients with symptoms, which constitute the majority of patients with intrathoracic stomach. Second, 18% represents a considerable lifetime risk of developing acute obstructive symptoms. Despite their advanced age, 85% of our study cohort was alive 5 years later and nearly 70% at 10 years. This attests to the longevity of current patients and underscores the length of time patients will be susceptible to complications of a watchful waiting approach. Finally, as our data show, the risk of emergent repair is considerable.

Complications associated with an acute presentation include obstruction, bleeding, strangulation, necrosis, and perforation. Patients may present with sudden onset of chest pain and/or obstructive symptoms, which can be associated with confusion, fever, and hemodynamic instability. Hill reported a mortality rate of 50% in 29 patients undergoing emergent repair in which preoperative decompression was not possible.[3] Beardsley and Thompson reported a mortality of 33% in a cohort of 15 patients with acutely obstructed hiatal hernia, pointing out that a delay in repair is the most important single factor that contributes to high morality.[4] Carter et al. reported on 25 patients with acute gastric volvulus showing 28% strangulation rate and 12% mortality.[9] Treacey and Jamieson reported 11% mortality (two of 18 patients) that underwent emergent repair.[10] Hallissey et al. identified a 25% mortality rate following repair after emergency admission in four patients and 5% mortality following elective repair in ten patients, advocating that elective surgery should be considered in all patients with paraesophageal hernia.[11] While advocating watchful waiting based on low incidence of acute presentation, Allen et al., when assessing patients that underwent emergent repair, found mortality in one of five patients (20%).[12] Menguy on the other hand demonstrated no mortality or major complications in 13 patients with emergent repair in a total cohort of 30 patients.[13] As can be seen in Table 6, mortality averaged 23% in published studies from the 1960s–1980s.

Table 6 Summary of Early and Modern Case Series that Report Emergent Repairs of an Intrathoracic Stomach

First author	Year	Operative repairs				Mortality				
		Total (N)	Elective (N)	Emergent (N)	Emergent (%)	Elective (N)	Elective (%)	Emergent (N)	Emergent (%)	Unspecified (N)
Early case series										
Beardsley[4]	1963	15	3	12	80.0	0	0.0	5	41.7	
Hoffman[18]	1968	23	0	23	100.0	0	n/a	9	39.1	
Hill[3]	1973	29	19	10	34.5	0	0.0	2	20.0	
Ozdemir[19]	1973	31	19	12	38.7	0	0.0	2	16.7	
Carter[9]	1980	25	0	25	100.0	0	n/a	3	12.0	
Treacy[10]	1987	54	45	9	16.7	0	0.0	1	11.1	
Menguy[13]	1988	30	17	13	43.3	0	0.0	0	0.0	
Haas[20]	1990	21	11	10	47.6	0	0.0	4	40.0	
Hallissey[11]	1992	24	20	4	16.7	1	5.0	1	25.0	
Allen[12]	1993	124	119	5	4.0	1	0.8	1	20.0	
					48.2 (mean)		0.7 (mean)		22.6 (mean)	
Modern case series										
Myers[21]	1995	37	29	8	21.6	0	0.0	0	0.0	
Trus[22]	1997	76	69	7	9.2					2
Altorki[14]	1998	47	33	14	29.8	0	0.0	1	7.1	
Gantert[23]	1998	55	50	5	9.1					1
Horgan[24]	1999	41	40	1	2.4					1
Wu[25]	1999	38	36	2	5.3	0	0.0	0	0.0	
Geha[15]	2000	100	80	20	20.0	0	0.0	2	10.0	
Mattar[26]	2002	136	133	3	2.2					3
Patel[27]	2004	240	235	5	2.1					3
Bawahab[16]	2009	20	0	20	100.0	0	n/a	0	0.0	
Current series	2009	127	103	23	18.1	1	1.0	5	21.7	
					20 (mean)		0.2 (mean)		6.5 (mean)	

More contemporary case series have reported lower mortality for emergent repair although most exclude, fail to mention, or are limited to small numbers of patients presenting emergently. Altorki and colleagues reported a 7% mortality (one of 14), Geha et al. reported 10% (two of 20), and Bawahab et al. reported no mortality in 20 patients following emergent repair.[14–16] Eighty-five percent of the patients in the latter report were repaired laparoscopically, demonstrating the feasibility of laparoscopic repair but possibly also reflecting a less ill and complicated study population. Based on the 1997 National Inpatient Sample, Stylopoulus et al. estimated a 5.4% operative mortality following emergency repair although the operative mortality of emergency surgery was 17% in a pooled analysis of six case series which compared to 1.38% following elective surgery averaged from a literature review of 21 studies.[2] The latter outcomes are similar to our findings.

Few population-based analyses have assessed the morbidity and mortality of either elective or emergent repair. Poulose and colleagues compared emergent and elective paraesophageal repair in 1,005 octogenarians using the 2005 Nationwide Inpatient Sample.[5] Patients undergoing elective repair had 2.5% mortality and an average length of stay of 7.0 days, compared to 15.7% mortality and average length of stay of 14.3 days following emergent repair. The authors concluded that elective repair of paraesophageal hernia regardless of symptoms may be warranted and possibly minimize mortality. Sihvo et al. reported a population based analysis from Finland. Of 563 patients undergoing either elective or emergent paraesophageal hernia repair over a 15-year period, mortality was 2.7%.[6] The absolute number of patients undergoing emergent and elective repair was not reported although there were 12 deaths after emergent and three after elective repair. The authors estimated that 13% of deaths could have been prevented by routine elective surgery. We recently reported an audit of the New York Statewide Planning and Research Cooperative System administrative database including nearly 5,000 admissions for an intrathoracic stomach over 5 years (2002–2006).[17] With or without surgical intervention, emergent admissions

had a higher mortality, longer length of stay, and higher hospital costs when compared to elective admissions. When assessing admissions with operative repair only, emergent operative admissions had nearly 5-fold higher mortality (5.1% vs. 1.1%) and doubled length of stay and hospital costs compared to elective operative admissions. These results further substantiate that emergent admissions drain hospital resources, and coupled with increased morbidity and mortality, consideration should be given to early elective repair.

There are several limitations to our study. Data collection was retrospective and as such subject to the biases associated with a retrospective review. Incomplete records did not allow us to document the time patients had symptoms prior to their presentation, an important piece of information to understand the incidence of acute presentation. It is difficult to know how many patients are truly asymptomatic in a specific study population, and being a referral center, we may be seeing a selected population of patients. The patients in our cohort presented for primary surgical repair, and those presenting with recurrence were excluded. The limited sample size within age groups prohibited us from determining if there is an advantage of elective repair in the elderly that would have offset the negative outcomes associated with emergent repair. Identifying significant outcomes of emergent and elective presentations within the elderly and different age groups might be possible with multicenter accrual of data. Long-term outcomes including hernia recurrence rates were not addressed in this study, which may impact the benefits of early elective repair. Further disease-specific long-term prospective follow-up with increased number of subjects and event rates would add strength to our study as well.

Conclusion

Emergent repair of an intrathoracic stomach is associated with a considerably higher mortality and morbidity than elective repair. The prevalence of emergent presentation remains considerable. We believe most patients with an intrathoracic stomach are best managed by elective repair, with the possible exception of those who are completely asymptomatic and/or with prohibitive comorbidities or very advanced age (>90 years). Those presenting emergently should undergo nasogastric tube decompression and resuscitation to reduce morbidity associated with the acute obstruction and potential sepsis, with repair prior to discharge.

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2009 SSAT PLENARY PRESENTATION

Duodenal Switch Provides Superior Resolution of Metabolic Comorbidities Independent of Weight Loss in the Super-obese (BMI≥50 kg/m^2) Compared with Gastric Bypass

Vivek N. Prachand · Marc Ward · John C. Alverdy

Abstract

Objective Increased body mass index is associated with greater incidence and severity of obesity-related comorbidities and inadequate postbariatric surgery weight loss. Accordingly, comorbidity resolution is an important measure of surgical outcome in super-obese individuals. We previously reported superior weight loss in super-obese patients following duodenal switch (DS) compared to Roux-en-Y gastric bypass (RYGB) in a large single institution series. We now report follow-up comparison of comorbidity resolution and correlation with weight loss.

Methods Data from patients undergoing DS and RYGB between August 2002 and October 2005 were prospectively collected and used to identify super-obese patients with diabetes, hypertension, dyslipidemia, and gastroesophageal reflux disease (GERD). Ali–Wolfe scoring was used to describe comorbidity severity. Chi-square analysis was used to compare resolution and two-sample *t* tests used to compare weight loss between patients whose comorbidities resolved and persisted.

Results Three hundred fifty super-obese patients [DS ($n=198$), RYGB ($n=152$)] were identified. Incidence and severity of hypertension, dyslipidemia, and GERD was comparable in both groups while diabetes was less common but more severe in the DS group (24.2% vs. 35.5%, Ali–Wolfe 3.27 vs. 2.94, $p<0.05$). Diabetes, hypertension, and dyslipidemia resolution was greater at 36 months for DS (diabetes, 100% vs. 60%; hypertension, 68.0% vs. 38.6%; dyslipidemia, 72% vs. 26.3%), while GERD resolution was greater for RYGB (76.9% vs. 48.57%; $p<0.05$). There were no differences in weight loss between comorbidity "resolvers" and "persisters".

Conclusions In comparison to RYGB, DS provides superior resolution of diabetes, hypertension, and dyslipidemia in the super-obese independent of weight loss.

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Introduction

Obesity has dramatically increased over the past several decades both in the USA and worldwide. According to a representative sample of nearly 14,000 individuals in the National Health and Nutrition Examination Survey, the prevalence of obesity among adults in the USA, defined as body mass index (BMI) ≥30 kg/m^2 [calculated as weight (kilograms) divided by the square of the height (meter)] increased from 13% in 1960 to 1962[1] to 32% in 2003 to 2004, with 3% of men and 7% of women classified as being severely obese (BMI≥40 kg/m^2) in the most recent estimate.[2] Strikingly, a disproportionate increase in the prevalence of superobesity (BMI≥50 kg/m^2) is evident when specifically examining trends in severe obesity, with a nearly tenfold increase in the prevalence of superobesity between 1986 and 2005 as compared to a twofold increase

in obesity (BMI≥30 kg/m^2) and fivefold increase in severe obesity (BMI≥40 kg/m^2) during this period.[3]

BMI is itself a strong predictor of overall mortality, with a progressive excess in mortality noted above the optimum BMI of 22.5–25. In a recent collaborative analysis of 900,000 adults enrolled in 57 studies, at a BMI of 30–35 kg/m^2, median survival was reduced by 2–4 years; at a BMI of 40–45 kg/m^2, it was reduced by 8–10 years. Furthermore, for each 5 kg/m^2 increase in BMI greater than 25, there was a nearly 30% increase in all-cause mortality due mainly to metabolic and vascular disease.[4] Indeed, the prevalence of metabolic comorbidities, including diabetes, hypertension, and dyslipidemia, increases significantly with increasing BMI.[5–7] The relationship between BMI and prevalence of comorbidities is not absolute, however: Not all severely obese or super-obese individuals have these conditions, and not all individuals with these conditions are overweight or obese. Furthermore, in comparison to individuals 40 years ago, the prevalence of hypertension and dyslipidemia (but not diabetes) as defined by levels of control in overweight and obese individuals has actually decreased,[8] although this appears to be due in large part to the increased use of anti-hypertensive and lipid-lowering medications. Despite these improvements in cardiovascular risk management, however, obesity-associated disability has actually increased by over 40% over the past decade.[9]

Even bariatric surgery, the most effective means of achieving significant and sustained weight loss in individuals with severe obesity,[10–13] may be less effective in achieving adequate weight loss as BMI exceeds 50 kg/m^2. Indeed, the initial concept of superobesity proposed by Mason et al.[14] was based on the observation that patients with BMI≥50 kg/m^2 undergoing vertical-banded gastroplasty often failed to achieve satisfactory weight loss after surgery, and this difference in weight loss outcome between patients with severe obesity and superobesity has since been demonstrated following Roux-en-Y gastric bypass (RYGB).[15–18]

The increase in the prevalence of superobesity, recognition of inadequate weight loss following RYGB in super-obese patients, and weight loss comparisons between bariatric operations in two recent meta-analyses[10,12] have prompted a growing interest in the biliopancreatic diversion with duodenal switch (DS) as a potentially advantageous procedure in the super-obese. The DS, developed by Hess and Hess[19] and Marceau et al.[20,21] is a hybrid operation that combines the DS of DeMeester et al.,[22] initially developed for the treatment and prevention of bile reflux, with the Scopinaro biliopancreatic diversion.[23] The greater technical complexity (particularly when performed laparoscopically) and perceived perioperative[24] and nutritional[25,26] risks of DS in comparison to RYGB, however, have limited the widespread adaptation of DS among bariatric surgeons. We

have previously demonstrated superior weight loss with the DS in direct comparison to RYGB without significant difference in morbidity and mortality in 350 consecutive super-obese patients.[27] As such, the added technical difficulty of the DS procedure and greater potential for nutritional deficiency of DS may be justified by the higher likelihood of significant and sustained weight loss.

Weight loss itself, however, is only one of the goals of bariatric surgery. An equally important outcome measure following a bariatric procedure is its impact on obesity-related comorbidities, particularly those associated with increased cardiovascular risk. Indeed, the cost-effectiveness of laparoscopic gastric bypass at 2 years after surgery is in large part predicated on a reduction in comorbidity-associated medication use, hospitalizations, and physician visits.[28] Numerous studies suggest an important linkage between weight loss and comorbidity improvement by showing that that a relatively modest amount of weight loss (10%) may result in significant improvement, and in some cases, resolution, of comorbidities.[29,30] Given that the observed weight loss following both DS and RYGB is often three to five times that amount, one would not anticipate substantial differences in comorbidity resolution between the two procedures. Furthermore, both RYGB[31] and DS[32–34] lead to dramatic improvement of obesity-related comorbidities. Nonetheless, given the differences that have been noted in the effects on comorbidities of the various bariatric procedures,[10,12] factors including the magnitude of weight loss and/or the physiology of the surgically altered anatomy may play an important role in their etiology. We herein report our follow-up comparison of comorbidity resolution and correlation with weight loss in super-obese patients following DS and RYGB.

Material and Methods

We conducted a retrospective review of an Institutional Review Board-approved, prospectively maintained database containing the demographic and anthropomorphic data of patients undergoing RYGB, biliopancreatic diversion with DS, and laparoscopic adjustable gastric banding (LAGB) between August 5, 2002 and November 10, 2005. The initial date was chosen, as it corresponds to the first DS performed at our institution. Patients underwent extensive multidisciplinary preoperative evaluation by a board-certified surgeon (VNP or JCA), dietician, and psychologist and were found to be appropriate candidates for bariatric surgery based on current NIH criteria [severe obesity (BMI≥40 kg/m^2 or 35–40 kg/m^2 with significant obesity-related comorbidities), history of multiple previous non-surgical weight loss attempts, adequate comprehension and support, and absence of

active substance abuse or poorly controlled psychologic disorders].[11]Eligibility for inclusion in this study included all consecutive patients undergoing standardized primary RYGB or DS with a preoperative BMI≥50 kg/m^2. Patients who had previous bariatric procedures or who underwent staged bariatric operations were excluded. Patients undergoing LAGB were excluded from analysis, as there were no super-obese patients who underwent banding during this 3-year period (the first LAGB at our institution was performed in March, 2005). The database was used to identify patients with preoperative diabetes (DM), hypertension (HTN), dyslipidemia (DL), and gastroesophageal reflux disease (GERD), and the Ali–Wolfe scoring scheme (AORC)[35] was used to describe comorbidity severity at the time of surgery and during follow-up and are shown in Table 1. DM, HTN, and DL were included in this study given their impact on cardiovascular risk, while GERD was chosen as an "internal control" given the recognized effectiveness of RYGB for the treatment of refractory GERD in severely obese patients who have failed other anti-reflux operations.[36] Comorbidity severity scoring was performed retrospectively based on chart review for visits that took place before the publication of the AORC scheme in 2006.

Procedure Selection

The relative advantages and disadvantages of the procedures were extensively discussed with the patient by the surgeon, and a general recommendation was made based on the severity of obesity, comorbidities present, and the patient's preference. While specific mention was made of the potential advantage of the DS with regards to weight loss in superobese patients, the final decision with regards to the procedure performed was made by the patient. In many instances, because the patient's insurance would not cover the DS, patients elected to proceed with the RYGB rather than attempt to appeal the decision of the insurance company, despite the

patient's preference for DS. The patient's primary care physician was notified in writing regarding the decision by the bariatric surgery team and the patient. Any necessary preoperative testing or treatments were performed. Mandatory preoperative weight loss or special diet was not routinely required.

Surgical Technique

Details regarding the techniques used to perform RYGB and DS have been previously described.[27] RYGB was performed in 152 super-obese individuals with a 40–50-cm biliopancreatic limb and a 100-cm ($n=27$) or 150-cm ($n=125$) Roux limb. The shorter Roux limb was used when mandated by insurance coverage. DS was performed in 198 super-obese individuals with a 100-cm common channel and 150-cm alimentary limb (distance from duodenoileostomy to ileoileostomy). Procedures were typically performed by an attending surgeon and senior surgical resident with a medical student operating the laparoscopic camera.

Intraoperative endoscopy with Roux (RYGB) or alimentary limb (DS) occlusion and air insufflation was used to test the integrity of the staple lines of the gastric reservoir and proximal anastomosis. A single 19-F Blake drain was placed near the proximal anastomosis extending up into the left upper quadrant, with removal taking place during the first postoperative visit (8–10 days postoperative). Patients were routinely admitted to the intermediate care unit with telemetry and continuous pulse oximetry after discharge from the recovery room and occasionally to the intensive care unit at the discretion of the surgeon and anesthesiologist. Patient-controlled intravenous narcotic analgesia was used for pain control. Low carbohydrate clear liquids at 30 mL/h were initiated on the morning of postoperative day 1, and enoxaparin 40–100 mg SQ bid was started and titrated to achieve a serum level just below therapeutic. Diet was

Table 1 Assessment of Obesity-Related Comorbidity Scale (adapted from Ali et al.[35])

Score	Diabetes	Hypertension	Dyslipidemia	GERD
0	Not present	Not present	Not present	Not present
1	Hyperinsulinemia without hyperglycemia	Borderline/intermittent/ diagnosis not confirmed	Borderline	Intermittent or variable symptoms, not requiring a response
2	Diabetes diagnosed, controlled by diet and exercise	Controlled by diet and exercise	Controlled by lifestyle changes: step 1, step 2 diet	Intermittent medication
3	Controlled by oral medications	Treatment with single medication	Controlled by low-dose medication	Regular medication (H^2 blockers or low-dose PPI)
4	Controlled by insulin	Treatment with multiple medications	Controlled by high-dose medication	High-dose PPI
5	Poorly controlled or severe complications	Poorly controlled or severe complications	Not controlled by medication	Meet criteria for antireflux operation or prior operation for GERD

advanced to pureed foods on postoperative day 2 as tolerated, and patients were discharged after demonstration of diet tolerance and return of bowel function. Enoxaparin was continued for 2–3 weeks after discharge.

Follow-up

Patients were seen 1.5 weeks postoperative for drain removal and 2.5 weeks postoperative for diet advancement and initiation of vitamin supplements (prenatal multivitamin, B12, and calcium citrate with vitamin D). Patients were seen by the surgeon and a bariatric dietician at each visit and by psychologists as needed. While the diet contents and progression were identical for both procedures, DS patients were instructed to achieve 75–85 g protein intake/day as opposed to 60–65 g protein/day for the RYGB patients. Subsequent follow-up appointments took place 1, 3, and 6 months, then yearly thereafter. Comorbidity assessments were performed at each postoperative visit and follow-up phone conversation, and nutritional parameters were measured at the 3-month, 6-month, and yearly visit, with supplementation adjusted accordingly. Resolution of comorbidity was defined as discontinuation of medications used for treatment with the absence of symptoms. All adjustments to medications used in the treatment of any comorbidity were made by the referring or primary care physician. Attempts were made by phone and by mail to contact patients who failed to keep follow-up appointments, moved, or whose insurance was no longer accepted at the University of Chicago Medical Center.

Statistical Analysis

Ideal body weight (IBW) was calculated using the formula IBW $= [(2.3 \times (\text{height in inches}) - 60)) + A) \times 2.2]$, where A is 45.5 for females and 50 for males, with excess body weight (EBW)=measured weight−IBW. Comparison of the demographic data was performed using two-tailed pooled t tests for continuous data (age, weight, BMI, and EBW) except length of stay, for which the Satterthwaite t test was used due to unequal variances. Chi-square analysis was used to compare the rate of resolution for each of these comorbidities except when a low number of observations required Fisher exact test, and two-sample t tests used to compare weight loss between patients whose comorbidities resolved and those whose comorbidities remained. Non-parametric Wilcoxon tests were used to compare the mean AORC score at various postoperative time points against their preoperative mean AORC score. Weights and comorbidity status were recorded at the time of clinic visit or telephone conversation. For purposes of analysis, weights and comorbidity status recorded between 4 and 8 months were grouped as "6 months postoperative," 9–15 months as

"12 months," 16–20 months as "18 months," 21–30 months as "24 months," and 31–60 months as "36 months." If more than one visit occurred for an individual patient during any of these periods, the latest visit was used and the others excluded.

Results

Three hundred fifty super-obese (BMI≥50 kg/m^2) patients underwent DS (n=198) or RYGB (n=152) over a 39-month period with equal 30-day mortality (DS, 1/198 (0.51%) and RYGB, 0/133, p=NS). Demographics of the two groups are shown in Table 2. Mean age and gender were similar in both groups, while mean preoperative weight (368.2 vs. 346.3 lbs, p=0.0002) and BMI (58.8 vs. 56.4 kg/m^2, p=0.0014) were significantly greater in the DS group compared to the RY group. The prevalence and severity of HTN, DL, and GERD was comparable in both groups (p=NS), while DM was less prevalent but more severe in the DS group.

The number of individuals for whom comorbidity scoring was available and their mean AORC score at each time point is shown in Table 3. Non-parametric Wilcoxon tests were used to compare the mean AORC score at various postoperative time points to the mean baseline AORC score, and all comparisons were found to be highly significant (p<0.05). Resolution rates for DM, HTN, and DL were greater for DS [DM: 18 months, 79.3% vs. 47.6%; 24 months, 91.2% vs. 50%; 36 months, 100% vs. 60%; HTN: 24 months, 56.5% vs. 28.6%; 36 months, 68.0% vs. 38.6%; DL: 36 months, 72% vs. 26.3%; p<0.05)], while GERD resolution was greater for RYGB (36 months, 76.9% vs. 48.57%; p<0.05; Table 4).

There were no statistically significant differences in mean weight loss noted between DS patients whose comorbidities resolved (AORC score 0) compared to DS patients whose comorbidities persisted (AORC score≥1). Similarly, no differences in weight loss were noted in RYGB patients whose comorbidities resolved and RYGB patients whose comorbidities persisted (data not shown). Finally, when comparing the weight loss of RYGB patients whose comorbidities resolved to the weight loss of DS patients whose comorbidities persisted, the weight loss for the DS patients whose hypertension and GERD did not resolve was greater than the RYGB patients whose hypertension and GERD did in fact resolve (Fig. 1).

Discussion

Given the exponential increase in the prevalence of super-obesity within the population of patients who may be

Table 2 Age and Gender Were Well-Matched

		DS	RYGB	p value
Number of patients		198	152	
Age (years)	Mean±SD	40.4±9.5	40.5±10.9	NS[a]
	Range	18–61	21–68	
Gender	(% F)	82.3%	84.2%	NS[c]
Weight (lbs)	Mean±SD	368.2±52.3	346.3±55.2	*0.0002*[a]
	Range	267.4–596.5	239.8–504.9	
BMI (kg/m^2)	Mean±SD	58.8±6.7	56.4±6.8	*0.0014*[a]
	Range	49.6–96.3	49.5–84.2	
EBW (lbs)	Mean±SD	233.9±42.5	215.9±43.9	*0.0001*[a]
	Range	162.2–408.1	159.9–379.5	
Diabetes	Number of patients (prevalence)	48 (24.2%)	54 (35.5%)	*<0.05*[a]
	Mean AORC score	3.27	2.9	*<0.05*[a]
Hypertension	Prevalence	133 (67.2%)	101 (66.5%)	NS[a]
	Mean AORC score	3.02	2.98	NS[a]
Dyslipidemia	Prevalence	62 (31.3%)	55 (36.2%)	NS[a]
	Mean AORC score	2.71	2.65	NS[a]
GERD	Prevalence	84 (42.4%)	51 (33.6%)	NS[a]
	Mean AORC score	2.54	2.52	NS[a]
Mortality	(%)	1/198 (0.51%)	0.0%	1.0000[b]
LOS (days)	(Mean±SD)	4.86±5.9	3.83±2.6	*0.0300*[d]
	Range	2–68	2–25	
LOS (days)	Median	4.00	3.00	
LOS>4		48 (24.24%)	30 (19.74%)	0.3154[c]

DS patients were heavier than RYGB in all measures. Mortality rate was not significantly different, but LOS was 1 day longer for DS. Equivalent proportions of patients had hospital stays> 4 days. The prevalence and severity of HTN, DL, and GERD was comparable in both groups (p=NS), while DM was less prevalent but more severe in the DS group. *p* values<0.05 are indicated in italics

SD standard deviation, *LOS* length of stay

[a] Pooled two-tailed *t* test

[b] Fisher's exact *p* value

[c] $\chi 2$ test

[d] Satterthwaite *t* test

Table 3 Patients Available for Follow-up

			Months postoperation					
			Pre	6	12	18	24	36
Diabetes								
Number of patients		DS	48	45	44	29	34	21
AORC			3.27	1.8	0.98	0.45	0.18	0
Number of patients		RY	54	43	37	21	28	20
AORC			2.94	2.4	1.54	1.33	1.29	1
Hypertension								
Number of patients		DS	133	123	116	79	85	75
AORC			3.06	2.59	2.04	1.7	1.16	0.83
Number of patients		RY	101	81	76	41	49	44
AORC			3	2.54	2.22	1.76	2.06	1.75
Dyslipidemia								
Number of patients		DS	62	55	52	40	40	25
AORC			2.71	2.31	1.65	1.18	0.68	0.56
Number of patients		RY	55	41	39	20	25	19
AORC			2.65	2.44	1.72	1.6	1.32	1.68
GERD								
Number of patients		DS	84	76	69	49	50	35
AORC			2.55	1.87	1.57	1.12	1.08	1.17
Number of patients		RY	51	43	34	20	26	26
AORC			2.53	1.58	1.12	0.8	0.88	0.58

The number of patients with the particular comorbidity and the mean AORC score at each time point for whom follow-up data is available is shown

Table 4 Resolution of Comorbidities Following DS and RYGB. Resolution of Comorbidity was Defined as Discontinuation of Medications Used for Treatment and the Absence of Symptoms of that Comorbidity

		Months postoperation				
		6	12	18	24	36+
DM						
%Resolved	DS	33.3	59.1	79.3	91.2	100
	RY	9.52	37.84	47.6	50	60
p value		0.05	0.25	0.05	0	0.04
HTN						
%Resolved	DS	7.4	24.1	32.9	56.5	68
	RY	8.8	19.7	31.7	28.6	38.6
p value		0.58	0.62	0.69	0.01	0
DL						
%Resolved	DS	7.27	32.7	45	70	72
	RY	10	33.3	35	52	26.3
p value		0.89	0.46	0.5	0.02	0.01
GERD						
%Resolved	DS	22.4	29	42.9	48	48.6
	RY	34.9	50	65	61	76.9
p value		0.14	0.06	0.1	0.93	0.04

Adjustments to medications used in the treatment of any comorbidity were made by the referring or primary care physician. DM, HTN, and DL resolution was greater for DS at 24 months and 36 months, while GERD resolution was greater for RYGB at 36 months

potential candidates for bariatric surgery, determining the "best" surgical treatment for super-obesity is an important task facing the bariatric surgical community. The optimal procedure should have acceptably low morbidity and mortality rates, result in significant and durable weight loss, and lead to improvement or resolution of obesity-related comorbidities as well as quality of life.

We have previously demonstrated that DS provides a significant advantage over RYGB when comparing weight loss, percentage of EBW lost, decrease in BMI, and likelihood of achieving at least 50% EBW loss without significantly increased perioperative morbidity and mortality.[27]

The main focus of this report is the comparison of comorbidity resolution following DS and RYGB. We demonstrate that DS provides greater resolution of DM, HTN, and DL, while RYGB provides better resolution of GERD. The finding regarding DM is particularly striking given the greater preoperative severity of DM in the DS group (AORC, 3.27 vs 2.9, $p<0.05$). Furthermore, the relative advantage for DS in the treatment of HTN and DL cannot be explained by a difference in preoperative comorbidity severity, given the equivalent AORC scores in DS and RYGB patients (Table 2).

We chose to focus on resolution, rather than improvement, of comorbidities in this study in part to attempt to better characterize this rather dramatic effect of bariatric surgery. Modest weight loss (8–10%) is clearly associated with significant improvement of cardiovascular disease-associated comorbidities but rarely leads to their resolution.[30] Additionally, it is the reduction in medication requirements, hospital admissions, and clinic visits associated with comorbidity resolution that is the primary contributor to the cost-effectiveness of bariatric surgery[28] and as such the comparative differences in comorbidity resolution may impact cost-effectiveness in different ways. For example, giving preferential consideration to DS in the setting of super-obesity and severe diabetes may be appropriate given the higher likelihood of successful weight loss and diabetes resolution.

It is important to recognize, however, that the term "resolution," particularly when applied in the context of metabolic obesity-related comorbidities, is controversial. "Remission" may in fact be a more broadly acceptable term to non-surgical medical specialists to describe these phenomena until longer term data become available. Furthermore, we did not obtain objective measurements of comorbidities (e.g., homeostasis model assessment–insulin resistance and euglycemic clamp for glucose homeostasis, 24-h pH study for GERD, etc.) to determine whether a
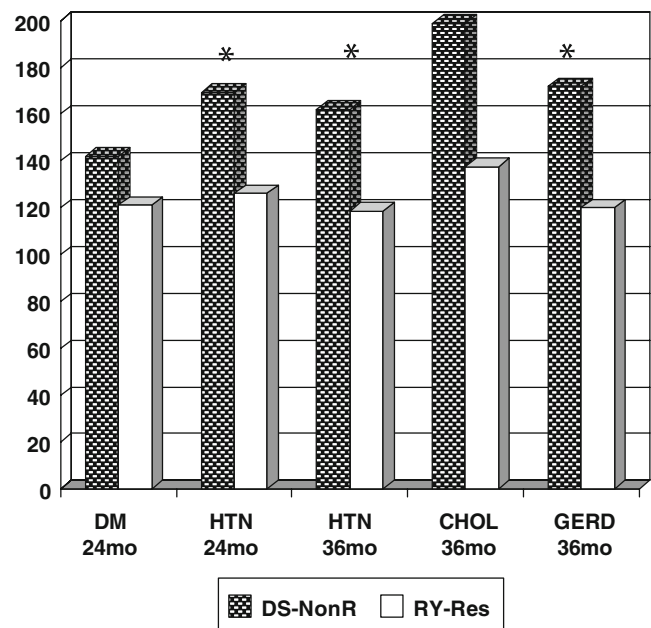
Figure 1 Weight loss comparison between RYGB "Resolvers" and DS "Non-resolvers". When comparing the weight loss of RYGB patients whose comorbidities resolved to the weight loss of DS patients whose comorbidities persisted, the weight loss for the DS patients whose hypertension and GERD did not resolve was greater than the RYGB patients whose hypertension and GERD did in fact resolve. *$p<0.05$.

comorbidity had in fact resolved physiologically. Indeed, reliance on the accuracy and appropriateness of medication discontinuation on the part of a broad range of referring physicians and primary care providers introduces potential for error in our data. Finally, the strong incentives to discontinue medications on the part of both the patient (financial, convenience) and the surgeon (improvement in measured outcome to reporting bodies) may inadvertently reduce adherence to evidence-based guidelines for tighter "triple endpoint" control of HbA1c, blood pressure, and triglycerides, as adherence to treatment recommendations, which have demonstrable benefit with regards to reduced cardiovascular risk, may require the continued use of medications.

The lack of a demonstrable difference in weight loss between patients whose comorbidities resolved and those whose comorbidities persisted was a surprising finding. Unfortunately, our data are underpowered to assess whether those patients whose comorbidities persisted had higher preoperative AORC scores and how demographic factors, such as sex, age, and race impact the response of comorbidities to surgery. Nonetheless, when comparing the weight loss of RYGB patients whose comorbidities resolved to the weight loss of DS patients whose comorbidities persisted, the weight loss for the DS patients whose hypertension and GERD remained unresolved was greater than the RYGB patients whose hypertension and GERD did in fact resolve. The greater GERD resolution seen with RYGB despite reduced weight loss compared to DS suggests differences in the physiologic effects of altered surgical anatomy independent of weight loss per se. In the absence of objective physiologic data, we speculate that the modest amount of acid produced in the small volume gastric pouch combined with minimal bile reflux given a Roux limb length exceeding 100 cm may account for the marked improvement in GERD seen following RYGB. In contrast, the gastric sleeve of the DS has greater acid-producing capacity compared to the RYGB pouch, and the small caliber of the sleeve may result in increased resistance to flow of acid from the proximal sleeve and clearance of refluxate in the distal esophagus. Similarly, there is currently great interest in the role that alterations in gut hormones such as ghrelin, leptin, peptide YY, and glucagon-like peptide-1 (GLP-1) play in surgical weight loss and comorbidity physiology. The latter two hormones are secreted by L cells in the distal small bowel and may be major factors in inducing satiety through central mechanisms as well as through delayed gastric emptying and increased intestinal transit time.[37,38] Additionally, GLP-1 is a potent incretin that lowers blood glucose levels by enhancing insulin secretion, reducing glucagon levels, and delaying gastric emptying.[39] RYGB has been shown to result in an increase in both hormones,[40–42] and it may be

that differences between the two procedures in their neurohormonal response may account for both the differences in weight loss and diabetes resolution following DS as compared to RYGB. As such, shifting attention from the effects of weight loss per se to the comparative physiology of the two operations promises to yield important insights into the mechanisms by which the procedures exert their effects as well as the pathophysiology (and potential development of non-surgical therapy) of these comorbidities.

Because the selection of the procedure performed was not randomized, a significant limitation of this study is selection bias. We generally recommended DS for all super-obese patients, particularly if their BMI was ≥ 60 kg/m^2 ($n=109$). Of the patients who ultimately underwent RYGB, about half did so because their insurer considered the DS to be "investigational" and they did not want to initiate a lengthy appeals process; about half did so because the DS was "too radical" or because an acquaintance or family member had a good outcome with RYGB. DS was not recommended in a few instances when patients had frequent or loose stools at baseline. Despite the lack of randomization, patient age and gender distribution did appear to be closely matched. Additionally, while there were variations in surgical technique with regards to the method of access and creation of anastomoses, the measured lengths used for small intestinal reconstruction were standardized to the extent possible. Finally, with the exception of a slightly greater daily protein requirement for DS patients, the perioperative management and follow-up regimen was purposefully kept the same for both procedures in an effort to minimize the influence that differences in postoperative care may have had on outcomes.

The loss of patients to follow-up is another factor that limits the quality of our data. While the rate of follow-up 1 year after DS and RYGB was 80% and 60%, respectively, at 3 years, the follow up was about 50% for both groups. This disappointing follow-up may have limited our ability to more accurately assess the likelihood of comorbidity resolution 2–3 years after surgery. While our follow-up rate is less than the 80–99% follow-up obtained in studies performed in the Canadian heath care system,[34,21] they are comparable to those seen in many American series.

Our previous direct comparison of short-term weight loss outcomes of DS to RYGB demonstrated that the DS provides superior weight loss in the super-obese compared to gastric bypass. This current study extends these findings by comparing the intermediate-term effects of these operations on the resolution of significant obesity-related comorbidities and demonstrates that weight loss per se may not be the primary determinant of comorbidity resolution. Further study and follow-up will be needed to confirm and extend the present findings, and a long-term assessment and comparison of nutritional outcomes

and quality of life will allow the development of an evidence-based rationale for procedure selection in this challenging patient population.

Conclusion

In comparison to RYGB, DS provides superior resolution of diabetes, hypertension, and dyslipidemia in the super-obese independent of weight loss.

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Discussant

Dr. J. Chris Eagon (Washington University, St. Louis): That was a wonderful presentation. I guess one of the questions I had was, "do you think that the duodenal switch operation is worse off in terms of GERD resolution because of the anatomical configuration of the sleeve?" Or is there some other effect there that is present that is making that difference?

Second of all, I was a little surprised about the relative lack of effect of gastric bypass compared to duodenal switch in terms of diabetes resolution.

Do you have any ideas about how to detect why that is the case? Are there some hormonal differences in the fact that the nutrients are being pushed a little bit farther downstream in the GI tract as a reason for that?

Discussant

Dr. Vivek Prachand (University of Chicago): Even though the sleeve gastrectomy does result in resection of a significant amount of the gastric parietal cell mass, I suspect that the amount of acid production in the remaining pouch or sleeve is substantially greater than the small 20-cc pouch that is made during gastric bypass.

Combining this increased acid production with the relative resistance to forward flow given the long tubular structure of the sleeve—thinking about Poiseuille's law—I think that there may be impaired esophageal clearance of acid. I think that both operations are very effective at controlling biliary reflux given the Roux limb length of greater than 100 cm.

With regards to the resolution of diabetes, I think that there are contributions both from decreased fat cell mass, as

well as the neurohormonal effects of these operations that contribute to the resolution.

It may very well be that the differential stimulation and increased release of GLP 1 and peptide YY with a greater amount of distal delivery of nutrients in the duodenal switch may, in part, account for the difference that we see.

Discussant

Dr. Michael Sarr: Are there some people you would not do a duodenal switch on, such as someone who is in the weight category but has severe gastroesophageal reflux?

Closing discussant

Dr. Vivek Prachand (University of Chicago): I think that is a patient that I would have serious reservations about performing a duodenal switch on. However, if they were a very bad diabetic, hypertensive, and so forth, then I still would probably lean more toward a duodenal switch than a bypass.

One of the questions that we do ask preoperatively is, "what is their typical bowel habit pattern beforehand?" If they are already having two to four bowel movements a day regularly, which is typically the pattern that we see after DS, I am also hesitant to offer duodenal switch to those patients.

Discussant

Dr. Michael Sarr: What about a distal gastric bypass? Do you think that these patients lose the same amount of weight as a duodenal switch? That operation would get rid of the reflux problem.

Discussant

Dr. Vivek Prachand (University of Chicago): I think your group has demonstrated that the weight loss is pretty similar to the duodenal switch and that might be a good option in a patient with reflux.

Discussant

Dr. Manfred Prager (Austria): How do the comorbidities contribute to the overall effect of the duodenal switch. Is it the length of the biliopancreatic and/or the nutritional limb? Or is it also that you have the duodenal-jejunal anastomosis and that you leave the pyloric valve?

Does the pyloric valve have a positive effect on the efficacy of the duodenal switch?

Discussant

Dr. Vivek Prachand (University of Chicago): I could speculate that, again, thinking about the distal gut hormones

and how they impact on gastric emptying, having an intact antropyloric mechanism may in part contribute to those sorts of effects. With regards to the biliopancreatic limb versus alimentary limb, as I mentioned, there are some groups that use fixed limb lengths as we do versus those that use proportionately tailored limbs. I think the answer is that we really do not know.

We chose to use fixed lengths because they are something that we could control, and be consistent with, and standardize. But I think that it is probably unrealistic and naive to think about the biliopancreatic limb as just being a passive conduit of biliopancreatic secretion when we know there is a lot of reabsorption and inactivation of enzymes that occurs.

2009 SSAT PLENARY PRESENTATION

Does LKB1 Mediate Activation of Hepatic AMP-Protein Kinase (AMPK) and Sirtuin1 (SIRT1) After Roux-en-Y Gastric Bypass in Obese Rats?

Yanhua Peng · Drew A. Rideout · Steven S. Rakita · William R. Gower Jr · Min You · Michel M. Murr

Abstract

Introduction Roux-en-Y gastric bypass (RYGB) improves steatosis and reduces liver triglycerides in obese rats. Sirtuin1 (SIRT1) and AMP-activated protein kinase (AMPK) are key metabolic regulators that reduce lipogenesis and increase fatty acid oxidation. LKB1 phosphorylates AMPK and may activate SIRT1. We hypothesize that RYGB in obese rats is associated with an upregulation of the LKB1–AMPK–SIRT1 signaling pathway.

Methods Obese Sprague–Dawley male rats underwent RYGB or sham. Liver tissue was obtained at 9 weeks postoperatively. Protein levels of SIRT1, LKB1, p-LKB1, AMPKα, p-AMPKα, and p-protein kinase C-ζ (PKC-ζ) were determined. Protein associations of LKB1 with each of SIRT1, AMPKα, and PKC-ζ were determined by co-immunoprecipitation. Data are mean±SD; for t test, $p<0.05$ was significant.

Results RYGB increased protein levels of hepatic AMPKα, p-AMPKα, and SIRT1 (all $p<0.001$ vs. sham); p-LKB1 but not LKB1 increased after RYGB ($p<0.001$ vs. sham). Physical interactions of LKB1–AMPK and LKB1–SIRT1 increased after RYGB ($p<0.001$ vs. sham). Although PKC-ζ mRNA and p-PKC-ζ did not change, interactions between LKB1 and PKC-ζ increased after RYGB ($p<0.001$ vs. sham).

Conclusion RYGB increases hepatic levels of SIRT1, AMPK, and p-AMPK as well as increasing interactions of LKB1 with AMPK or SIRT1. p-PKC-ζ may play an intermediary role in the interaction between AMPK and SIRT. These findings demonstrate key signaling changes in powerful metabolic regulators that may account for the resolution of steatosis after RYGB.

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Introduction

Obesity and its related disorders are a fast-growing epidemic. Obesity also induces liver injury; at least 95% of patients with class III obesity (body mass index (BMI)≥ 40 kg/m^2) exhibit steatosis, steatohepatitis, or fibrosis on routine liver biopsies.[1] Additionally, preliminary data on liver biopsies of 100 patients after surgically induced weight loss show significant improvement in the histological features of steatosis and steatohepatitis and suggest that the progression of fibrosis is halted.[2,3]

In a rat model, we demonstrated that high fat diet induces steatosis and that Roux-en-Y gastric bypass (RYGB) induces sustained weight loss and improves steatosis.[4–6] We further confirmed that RYGB decreases hepatic triglycerides and downregulates hepatic lipogenic signaling.[7,8] Furthermore, we have demonstrated that AMP-

activated protein kinase (AMPK) and mammalian sirtuin1 (SIRT1) are upregulated in the livers of obese rats that underwent RYGB.[8]

AMPK is a metabolic energy sensor of AMP/ATP ratio in eukaryotes that maintains energy stores and enhances oxidative metabolism.[9] Therapies designed to increase AMPK are used in the treatment of type II diabetes and associated metabolic disorders.[9–12] Additionally, AMPK is a heterotrimeric protein kinase that regulates lipid and glucose metabolism through direct phosphorylation of its substrates and indirect control over gene transcription.[13]

SIRT1 is an NAD^+-dependant class III protein deacetylase that is localized exclusively in the nucleus and acts as a master metabolic regulator.[14–17] Increased SIRT1 and AMPK are associated with decreased lipogenesis and increased fatty acid oxidation,[17] and both play a key role in protecting hepatocytes against alcohol-induced fatty liver.[18] While both SIRT1 and AMPK have similar beneficial actions and share signaling pathways, a direct interaction has not been demonstrated between these two enzymes.[9,12,14] Nonetheless, LKB1 (a serine/threonine kinase also called STK11) has been proposed as a link between these two powerful metabolic regulators.[12]

AMPK can be activated by an increase in the cellular AMP/ATP ration and is phosphorylated at the Thr172 site by LKB1, Ca^{+2}/calmodulin-dependent protein kinase β, and transforming growth factor β-activated kinase-1.[12,19] LKB1 itself can be activated by protein kinase C-ζ (PKC-ζ) through phosphorylation at Ser428.[20] Given these complex interactions, we hypothesize that activation of AMPK and SIRT1 after RYGB will be associated with activation of LKB1.

Materials and Methods

Animals and Animal Care All experiments were approved by the Institutional Animal Care and Use Committee of the University of South Florida College of Medicine.

High Fat Diet and RYGB Model Four-week-old Sprague–Dawley rats were maintained in light- and temperature-controlled environments (12:12-h light/dark, 20–24°C). Rats were fed regular chow containing 5% fat by weight or high fat diet (Harlan) containing 60% fat for 14 weeks to induce obesity.[4] Subsequently, obese rats were randomized to RYGB or sham procedure as previously described.[4] Liver tissues were obtained at 9 weeks postoperatively.

RT-PCR for mRNA Measurement PKC, SIRT1, LKB1, and AMPK mRNA were measured with semiquantitation

reverse transcriptase polymerase chain reaction (RT-PCR) and real-time RT-PCR. The methods are described in our and others previous reports.[21,22]

Immunoblotting Liver cells were lysed in radio immuno-precipitation assay buffer (phosphate-buffered saline (PBS) with 0.1% sodium dodecyl sulfate (SDS), 1% Nonidet P-40 (NP40), 0.5% sodium deoxycholate); 50–100-μg samples of protein were fractionated by 10% SDS-polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose membrane, blocked for 1 h with PBS (5% instant nonfat dry milk, 0.1% Tween-20), and then incubated for 2 h with antibodies (0.05 μg/ml) to either LKB1, p-LKB1, Sirt1, AMPK, p-AMPK, PKC-ζ, p-PKC-ζ, and β-actin. Histone-1 (Santa Cruz, CA, USA) was used for nuclear extract loading control. Bound primary antibody was detected by incubating with horseradish peroxide goat antimouse or antirabbit IgG (0.0125 μg/ml). Membranes were developed using Super Signal (Pierce, Rockford, IL, USA) ECL reagent and quantified by densitometry.

Co-immunoprecipitation and Western Blotting Co-immunoprecipitation was used to determine if LKB1, AMPK, Sirt1, or PKC interact physically. Briefly, liver tissue was lysed (50 mm Tris-HCl, pH 8.0, 5 mm ethylenediaminetetraacetic acid, 150 mM NaCl, 0.5% NP40, 1 mm phenylmethylsulfonyl fluoride); 1,000 μg of protein was immunoprecipitated with LKB1 antibody and protein A-Sepharose beads. The beads were then washed with lysis buffer; the immunoprecipitate was fractionated by 10% SDS-PAGE and transferred to Hybond ECL nitrocellulose membrane. Subsequently, AMPK, SIRT1, or PKC-ζ antibodies were used for blotting with the co-immunoprecipitated protein. Gels were quantified using densitometry.

Immunofluorescent Staining for Protein Colocalization Formalin-fixed liver sections were deparaffinized/hydrated with xylene, ethanol, PBS, and treated with 0.1–0.2% trypsin in 0.4% $CaCl_2$ for 1 h and then incubated with either F4/80 (macrophage marker), SIRT1, LKB1, AMPK, or PKC-ζ antibodies (1:200 in PBS plus 10% normal goat serum) for 2–4 h. The slides were washed with PBS + 0.1% Triton X-100, incubated with fluorescent isothiocyanate goat antimouse or rabbit IgG and mounted with antifade solution containing 4′,6-diamidino-2-phenylindole (DAPI). The slides were examined by Nikon microscope, and the images were merged by Image-pro-express software (Image Processing Solutions Inc., North Reading, MA, USA).
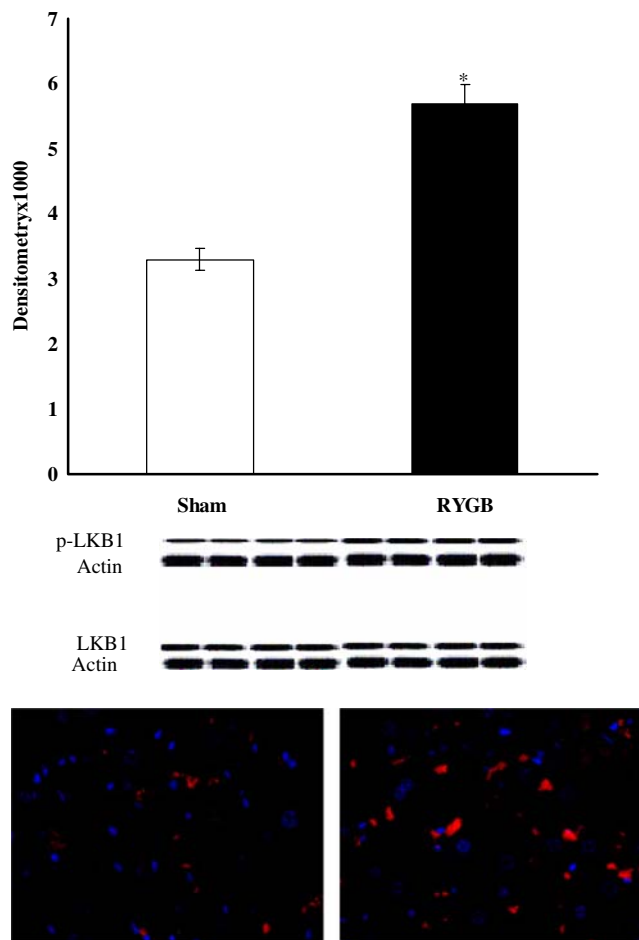
Data Analysis All experiments were repeated at least in triplicates. t test was used to compare means; $p < 0.05$ was significant. Data are mean±standard deviation.

Results

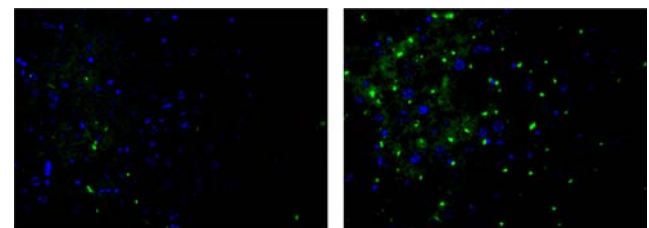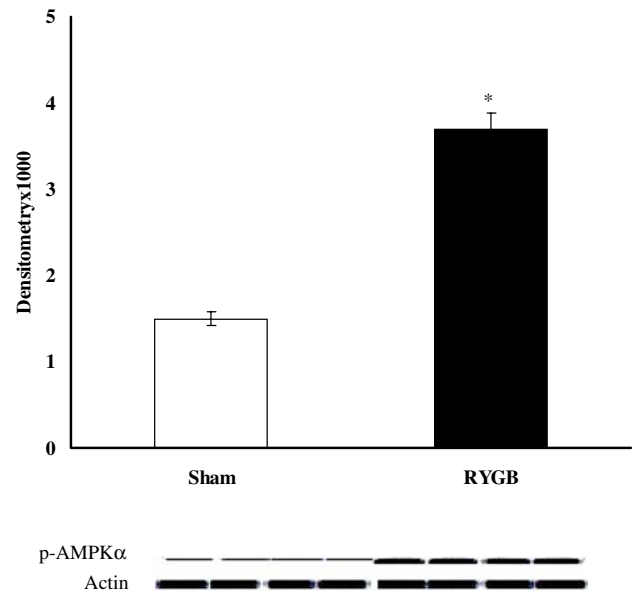*Expression of LKB1/p-LKB1* RYGB increased p-LKB1 protein ($5{,}674 \pm 125$ vs $3{,}265 \pm 89$; $p < 0.001$ vs sham; Fig. 1); however, total LKB1 expression was not changed ($p > 0.05$). Additionally, LKB1 and p-LKB1 localized mostly in the cytoplasm of liver cells after RYGB, while they localized mostly to the nucleus in the sham group (Fig. 1) suggesting that RYGB is associated with activation and transportation of LKB1 to the cytoplasm.

AMPKα and p-AMPKα RYGB increased the expression of AMPKα and its phosphorylated form (p-AMPKα) compared to sham rats (AMPKα $5{,}431 \pm 150$ vs. $2{,}323 \pm 117$; p-AMPKα $3{,}652 \pm 120$ vs. $1{,}534 \pm 60$; all $p < 0.001$ vs sham; Fig. 2).

SIRT1 RYGB increased SIRT1 compared to sham rats (protein $4{,}567 \pm 47$ vs. $2{,}675 \pm 36$, $p < 0.001$ vs sham control; Fig. 3). By immunofluorescent staining, the majority of
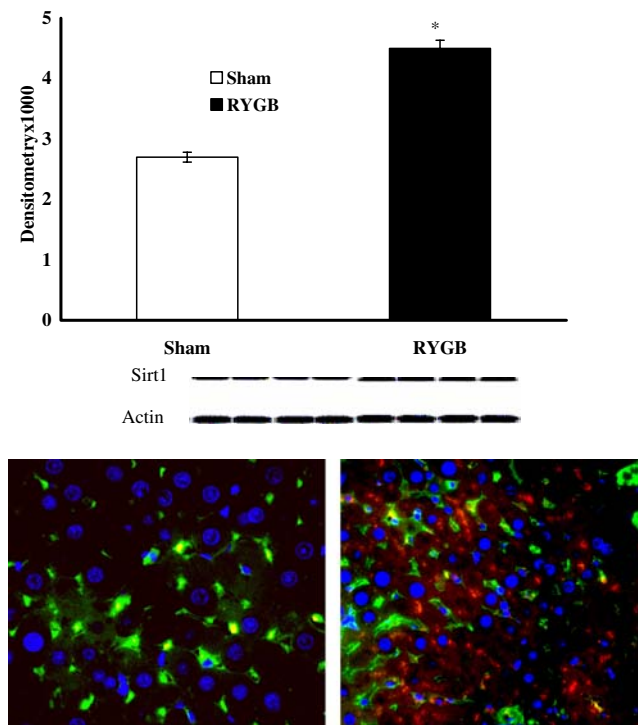

Figure 2 RYGB increases p-AMPKα expression in rat liver compared to sham control, *$p < 0.001$ vs sham; representative gels are shown *below bar graph*. Additionally, RYGB (*right*) increases immunostaining for p-AMPK (*green*) in liver sections compared to sham (*left*); DAPI is *blue*.

cells that stained for SIRT1 were hepatic parenchymal cells; there was a lesser degree of staining of SIRT1 in non-parenchymal cells that also stained positive for the macrophage marker F4/80 (Fig. 3), thereby suggesting that SIRT1 is mostly localized in hepatocytes. RYGB had no effect on SIRT1 mRNA (data not shown; $p > 0.05$).

PKC-ζ and p-PKC-ζ/λ Phosphorylated PKC-ζ/λ levels were lower after RYGB compared to sham control ($2{,}135 \pm 20$ vs. $3{,}742 \pm 30$, $p < 0.001$); however, RYGB did not change total PKC-ζ/λ protein or mRNA levels ($p > 0.05$; Fig. 4).

Physical Interactions of LKB1 Using co-immunoprecipitation techniques, RYGB increased the physical interactions between LKB1 and AMPK, LKB1 and SIRT1, as well as LKB1 and PKC-ζ/λ (LKB1/AMPK $6{,}352 \pm 142$ vs $2{,}132 \pm 87$; LKB1/SIRT1 $4{,}563 \pm 45$ vs. $1{,}543 \pm 22$; LKB1/PKCζ/λ $4{,}356 \pm 102$ vs $2{,}354 \pm 78$, respectively; all $p < 0.001$ vs. sham). However, there were no direct interactions observed between AMPK–SIRT1 and

Figure 1 RYGB upregulates p-LKB1 level in rat liver compared to sham control, *$p < 0.001$ vs sham; representative gels are shown *below bar graph*. Additionally, RYGB (*right*) increases immunostaining for p-LKB1 (*red*) in liver sections compared to sham (*left*); DAPI is *blue*.

Figure 3 RYGB increases SIRT1 expression in rat liver compared to sham control, *p<0.001 vs sham; representative gels are shown *below bar graph*. Additionally, RYGB (*right*) increases immunostaining for SIRT1 (*red*) as well as the macrophage marker F4/80 (*green*) in liver sections compared to sham (*left*); DAPI is *blue*.

AMPK–PKC-ζ/λ in both sham and RYGB groups, thereby suggesting that LKB1 may be the link between activation of AMPK and SIRT1 (Fig. 5).

Discussion

We have viewed excess adiposity in the liver as an imbalance between lipogenesis and fatty acid oxidation and therefore focused our laboratory efforts to understand mechanistic changes in these metabolic processes in response to surgically induced weight loss. Our data are the first evidence that lends insight to the regulation of the two powerful metabolic regulators SIRT1 and AMPK after surgically induced weight loss. Our findings that RYGB upregulates hepatic SIRT1 expression and AMPK activation without any direct physical association between the two enzymes are novel and consistent with other models of alcohol injury and in vitro studies.

Our results show that SIRT1 and AMPK expression increases after RYGB procedure on obese rats; this may be partially because of decreasing food intake at the beginning of weeks after postsurgical procedure and rapid weight loss. LKB1 is a kinase that is known to function as an upstream

activator of AMPK; the previously designated AMPK kinase that has been purified from rat liver corresponds to LKB1; additionally, blocking LKB1 abolishes AMPK activation.[23] In our current model of diet-induced obesity and RYGB, hepatic LKB1 is activated after RYGB and may be the link between activation of SIRT1 and AMPK. These two master metabolic controllers have direct and powerful effects on lipogenesis and fatty acid oxidation, and therefore, dysregulation of these enzymes induces excess adiposity of liver parenchymal cells. Specifically, dysfunction of hepatic AMPK is a key factor in the accumulation of lipids in the liver and the hyperlipidemia associated with diabetes.[12] Additionally, sterol regulatory element binding protein-1c (SREBP-1c) which controls gene expression of fatty acid and triglyceride synthesis is negatively regulated by AMPK.[24]

Recently published data confirm that metformin (an antidiabetic drug) and polyphenols lower systemic and hepatic lipids via activating LKB1–AMPK signaling pathway.[16,17] The beneficial impact of polyphenols on lipid accumulation is mediated by activation of SIRT1 or AMPK.[12,18] Moreover, the activation of SIRT1 improves
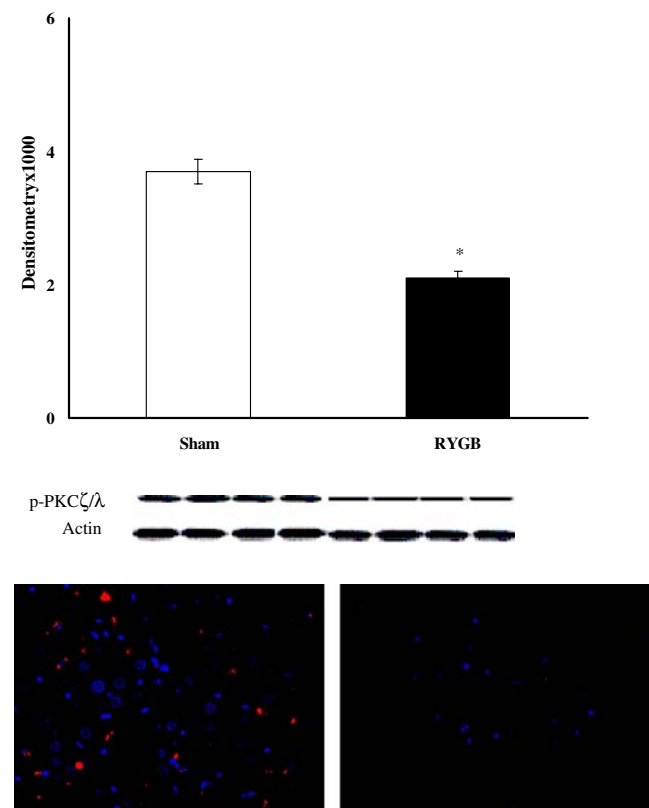
Figure 4 RYGB downregulates p-PKC ζ/λ expression in rat liver compared to sham control, *p<0.001 vs sham; representative gels are shown *below bar graph*. Additionally, RYGB (*right*) decreases immunostaining for p-PKC ζ/λ (*red*) in liver sections compared to sham (*left*); DAPI is *blue*.
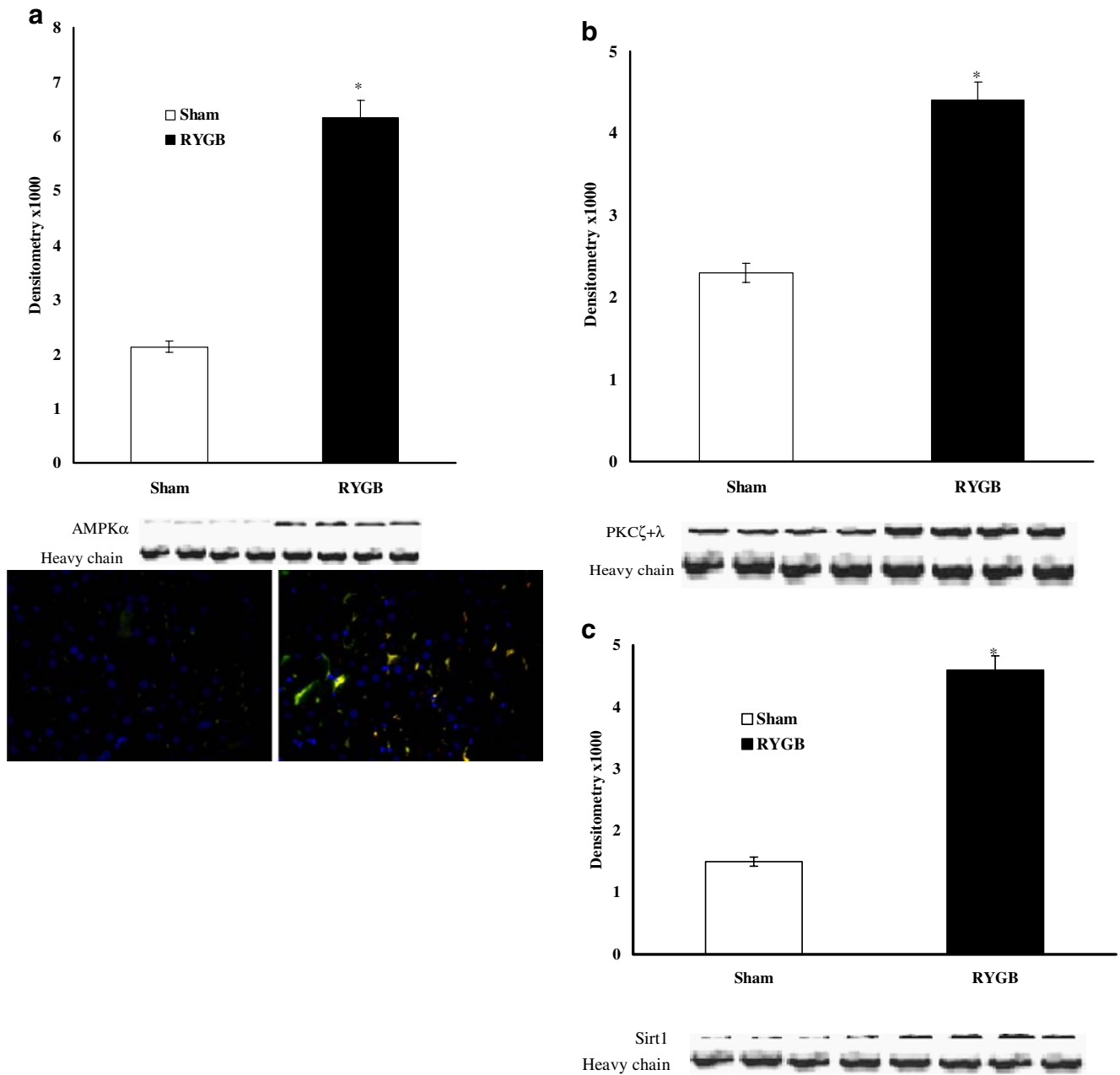
Figure 5 RYGB increases interactions among LKB1 and AMPK, LKB1 and PKC, and SIRT1 and LKB1 in rat liver compared to sham control, *all $p<0.001$ vs sham. Co-IP Western blotting representative gels are shown *below bar graph*. **a** RYGB (*right*) increases co-immunofluorescence of LKB1 (*red*) associated with AMPK (*green*); the two colors combine to create *yellow*; DAPI is *blue*. **b** RYGB (*right*) increases co-immunoprecipitation of LKB1 associated with PKC-ζ/λ compared to sham. **c** RYGB (*right*) increases co-immunoprecipitation of LKB1 associated with SIRT1 compared to sham.

insulin sensitivity and protects hepatocytes against alcohol-induced lipid accumulation.[17]

SIRT1 exhibits its effect by deacetylating its target molecules; similar to AMPK, SIRT1 inhibits the transcriptional activity of SREBP-1c and therefore decreases lipogenesis. Moreover, SIRT1 activates peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) by deacetylation thereby increasing fatty acid oxidation via upregulation of medium-chain acetyl-coenzyme A (CoA) dehydrogenase.[25,26] PGC-1α can also be phosphorylated by AMPK leading to increased fatty acid oxidation.[27]

This dual control of de novo fatty acid synthesis and fatty acid oxidation by AMPK and SIRT is an area of intensive investigation. Shaw et al.[28] demonstrated that there is near complete loss of AMPK activity in liver-

specific LKB1 knockout mice that was associated with hyperglycemia, increased gene/protein expression of lipogenic enzymes, and loss of the ability of metformin to activate AMPK and lower blood glucose level.[29,30]

Hou et al.[12] suggested that SIRT1 functions as an upstream regulator for LKB1/AMPK signaling and that it is essential for regulating hepatocyte lipid metabolism; studies in HepG2 hepatocytes and mice livers that utilized pharmacological and genetic stimulators or inhibitors of SIRT1, LKB1 natural knock out cell line, and AMPK dominant negative adenovirus have demonstrated that stimulation of AMPK depends on SIRT1 activity and that LKB1 is required for activation of AMPK by polyphenols and SIRT1. We demonstrated physical interactions between LKB1 and AMPK and between LKB1 and SIRT1; however, we did not demonstrate any interaction between AMPK and SIRT1.

Activated LKB1 increases the phosphorylation and activation of AMPK. AMPK is also activated by different stimuli which include pathological stresses, such as oxidative damage, hypoxia, glucose deprivation as well as physiological stimuli such as exercise, muscle contraction, and hormones such as leptin and adiponectin.[31] AMPK plays a critical role in hepatocyte lipid metabolisms through acetyl-CoA carboxylase and fatty acid synthase (FAS) and their effect on fatty acid oxidation and synthesis.[12,19,32,33]

In addition to its role in regulating LKB1, SIRT1 associates with and deacetylates substrates, such as PGC-1α, FOXO1, and PPARα/γ that are critically important in lipid and glucose metabolism in hepatocytes. Moreover, PGC-1α is also directly phosphorylated by AMPK,[14] and there is evidence that AMPK indirect activation by SIRT1 also protects against activation of FAS and lipid accumulation caused by high glucose.[12]

Therefore, SIRT1 likely regulates hepatic lipid homoeostasis through AMPK-mediated phosphorylation of PGC-1α. Recently, Canto et al.[10] claimed that AMPK controls the expression of genes involved in energy metabolism in the mouse skeletal muscle through another metabolic sensor, the NAD^+-dependent SIRT1; AMPK enhances SIRT1 activity by increasing cellular NAD^+ levels resulting in the deacetylation and modulation of the activity in downstream targets of SIRT1 which include the PGC-1α and FOXO1/3 transcription factors. Additionally, AMPK is required for the rosiglitazone-mediated attenuation of both reactive oxygen species production and NADPH oxidase NOX2 protein expression in Kupffer cells treated with free fatty acids.[8]

One possible explanation of these physical interactions is that AMPK and SIRT1 are activated by different stimuli that follow different pathways. However, a more plausible explanation given our data is that LKB1, which associates with both AMPK and SIRT1, is activated by an upstream regulator.

Atypical PKC-ζ plays an essential role in metformin-enhanced AMPK activation by phosphorylating LKB1 at Ser428 which is required for LKB1 transport to the cytosol where it phosphorylates AMPK. Because LKB1 can be phosphorylated at Ser428 by many stimuli, it may be the common pathway required for AMPK and SIRT1 activation.[34]

RYGB decreases p-PKC-ζ/λ expression; nonetheless, RYGB increases interaction of PKC-ζ with LKB1 suggesting that it may be involved indirectly in control of LKB1 activity. Recent analysis revealed that with LKB1 activator peroxynitrite, PKC-ζ directly phosphorylated LKB1 at Ser(428) in vitro and in intact cells, resulting in increased PTEN phosphorylation at Ser(380)/Thr(382/383), and peroxynitrite enhanced PKC-ζ nuclear import and LKB1 nuclear export. They conclude that PKC-ζ mediates LKB1-dependent Akt inhibition, resulting in endothelial apoptosis.[35]

Same group also reported that PKC-ζ can regulate AMPK activity by increasing the Ser428 phosphorylation of LKB1, resulting in association of LKB1 with AMPK and consequent AMPK Thr172 phosphorylation by LKB1.[36] However, Ussher et al. shows that PKC-ζ plays a very minor role in the regulation of AMPK in cardiac and skeletal muscle and may actually be a downstream target of AMPK in skeletal muscle.[37] We only know that PKC-ζ and LKB1 have directly interaction each other. So far we do not know what is the real effect of PKC-ζ on LKB1 phosphorylation and activity in obese rats after RYGB. We will continue to study.

Our findings that RYGB enhances SIRT1 protein expression without changing its transcriptional activity are supported by other reports.[38] A mechanism for activation of LKB1 by SIRT1 includes activation of LKB1 by deacetylation of key lysine residues and facilitating its movement from the nucleus to the cytoplasm.[39]

Based on this study, we are proposing that RYGB may improve steatosis by increasing the phosphorylation and activation of AMPK; consequently, this leads to the phosphorylation of PGC1α, the upregulation of NAD^+ or $NADP^+$ levels, and the activation of NAD^+-dependant SIRT1. LKB1 can be deacetylated by SIRT1, and in turn, LKB1 enhances AMPK activation. The role of PKC-ζ/λ in associating with LKB1 may provide further insight to upstream regulators in lipid accumulation and surgically induced weight loss.

Although phosphorylated PKC-ζ, LKB1, and AMPK proteins increased, we have not seen the changes in the mRNA transcriptions (data not shown). Further studies are needed to ascertain the proposed pathway; we need to provide direct evidence for activation of AMPK, LKB1, PKC, and SIRT1 with both kinase and acetylation assays, as well as inhibitor/knock out and overexpression studies.

Since LKB1 directly phosphorylates and activates AMPK, AMPK regulates lipid, cholesterol, and glucose metabolism in central metabolic tissues, such as liver,

muscle, and adipose tissue,[40] and upstream components of LKB1, such as adiponectin, leptin, and other gastrointestinal hormones, may be the important regulators for LKB1 expression and activations.[41,42] Mapping upstream signaling pathways of LKB1/AMPK after RYGB rats is more likely involved in adipose tissue and liver.

We will investigate putative signaling between adipocytokines and the liver. Notwithstanding, these data provide important insight into the metabolic changes resulting from surgically induced weight loss.

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Discussant

Dr. Kevin E. Behrns (Gainesville, FL): I think it is important to note that this is a very timely topic, hepatic metabolism in obese patients and especially how it is affected by bariatric procedures. I would challenge your conclusion a little bit in that you say that LKB1 is a direct link to AMPK, but I think you need to perform some inhibitor studies to show that it is a direct link. So I would ask you how you could do this. This is obviously an in vivo model, but I think that you could do it either through primary hepatocytes or an adenovirus expressing siRNA. So I would ask if you thought about that.

Then you started the talk by showing us that you have liver biopsy specimens at 660 patients. So this is a perfect translational model. So how would you take the information that you have got from your in vivo model of rat gastric bypass and apply that to the biopsy specimens that you have on those patients?

Closing Discussant

Dr. Drew A. Rideout (Tampa, FL): To address the inhibitor study, it is in vivo, which makes it much more difficult. There are a couple of different possibilities. You can use knockout or overexpression models. Those tend to be mice models. Creating a gastric bypass is very challenging in the rat and mouse, but we are working on doing so in a mouse at this point. The other option is to perfuse the liver. As you mentioned, we can administrate an inhibitor via perfusion and then isolate hepatocytes or Kupffer cells and analyze for changes. We have also used a Kupffer cell line to access some of these changes in the Kupffer cells; thus, there are some in vitro studies that can be done using overexpression. These are future studies that we are working on.

As far as the translational studies, we do have follow-up. We have biopsies of 100 patients who had undergone gastric bypass, and they do show intrusion of steatosis and what appears to be a halting of fibrosis, and so now we have a large amount of biopsies before and quite a few biopsies after surgery. We need to set up further studies to look at those issues, which are obviously very small, but to look at those tissues and see if these changes apply as well as in this animal model.

Outcome Based on Management for Duodenal Adenomas: Sporadic Versus Familial Disease

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Abstract

Introduction Management and outcomes for duodenal adenomas may vary based on etiology, familial versus sporadic. We reviewed the records of patients managed at our institution for duodenal adenomatous polyps for the 20-year period ending July 2006.

Discussion Methods of polyp resection (endoscopic, local surgical resection, or definitive surgical resection) within both sporadic and familial patient groups were compared. Patients with known cancer were excluded. Two hundred seventy-eight patients with duodenal polyps were followed during this time period: 110 patients (39.6%) with sporadic polyps and 168 (60.4%) with familial adenomatous polyposis (FAP). Sporadic patients presented at a mean age of 66.5 years. Endoscopic resection was attempted in 44 patients (40%) with morbidity in 9% and local recurrence rate of 52% with a mean follow-up of 43 months. Surgical resection was performed in 46 patients (42%): 27 by definitive resection and local resection in 19. At a mean follow-up of 41 months, there were no local recurrences in the patients treated by definitive resection and six recurrences (32%) after local resection. Morbidity was 39%. There was a significant difference in local recurrence when comparing definitive resection to both endoscopic and local resection ($p<0.001$, $p=0.002$, respectively), but no significant difference between endoscopic and local excision ($p=0.13$). Cancer was discovered in the surgical specimens of 11 patients (24%) with benign preoperative biopsies. FAP patients began surveillance at a mean age of 39.5 years, and mean surveillance duration was 100 months. Endoscopic resection/ablation was attempted in 40 patients (24%) with a morbidity of 7.5%. With a mean follow-up of 77.5 months, the local recurrence rate was 72.5%. Surgical resection was performed in 50 patients (30%) with a mean follow-up of 44 months. Definitive resection was performed in 47 and local excision in three with local recurrence rates of 9% and 100%, respectively. Surgical morbidity was 48%. Local recurrence was significantly lower following definitive resection compared to endoscopic or local resection ($p<0.001$), but there was no difference in local recurrence between the latter two groups ($p=0.29$). Four patients (8%) undergoing surgery were discovered to have invasive cancer despite benign endoscopic biopsies. In summary, endoscopic and local surgical management for both sporadic and familial duodenal polyps are associated with a high rate of local recurrence. Definitive resection in the form of pancreaticoduodenectomy, pancreas-sparing duodenectomy, or segmental duodenectomy offers the best chance for polyp eradication and prevention of carcinoma, regardless of polyp etiology.

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Introduction

Duodenal carcinoma is a rare neoplasm accounting for less than 1% of all gastrointestinal tract cancers. Among patients with resectable disease, estimated 5- and 10-year disease-specific survival following R0 resection is 68% and 56%, respectively.[1] Similar to colorectal cancer, duodenal polyps are thought to follow an adenoma-to-carcinoma sequence, thus providing a rationale for preventive intervention. The risk to develop duodenal cancer and the ability to prevent its occurrence with any form of intervention may be vastly different in sporadic and familial duodenal polyps. Patients with familial adenomatous polyposis (FAP) syndrome comprise a unique subset of patients with duodenal polyps. They have a cumulative lifetime risk of approximately 5% for the development of duodenal cancer, which is currently the most frequent cause of cancer-associated mortality.[2,3] This cancer risk can be as high as 36% for patients with advanced duodenal polyposis. In the case of both sporadic and FAP-associated duodenal adenomas, surveillance and management has traditionally consisted of a variable combination of endoscopy and surgery. Farnell et al. reported a series of patients with villous adenomas with a high rate of recurrence following local excision.[4] Similar to other series, their report combined the outcomes for sporadic and familial disease, including patients with known invasive cancer. Other series have demonstrated that the local recurrence rate following local excision in patients with FAP is high, resulting in a lack of downstaging or alteration in the risk to develop cancer.[5] It is presumed that the ability to alter the risk of cancer for any patient with adenomatous polyps requires an intervention that achieves sustained eradication. This has led our group and others to adopt a more aggressive approach in patients with advanced stage FAP.[6,7] This type of surgical approach, with its attendant complications, may not be justified in all patients with duodenal polyps, particularly if the polyps occur sporadically. For these reasons, we have reviewed our management and outcomes for duodenal adenomas based on their etiology, familial versus sporadic.

Methods

After institutional review board approval, the medical records of patients managed at our institution for duodenal adenomas for the 20-year period ending July 2006 were reviewed. Patients were identified using a pathology database from endoscopic biopsies. Both electronic and paper chart medical records were used to gather patient information including age, gender, and sporadic versus familial disease. FAP patients were collected from the Cleveland Clinic inherited colorectal cancer registry, all of whom had undergone prior total colectomy. Adenoma characteristics studied included number, size, location, histology (tubular adenoma (TA), villous adenoma (VA), and tubulovillous adenoma (TVA)), and degree of dysplasia (low-grade dysplasia, high-grade dysplasia (HGD), carcinoma in situ (CIS)). Patients found to have invasive cancer on preoperative biopsy were excluded from analysis. Burden of duodenal disease in FAP patients was characterized according to the Spigelman classification system (Table 1).

Endoscopic duodenal surveillance in FAP patients was performed according to a standard protocol: forward and side-viewing endoscopic examination; biopsy of the papilla; directed biopsy of >1 cm, enlarging, or ulcerated duodenal polyps; and documentation of endoscopic findings on a standardized collection form at the time of the procedure.[9] Endoscopic polypectomy, mucosal resection, fulguration, and argon plasma coagulation were employed selectively and were not based on a standardized treatment protocol. Endoscopic ultrasound and endoscopic retrograde cholangiopancreatography were used selectively to evaluate depth invasion and pancreatic/common bile duct involvement, respectively. Endoscopic surveillance was recommended to be performed every 5 years for stage 0, 3 years for stages I and II, 1 year for stage III, and 3–6 months for stage IV.

FAP patients with Spigelman stage IV (and papilla-dominant stage III) disease and patients with sporadic polyps judged by a therapeutic endoscopist at our institution not to be a candidate for endoscopic excision were referred for surgical management. Surgical treatments consisted of pancreaticoduodenectomy (PD), pancreas-sparing duodenectomy (PSD), segmental duodenectomy, or transduodenal polypectomy/ampullectomy. The type of operation was based on polyp characteristics (location,

Table 1 Spigelman Classification of the Severity of Duodenal Adenomatosis[8]

	Number of points		
	1	2	3
Number of polyps	1–4	5–20	>20
Polyp size (mm)	1–4	5–10	>20
Histology	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Stage 0, 0 points; stage I, 1–4 points; stage II, 5–6 points; stage III, 7–8 points; stage IV, 9–12 points

presence of cancer) and experience of the surgeon. *Local* surgical treatment was defined as either transduodenal polypectomy or ampullectomy, whereas *definitive* treatment was defined as PD (standard and pylorus preserving), PSD, or segmental duodenectomy.

Postoperative mortality was defined as death within 30 days of surgery. Morbidity included anastomotic leak, gastroparesis, surgical site infection, bleeding requiring reoperation or other intervention, and respiratory failure requiring mechanical ventilation and/or monitoring in an intensive care unit.[10] Patients were followed clinically and with continued endoscopic surveillance in the case of FAP patients. Recurrences were classified as local if at the site of prior polyp resection or at the neo-ampulla or duodenal cuff following PSD.

Patient groups were compared with respect to continuous variables such as age and number of endoscopies using a two-tailed *t* test with a null hypothesis of a no difference in means and unequal variances. Group comparisons with respect to discrete variables such as the necessity for surgery and/or recurrence were performed using a chi-square test. Groups compared included those defined by disease and surgical groups. A level of $\alpha = 0.05$ was used to define statistical significance. When pairwise comparisons of groups were performed, a Bonferroni correction was applied to adjust for the size of the main grouping.

Results

Two hundred seventy-eight patients were treated for duodenal adenomas at our institution over the past 20 years. The characteristics of sporadic and FAP patients are depicted in Table 2. One hundred ten patients (39.6%) were treated for sporadic adenomas and 168 (60.4%) were under surveillance for FAP. Patients with sporadic disease presented at a mean age of 66.5 years (range 35–86) while surveillance of the FAP cohort was started at a mean age of 39.5 years (range 13–84; $p < 0.001$). Forty-six patients (41.4%) in the sporadic group ultimately underwent surgery compared to 50 (29.8%) in the FAP group ($p = 0.04$). The

mean number of endoscopies performed (including pre- and postoperative) in the sporadic group was 3.1 versus 6.1 in the FAP group ($p < 0.001$).

Within the sporadic group, six patients had multiple polyps (5%) with the remainder having solitary lesions. Polyp location was as follows: bulb in eight (7%), D2 in 51 (46%), D3 in ten (9%), D4 in one, and ampulla in 27 (25%). Polyps involved multiple duodenal segments in ten (9%) patients, and in three patients, polyp location was not specified by the endoscopist. Initial endoscopic biopsy among patients with sporadic polyps is depicted in Table 3. Adenoma histology on endoscopic biopsy revealed TA in 41.8%, TVA in 40%, and VA in 17.3%. High-grade dysplasia or carcinoma in situ was present in 11.8%. Spigelman stage among FAP patients at initial endoscopy was as follows: 72 (43%) stage O/I, 38 (23%) stage II, 28 (17%) stage III, and 29 (17%) stage IV.

Tables 4 and 5 depict treatment and outcomes for sporadic and familial polyps. Among patients with sporadic disease, endoscopic resection or ablation procedures were attempted in 44 patients (40%). There were four complications (9%) following endoscopic resection including two cases of nonnecrotizing pancreatitis and two perforations, one of which required operative repair. Twenty-three patients (52%) with sporadic polyps initially treated with endoscopy developed a local recurrence following a mean follow-up of 43 months. Mean interval to recurrence was 5.8 months: Seven patients underwent at least two subsequent endoscopic resections with complete eradication of the recurrent polyp, and seven underwent surgical resection. One of these seven patients underwent PD and was found to have invasive cancer following 4 months of aggressive endoscopic therapy for biopsy-proven VA with

Table 2 Characteristics of Sporadic Versus FAP Patients

	Sporadic	FAP	*p* value
Number of patients	110	168	
Age (years)[a]	66.5	39.5	<0.001
Endoscopic procedures			
Total	339	994	
Mean (per patient)	3.1	6.1	<0.001
Surgery (%)	46 (41.4)	50 (29.8)	0.04

[a] Clinical presentation or inception of endoscopic treatment/surveillance

Table 3 Location and Initial Endoscopic Biopsy of Sporadic Duodenal Adenomas

N=110	Type	Number (%)
Histology	TA	46 (41.8)
	TVA	44 (40)
	VA	19 (17.3)
Dysplasia	Low grade	97 (88.1)
	HGD/CIS	13 (11.8)
Polyp location	Bulb	8 (7)
	D2	51 (46)
	D3	10 (9)
	D4	1 (1)
	Ampulla	27 (25)
	Multiple segments	10 (9)
	Not known	3 (3)

One patient did not have an endoscopic biopsy

HGD high-grade dysplasia, *CIS* carcinoma in situ

Table 4 Treatment and Outcomes of Duodenal Adenomas in Sporadic Versus FAP Patients

		Treatment			Local recurrence			Carcinoma[a]
			Surgery			Surgery		
	N	Endo	Local	Definitive	Endo	Local	Definitive	
Sporadic	110	44 (40%)	19 (17%)	27 (24%)	23 (52%)	6 (32%)	0	11 (24%)[b]
FAP	168	40 (24%)	3 (2%)	47 (28%)	29 (73%)	3 (100%)	4 (9%)	4 (8%)[b]

[a] Excludes those with diagnosis of carcinoma on preoperative biopsy

[b] Among patients undergoing surgery

HGD. Nine patients with recurrent polyps have been managed with continued endoscopic surveillance and treatment with no evidence of invasive carcinoma.

Surgery was performed in 46 sporadic patients (41%) and consisted of definitive resection in 27 (59%): PD in 17, PSD in one, and segmental duodenectomy in nine versus local treatment by transduodenal resection in 19 (41%). At a mean follow-up of 41 months, there were no local recurrences in the definitive surgery group, and six (32%) patients recurred in the group treated with local resection. Three of these patients have been successfully managed endoscopically, one with polyp eradication and two with persistent stable disease. One patient originally had a transduodenal resection for a benign polyp followed by multiple endoscopic treatments for recurrences which ultimately developed into a duodenal carcinoma. The two remaining patients underwent transduodenal excision of polyps which were benign on preoperative endoscopic biopsy but demonstrated invasive cancer on permanent surgical pathology. Both patients declined radical reresection and died of recurrent disease. Overall, adenocarcinoma was identified in the surgical specimens of 11 patients (24%) who had benign preoperative endoscopic biopsies. Six of these demonstrated either high-grade dysplasia or carcinoma in situ preoperatively, and the others were at

least 3 cm in size. Size of polyps containing invasive cancer was 4.4 versus 3.5 cm for those without cancer ($p=0.29$ on Wilcoxon rank sum test). Nine patients were treated with definitive resection and two by local resection (discussed above). Six of these patients developed recurrences: four with metastatic disease following definitive resection and two with local recurrence following transduodenal resection. When comparing local recurrence rates, there was not a significant difference between endoscopic and local surgical resection (52% versus 32%), whereas both of these modalities entailed a higher local recurrence rate than seen with definitive surgical resection (0%; $p<0.001$, $p=0.002$; Table 4). Complications developed in 18 of the 46 patients who underwent surgical resection (39%), including four pancreatic leaks, three gastrojejunal/duodenal leaks, five wound infections, gastroparesis in nine, ileus in two, and pseudoaneurysm in one. More than one complication developed in six patients. Complication rates for definitive versus local resection were 59.3% and 10.5%, respectively ($p=0.002$). There was one mortality due to aspiration after pancreaticoduodenectomy (Fig. 1).

**Table 5** Local Recurrence According to Etiology and Treatment

	Treatment	Local recurrence (%)	p value
Sporadic	Endo	52	0.13
	Local	32	
	Endo	52	<0.001
	Definitive	0	
	Local	32	0.002
	Definitive	0	
FAP	Endo	73	0.29
	Local	100	
	Endo	73	<0.001
	Definitive	9	
	Local	100	<0.001
	Definitive	9	

Proposed Algorithm for Management of Sporadic Duodenal Adenomas

HGD = high grade dysplasia; CIS = carcinoma in situ
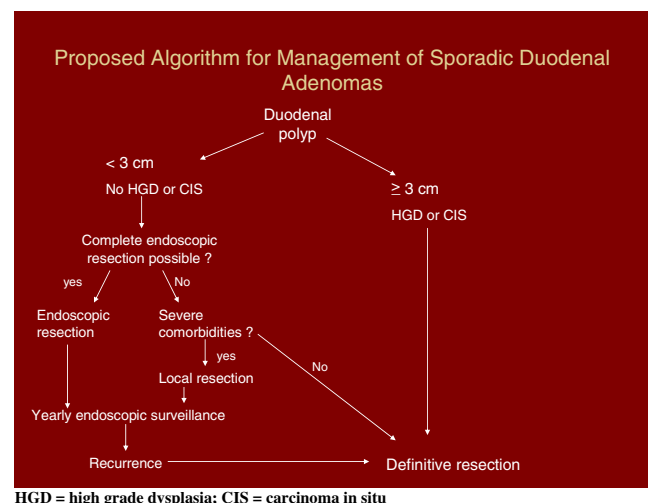
Figure 1 Proposed algorithm for management of sporadic duodenal adenomas. *HGD* high-grade dysplasia, *CIS* carcinoma in situ.

FAP patients underwent endoscopic surveillance for a mean duration of 100 months. Endoscopic resection or ablation was attempted in 40 (23%) patients. Four complications developed in three patients (7.5%) including mild pancreatitis in one patient, two perforations requiring surgical repair, and a duodenal stricture following photodynamic therapy which required endoscopic dilation. One patient with stage III disease was discovered to have invasive cancer in a polyp during endoscopic surveillance and died from metastatic disease without surgery. There were a total of 29 (72.5%) recurrences documented at a mean follow-up interval of 12.8 months. Fifteen patients with recurrent polyps ultimately had surgery for Spigelman stage III or IV disease. In total, surgical resection was performed in 50 patients: PD in 12, PSD in 31, segmental duodenectomy in four, and transduodenal resection in three patients. Final pathology demonstrated invasive cancer in four patients (8%) and included two PDs, one PSD, and one segmental duodenal/jejunal resection. At a mean follow-up of 44 months, there were four local recurrences (9%) in the definitive surgery group (neo-ampulla or duodenal cuff) and one metastatic recurrence in a patient found to have duodenal cancer at the time of surgery. All three FAP patients who underwent transduodenal polyp resection recurred (100%). There was no significant difference in the rate of recurrence following local surgical versus endoscopic resection (100% versus 73%, $p=0.29$). The lower rate of local recurrence following definitive surgical resection as compared to endoscopic or local resection reached statistical significance ($p<0.001$). Complications following surgery developed in 24 patients (48%) including delayed gastric emptying in five patients (10%), nine anastomotic leaks (18%), two cases of pancreatitis, two wound infections, two minor pancreatic duct leaks, two cases of pulmonary thromboembolism, one chyle leak, one intra-abdominal abscess, and one reoperation for bleeding. No complications developed in patients who underwent local surgical resection procedures. There were no postoperative mortalities (Fig. 2).

Discussion

The management of duodenal adenomatous polyps is guided by the principle that they have the potential for malignant transformation, a concept similar to polyps in the colon. The goal of any treatment is to prevent progression to carcinoma and address local complications such as bleeding and obstruction. This study demonstrates that endoscopic and local surgical management for both sporadic and familial duodenal polyps are associated with a high rate of local recurrence. Definitive surgical therapy in the form of PD, PSD, or segmental duodenectomy offers the best chance for

HGD = high grade dysplasia; EGD = esophagogastroduodenoscopy
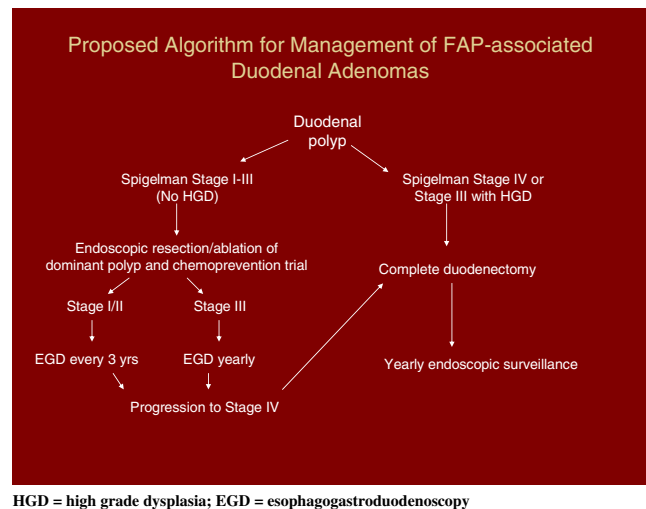
Figure 2 Proposed algorithm for management of FAP-associated duodenal adenomas. *HGD* high-grade dysplasia, *EGD* esophagogastroduodenoscopy.

polyp eradication and potential prevention of carcinoma, regardless of the etiology of the polyp.

We demonstrated that endoscopic resection/ablation of sporadic adenomatous polyps was associated with a 52% rate of local recurrence, albeit with a low morbidity. This modality is ideally applied to small polyps in patients that are willing to comply with endoscopic surveillance. Local surgical resection in the form of transduodenal polypectomy carried a similarly high local recurrence rate of 32%, which is comparable to that demonstrated in other series.[4,11] Aside from local recurrence, the possibility of occult malignancy in a polyp must also be taken into account. In a series of 30 patients with sporadic ampullary adenomas, Meneghetti et al. identified six cases of invasive cancer and a frozen section false negative rate of 15% for the detection of adenocarcinoma.[11] An even higher rate of 65% for the presence of occult invasive adenocarcinoma was reported by Jordan et al. in their series.[12] We found a 24% rate of invasive carcinoma among patients submitted to surgical resection with benign endoscopic biopsies. This risk was higher for polyps ≥3 cm and those demonstrating HGD or CIS, and thus, these should be treated by definitive resection. Frozen section analysis may unfortunately be misleading in a certain percentage of cases but is helpful when invasive cancer is identified. Definitive surgical resection yielded better local control although with a higher complication rate. One must therefore weigh local recurrence risk against the potential morbidity of surgery. Our data would support a limited role for local surgical excision of sporadic duodenal polyps. Factors that influence this decision are medical comorbidities, polyp size and location, and clearly defined margins. For a villous adenoma, involving the lateral wall of the second portion of the

duodenum transduodenal resection makes sense as opposed to a pancreaticoduodenectomy. However, for extensive lesions, at least a segmental resection including the ampulla is a better long-term option. Endoscopic surveillance is mandatory following any form of polyp resection whether in the setting of sporadic or familial disease.

In our FAP cohort, we demonstrate a high rate of local recurrence following both endoscopic and local surgical resection with a remote chance of downstaging. High rates of recurrence following local excision of FAP-associated duodenal polyps have previously been reported.[5] Definitive surgery in the form of PD or PSD resulted in a low local recurrence rate of 9%. We previously reported our experience with PSD as an effective treatment and potential alternative to PD in the management of advanced duodenal polyposis.[6] Sparing of the pylorus may help attenuate deleterious effects on bowel function that can be amplified by the uniform absence of the colon in this patient population.[13] In contrast to PD, PSD offers the advantage of allowing for more complete postoperative endoscopic surveillance, especially of the neo-ampulla. Whereas the need for complete duodenectomy has been well established in the setting of stage IV disease, the optimal form of treatment in less advanced stages is controversial.[14,15] Despite a recurrence rate of 73% following endoscopic resection in the FAP cohort, carcinoma developed in only one of these patients. Therefore, for patients with stages I–III polyposis that are part of a regular surveillance program, which is viewed as diagnostic and rarely therapeutic, local surgical resection makes little sense given the natural history of the disease and the potential for complicating any future surgical procedures. Definitive resection in the form of PD or PSD should be considered for stage IV disease or polyps not amenable to endoscopic resection.

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Discussant

Dr. Margo Shoup (Loyola University, Chicago): Your topic is called duodenal adenomas. But really what you are talking about is a combination of duodenal adenomas and ampullary adenomas, and they are really two different entities.

I think all of us would agree that we would treat a 2-cm adenoma on the lateral wall of the duodenum very differently than we would treat a 2-cm ampullary adenoma in somebody with obstructive jaundice.

So that leaves my question. If I understood your numbers right, in the sporadic patients, 40% of them underwent surgery and a quarter of those actually had cancer at the time of final pathology.

My question to you is, which one of those were ampullary and which one of those were duodenal? In other words, if you look at the sporadic patients, what percentage of ampullary adenomas actually had adenocarcinoma in them, and what percentage of duodenal adenomas had adenocarcinoma? And if you have those numbers, which percentage actually had recurrence after local resection as well?

My second point is, in the familial group, you said that the way it was picked up was because they had surveillance. Why were the sporadic patients undergoing EGD?

It looks like 75% of those patients had tumors either in the second portion of the duodenum or in the ampulla.

So my question is, were they symptomatic? That would make sense, and if so, how did symptoms relate to the incidence of carcinoma and patients undergoing definitive resection, or to recurrence in those undergoing local resection?

Thank you for the opportunity to discuss this paper.

Closing Discussant

Dr. Michael D. Johnson: I did not show it up there, but most of the polyps were in the second portion of the duodenum or the ampulla. Proportionately, more of the recurrences and cancers were in the second portion of the duodenum and the ampulla.

Unfortunately, I did not look specifically at whether those differences were significant. But I think it kind of makes sense that a lot of people are going to tend to be a little more aggressive endoscopically with those lesions in order to avoid having to perform a Whipple operation.

The familial group underwent duodenal surveillance according to an institutional protocol. Most of the patients in the sporadic group were not symptomatic from the polyps, per se, but were undergoing endoscopy for reflux symptoms or other vague abdominal complaints. A minority of patients underwent endoscopic examination due to things such as anemia or jaundice which could be attributed to the polyp in question.

We did not specifically look at factors that predicted whether or not those adenomas were cancerous aside from looking at size which did not turn out to be a predictive variable. But that would be a good thing to look at in terms of predicting which ones of these can be safely managed endoscopically without worrying about them advancing to cancer.

Discussant

Dr. Michael Sarr (Rochester, MN): Where was the recurrence in the four patients with FAP that had a formal, anatomic resection?

Closing Discussant

Dr. Michael D. Johnson: They recurred either at the neo-ampulla or the duodenal cuff. We did not consider polyps in the advanced jejunal limb to be a recurrence because those were not really addressed by the initial operation. So it was about half and half, the duodenal cuff and the neo-ampulla in terms of recurrence.

Discussant

Dr. Richard McCallum (El Paso): Whenever we think about duodenal polyps or any ampulla lesion, we think about Gardner's syndrome.

How many patients do you think were hiding under the covers that had a familial polyposis history or a positive family history and had Gardner's syndrome presenting with a duodenal polyp?

Closing Discussant

Dr. Michael D. Johnson: It is possible that a few of those people that we categorized as sporadic may have had a familial syndrome other than FAP.

Defining Criteria for Selective Operative Management of Pancreatic Cystic Lesions: Does Size Really Matter?

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Abstract

Introduction Proposed criteria for resection of pancreatic cystic lesions have included symptoms, size (>3 cm), and suspicious features by endoscopic ultrasound (EUS). The objective of this study was to evaluate risk factors for malignancy in a large series of patients undergoing resection of suspected pancreatic cystic neoplasms.

Methods Medical records of patients selected for resection of pancreatic cystic lesions at Duke University Medical Center from 2000 to 2008 were reviewed. Lesions with solid components on cross-sectional imaging were excluded. Malignancy was defined as invasive or in situ carcinoma.

Results After review, 101 patients were confirmed to have undergone resection for suspected cystic neoplasms of the pancreas. Preoperative EUS was performed in 71 patients. Sixteen patients (16%) had malignant lesions (preoperative size 1.5–5.9 cm). There was no clear association between size and malignancy. Male gender, biliary ductal dilatation (BDD), pancreatic ductal dilatation (PDD), and suspicious cytology (but not age, symptoms, or size) were associated with increased risk of malignancy. When factors available for all patients were incorporated into a multivariate model, only BDD and PDD were independent risk factors for malignancy. Only one patient with malignancy had neither BDD nor PDD but did have solid components by EUS.

Conclusions In patients selected for resection, size was not an independent risk factor for malignancy. While size might be appropriate for stratification of asymptomatic patients with simple cysts, size should not be used as a selection criterion for patients who have cysts with solid components or with associated BDD or PDD.

Abbreviations

ERCP	Endoscopic retrograde cholangiopancreatography
EUS	Endoscopic ultrasound
MRCP	Magnetic resonance cholangiopancreatography
FNA	Fine needle aspiration
BDD	Biliary duct dilatation
PDD	Pancreatic duct dilatation
SCN	Serous cystic neoplasm
MCN	Mucinous cystic neoplasm
IPMN	Intraductal papillary mucinous neoplasm
SPN	Solid pseudopapillary neoplasm
PET	Positron emission tomography

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Introduction

In 1978, Compagno and Oertel made the first distinction between serous and mucinous cystic neoplasms of the pancreas.[1,2] Since then, there has been great interest in further classifying these cystic lesions both histologically and clinically. The behavior of cystic neoplasms ranges widely, not only between serous and mucinous neoplasms but also between subgroups of mucinous neoplasms. The

importance of further investigation of cystic lesions of the pancreas has increased over time as these lesions are recognized more frequently in symptomatic and asymptomatic patients due to the increased use of cross-sectional imaging.[3]

Cystic lesions comprise 15% of all pancreatic tumors.[4–6] The former can be classified into three categories: primary cystic neoplasms, pseudocysts, and solid tumors with cystic degeneration. The primary cystic neoplasms can be further classified as serous cystic neoplasm (SCN), mucinous cystic neoplasm (MCN), intraductal papillary mucinous neoplasm (IPMN), solid pseudopapillary neoplasm (SPN), and cystic neuroendocrine tumor (NET). The distinction of SCN—which have extremely low (if any) malignant potential[7,8]—from MCN, IPMN, SPN, and NET—which do have definite malignant potential—is paramount. Radiographically, these lesions sometimes have characteristic features sufficient to classify them as SCN versus one of the premalignant tumor types. However, adjuncts to cross-sectional imaging, such as endoscopic retrograde cholangiopancreatography (ERCP),[9] endoscopic ultrasound (EUS) with cyst fluid analysis,[10,11] and, more recently, positron emission tomography (PET),[12,13] are frequently employed to more accurately characterize these lesions with respect to subtype and risk of malignancy.

The management of cystic lesions of the pancreas remains controversial. Although some authors have previously proposed that almost all cystic lesions should be resected,[14] most recent studies have supported a more selective approach to resection.[15–18] Proposed selection criteria for resection have included symptoms, cyst size, cyst growth, suspicious features on imaging (e.g., solid components, septations, pancreatic ductal dilatation, biliary ductal dilatation), and cyst fluid analyses (e.g., presence of mucin, high viscosity, elevated carcinoembryonic antigen (CEA) levels, and presence of K-ras mutations).

At our institution, we utilize many of these factors in making decisions about whether to resect cystic lesions of the pancreas. The objective of this study was to help refine our criteria for resection by identifying risk factors for malignancy in patients with suspected pancreatic neoplasms. We retrospectively identified all patients who underwent resection of pancreatic cystic lesions over an 8-year period and assessed the association between several of the factors listed above and the presence of malignancy.

Methods

The medical records of all patients undergoing pancreatic resections (n=548) at Duke University Medical Center from 2000 to 2008 were reviewed. Only patients undergoing resection of suspected cystic neoplasms (n=101) were included in this study; patients with solid components on cross-sectional imaging were excluded.

Basic demographics such as age, gender, and race were recorded. Lesions were categorized as symptomatic if they were detected during the evaluation of jaundice, abdominal, or back pain, pancreatitis, or gastrointestinal symptoms not attributable to another cause. The Gastroenterology Service at Duke University performed all ERCP and EUS procedures. Anatomical findings of the lesion including size, location, biliary duct dilatation, and pancreatic duct dilatation (>4 mm) were collected. When data from both cross-sectional imaging and EUS were available, the EUS data were utilized, as we considered these data to be more accurate. All specimens including fluid and cells obtained by fine needle aspiration (FNA), endoscopic brushings, and final operative specimens were analyzed by the Department of Pathology using standard techniques. The FNA findings were categorized as mucin present (without cells), benign nonmucinous cells, benign mucinous cells, atypical cells suspicious for malignancy, malignant cells, or other (including NET and SPT). All surgeries were performed by the senior authors of this paper (B.M.C, D.S.T, T.N.P, and R.R.W). The postoperative pathology report included histologic diagnosis, total tumor size, total number and number of positive lymph nodes, margin status, and presence and size of invasive components. Malignancy was defined as invasive or in situ carcinoma.

All statistical analyses were performed by a statistician (SS) using SAS 8.2 (Cary, NC, USA). Two-sample t test was used to compare means of continuous variables. Wilcoxon rank sum test was used for nonparametric data. The Wald chi-square test in logistic regression model was used for univariate and multivariate analysis. All statistical tests controlled type I error at 5%. The Institutional Review Board at Duke University Medical Center approved all aspects of this research.
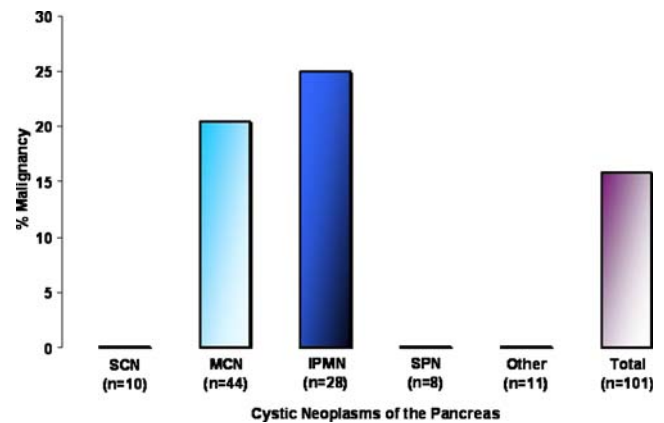
Results

Over the 8-year period, 101 patients underwent resection for suspected cystic neoplasms of the pancreas. Patient and preoperative cyst characteristics are listed in Table 1. Only four patients had initially been observed (and offered resection based on a change in the lesion on serial imaging). ERCP was performed in 31 patients and EUS with or without FNA was performed in 73 patients. Cytology was obtained in 50 patients (Table 2). Of four patient with malignant cells on cytology, final pathology confirmed malignancy in two patients. One patient with malignant cytology received neoadjuvant therapy and had no residual invasive cancer on final pathology. Another patient had a benign IPMN with high-grade dysplasia.

Table 1 Descriptive Characteristics of 101 Patients Who Underwent Resection for a Primary Pancreatic Cystic Neoplasm

Variable	Total (N=101)
Sex	
Male	34%
Female	66%
Race	
White	86%
Black	14%
Age (years)	
Mean (SD)	58 (13.8)
Min, median, max	18, 57, 79
Symptoms	
Yes	82%
No	18%
Location	
Proximal (head, neck, uncinate)	43%
Distal (body, tail)	57%
Biliary duct dilatation	
Yes	8%
No	92%
Pancreatic duct dilatation	
Yes	28%
No	72%
Preoperative size of cyst (cm)	
Mean (SD)	3.65 (2.69)
Min, median, max	0.9, 2.9, 18.5

Figure 1 Percent malignancy among the subtypes of primary pancreatic cystic neoplasms.

rate of malignancy among different racial groups. Patients with malignant lesions were older, but this difference did not achieve statistical significance. A large proportion of the resected lesions in this series were symptomatic (82%), and we did not identify a difference in malignancy rates between asymptomatic and symptomatic lesions. Over half of all lesions were located in the body or tail of the pancreas, and there was not a significant difference in malignancy rate between proximal and distal lesions. Imaging findings of either biliary duct (BDD) or pancreatic duct dilatation (PDD) proved to be significantly associated with malignancy. Sixty percent of patients with BDD and 30% of patients with PDD had malignant lesions, both of which were significant with P values less than 0.01. Notably, only one patient with malignancy had neither BDD nor PDD but did have solid components by EUS.

The mean benign cyst size was 3.7 cm, and the mean malignant cyst size was 3.3 cm. All cystic lesions were grouped based on preoperative size, and malignancy rates were compared. As shown in Fig. 2, there was no apparent relationship between size and risk of malignancy in lesions selected for resection.

Factors with a P value of less than 0.1 by univariable analysis as well as preoperative size were included in a multivariate model (Table 4). Tumor location was not included in this model due to its clear association with

Thirteen of 16 patients with mucin or benign mucinous cells on cytology were confirmed to have mucinous neoplasms. The overall rate of malignancy was 16%. There were no malignant SCN, SPN, or miscellaneous cysts in this series, whereas 20% of MCNs and 25% of IPMNs were malignant (Fig. 1).

A univariable analysis was performed to identify predictors of malignancy (Table 3). Twenty-six percent of male patients had malignant lesions as compared to 10% of female patients ($P<0.05$). There was no difference in the

Table 2 Pancreatic Cyst Cytology of 50 EUS FNA Samples

Pancreatic cyst cytology from EUS

Final pathology	Benign mucinous	Malignant	SPN	Other
Total (n=50)				
Benign nonmucinous (n=17)	10	0	0	7
Mucin or benign mucinous cells (n=16)	9	4	0	3
Suspicious (n=3)	2	0	0	1
Malignant (n=6)	2	4	0	0
SPN or NET (n=8)	1	0	6	1

EUS endoscopic ultrasound, *SPN* solid pseudopapillary neoplasm, *NET* neuroendocrine tumor

Table 3 Univariable Analysis of Patient and Pancreatic Cyst Variables Predictive of Malignancy

Predictors of malignancy in univariable analysis				
Variable	N (% malignant)	Odds ratio	Confidence interval	P value[b]
Sex				
Male	9 (26)	3.09	1.03, 9.20	0.043
Female	7 (10)			
Race				
White	15 (17)	2.9	0.36, 24.23	0.312
Black	1 (7)			
Age	Per 1-year *increase*	1.02	1.00, 1.09	0.072
Symptoms				
Yes	13 (16)	0.99	0.25, 3.91	0.994
No	3 (16)			
Location[a]				
Proximal	10 (23)	2.63	0.87, 7.91	0.086
Distal	6 (10)			
Preoperative size of cyst	Per 1-mm increase	0.94	0.74, 1.18	0.577
Biliary duct dilatation				
Yes	5 (62)	12.42	2.60, 59.34	0.002
No	11 (12)			
Pancreatic duct dilatation				
Yes	9 (32)	5.78	1.73, 19.35	0.004
No	7 (8)			

[a] Proximal = head, neck, uncinate; distal = body, tail

[b] *P* value from Wald chi-square test in logistic regression model

biliary ductal dilatation. Age, sex, nor preoperative cyst size was an independent predictor of malignancy. However, both biliary ductal and pancreatic ductal dilatation were significant predictors of malignancy among patients who underwent resection of suspected pancreatic cystic neoplasms.

Discussion

The evaluation and management of pancreatic cystic lesions continues to evolve. Patient history and high-quality cross-
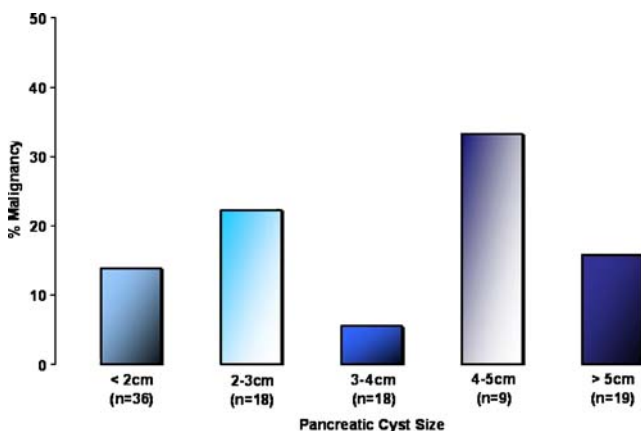
Figure 2 Percent malignancy stratified by size. Both small and large cysts had comparable malignancy rate.

sectional imaging generally allow us to differentiate primary cystic neoplasms from pseudocysts or solid tumors with cystic degeneration. The focus of surgeons, radiologists, and gastroenterologists has long been on distinguishing primary cystic neoplasms with malignant potential (MCN and IPMN) from cystic neoplasms with no or very low malignant potential (SCN).[19] For healthy patients with a long life expectancy, the diagnosis of a mucinous neoplasm is considered an indication for resection. However, for many of our patients—particularly elderly patients with incidentally identified cystic lesions—the risks of resection may exceed the risks of malignancy. Therefore, there has been increasing focus on distinguishing mucinous neoplasms with a high risk of being malignant (or becoming malignant in the near future) from those with a low risk of malignancy. This study was designed to evaluate the preoperative factors that were associated with malignancy in resected cystic lesions. This study specifically did not review those patients with cystic lesions that were managed by observation with serial cross-sectional imaging.

Upon reviewing all resected pancreatic cystic lesions, variables such as age, the presence of symptoms, and preoperative size did not predict malignancy in our study. Age has been an inconsistent risk factor supported by some.[16,17,20,21] Similarly, symptoms—depending on how they are defined—have not been consistently associated with malignancy. One large retrospective study found premalignant and malignant lesions were more likely to

Table 4 Multivariable Analysis of Patient and Pancreatic Cyst Variables Predictive of Malignancy

Predictors of malignancy in multivariable analysis

Variable	Adjusted odds ratio	Confidence interval	P value
Sex			
Male	1.12	0.267, 4.703	0.7
Female			
Age (per 1-year increase)	1.02	0.949, 1.078	0.877
Preoperative size of cyst (per 1-mm size increase)	0.865	0.583, 1.283	0.469
Biliary duct dilatation			
Yes	20.20	3.23, 126.43	0.001
No			
Pancreatic duct dilatation			
Yes	7.63	1.84, 31.57	0.005
No			

*P value from Wald chi-square test in logistic regression model

have symptoms of abdominal pain, nausea, or vomiting.[16] Another found malignant lesions to be associated with obstructive jaundice and weight loss but not abdominal pain.[21] Yet another large study found no association between malignancy and any of the symptoms.[17] Size, however, is generally considered to be one of the most important risk factors, with very low rates of malignancy associated with tumors less than 3 cm.[22] Meanwhile, a review of patients undergoing serial cross-sectional imaging and subsequent resection failed to show a significant relationship between cyst size and malignancy,[16] but several reviews of resected IPMNs have demonstrated preoperative cyst size as a significant predictor for malignancy.[8,17] Our study was a heterogeneous but highly selected series of patients, and this apparent discrepancy may be attributable to our inclusion of all resected cystic lesions rather limiting our analysis to one histologic type of cystic neoplasm.

The intent of this study was to evaluate the factors available to surgeons preoperatively when they are making decisions about whether to resect cystic lesions of the pancreas. In this series of patients selected for resection, size was not an independent risk factor for malignancy. BDD and PDD, however, were strong predictors of malignancy. Jaundice clearly has been associated with increased risk of malignancy and worse outcome.[21,23] PDD may be a surrogate marker for main-duct IPMN, which is believed to have greater malignant potential than branch-duct IPMN.[21,24,25] While size might be appropriate for stratification of asymptomatic simple cysts, patients who have cysts with solid components or suspected mucinous neoplasms with associated BDD or PDD should be offered resection regardless of size. Conversely, in patients without other risk factors, we may be able to observe cysts larger than 3 cm.

Endoscopic diagnostic modalities are useful adjuncts to cross-sectional imaging in many cases. ERCP has a significant, albeit limited role in the management of pancreatic cystic lesions. For patients with cystic lesions at our institution, ERCP is utilized primarily as a therapeutic modality for biliary decompression in jaundiced patients, and its role as a diagnostic modality is reserved for patients who present with BDD or PDD without an associated lesion on cross-sectional imaging. However, it remains the most sensitive diagnostic modality to identify a direct communication between the pancreatic duct and a cyst.[22,26] IPMN has certain findings exclusive to ERCP that are considered diagnostic. For instance, visualization of mucus protruding through either the papilla or pancreatic duct correlates highly with the diagnosis of IPMN.[27] In another similar diagnostic study, MRCP may be superior to ERCP in identifying IPMN by improved visualization of the extent of ductal involvement and internal architecture as well as having fewer procedural related risks.[9,28]

EUS appears to be the more valuable endoscopic technique by allowing for high-resolution imaging of the pancreas and acquisition of tissue and fluid by FNA. Controversy exists regarding the ability of EUS to distinguish malignant from benign lesions based on cyst morphology alone.[29] IPMN are detectable by EUS with a high sensitivity, but EUS alone fails in distinguishing benign and malignant lesions.[30] The presence of mucin or mucinous cells by FNA, however, is highly specific for mucinous neoplasms. Furthermore, the degree of cytologic atypia seen by FNA in IPMN has been shown to be predictive of malignancy.[31] The combination of EUS and FNA appears the most promising in predicting lesions requiring resection with reported sensitivies and specificity of 97% and 100%, respectively.[32] However, this ability of cytology to predict malignancy and guide resection has
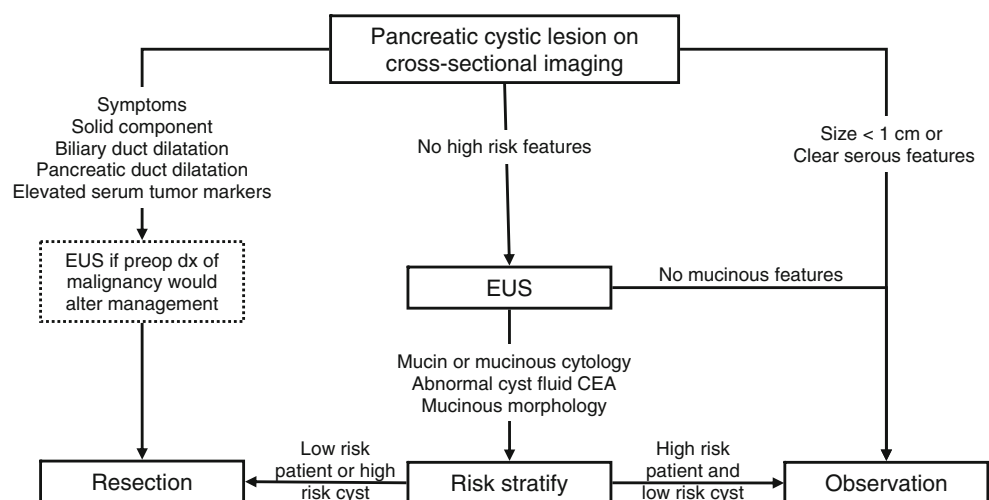
been brought into questions by similar studies with less convincing results, and overall, its role still remains unclear.[10,33] Aspirated cyst fluid analysis has been reported to improve diagnostic accuracy by measurement of tumor markers (CA15–3, CA19–9, CA72–4, CA-125, CEA); CEA—with a cutoff of 192 ng/ml—had the highest sensitivity (73%) and specificity (84%) in differentiating mucinous versus nonmucinous cysts.[10] More recently, molecular studies of cyst fluid have shown that K-ras mutations, a common tumor suppressor gene mutation, are more prevalent in malignant lesions.[34] Although we found EUS to be useful in the evaluation of cystic lesions of the pancreas, cyst fluid CEA levels were not routinely collected during the earlier years of the study period. Therefore, these data were not available for a sufficient number of patients to include this factor in our analysis.

A major limitation of this study is that only patients who underwent resection were included in the review, thus lacking a "denominator" (i.e., both resected lesions and those followed with serial imaging). The findings of this study do not reflect those of all patients seen in the clinic with a cystic lesion of the pancreas. Although it is true that most of the lesions in this series were symptomatic and would have been resected regardless of the presence of other risk factors, our aim was to identify other risk factors for malignancy that may be generalized to asymptomatic patients. However, we do believe this study supports the selective operative management of cystic pancreatic lesions and, furthermore, the role of EUS in more accurately evaluating cystic lesions for associated solid components, pancreatic ductal dilatation, cytology, and cyst fluid analyses.

We propose a conservative algorithm for the management of pancreatic cystic lesions based not only on our data but also on data from other large series (Fig. 3). We are making the assumption that there is no recent history of acute pancreatitis and that clinical suspicion for pseudocyst is low. We have chosen 1 cm as a size cutoff, below which

the risk of malignancy is exceedingly low. For subcentimeter cystic lesions without solid components on high-quality cross-sectional imaging or for lesions with features clearly consistent with SCN, we recommend no further workup, although continued observation is warranted. Lesions of any size that are symptomatic, have solid components on cross-sectional imaging, or are associated with biliary ductal dilatation, pancreatic ductal dilatation, or elevated serum tumor markers[35] should be considered indications for resection. Although the guidelines proposed by the International Association of Pancreatology suggest that all suspected main-duct mucinous neoplasms and all mucinous neoplasms greater than 3 cm should be resected, our data support the recommendation that all suspected main-duct mucinous neoplasms—based on the presence of biliary or pancreatic ductal dilatation—should be resected, but our data and those of others suggest that some mucinous lesions greater than 3 cm—without biliary or pancreatic ductal dilatation—may be observed.[36] For patients with indications for resection on cross-sectional imaging, EUS is not necessary unless the preoperative diagnosis of malignancy would alter management. For example, some patients at our institution would be offered preoperative (neoadjuvant) therapy if there is biopsy-proven adenocarcinoma. For patients without high-risk features on cross-sectional imaging, EUS is useful for more accurately assessing for solid component and pancreatic ductal dilatation, distinguishing between mucinous and nonmucinous lesions and—to a limited extent—for stratifying risk of malignancy. The sensitivity and specificity of cyst fluid CEA level depend on the cutoff values used.[10] To maximize the sensitivity of CEA and minimize "missed" mucinous neoplasms, we propose using a value greater than 5 ng/ml—in combination with cytology and morphological features—to identify patients with possible mucinous neoplasms. For healthy "low-risk" patients, the diagnosis of even a possible mucinous neoplasm may be considered an

Figure 3 Proposed algorithm for evaluation and management of cystic lesions of the pancreas.

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Discussant

Dr. James Moser (University of Pittsburgh Medical Center, Pittsburgh, PA): Thank you for asking me to discuss your excellent presentation. It is a real pleasure to comment on such a well-written manuscript on a topic of such clinical importance.

Risk stratification for cystic pancreatic lesions is still largely based on a mountain of circumstantial evidence rather than genetic predictors of aggressive biology. Our pancreatic cancer program at Pittsburgh has been very dedicated to the use of molecular prognostic factors obtained via EUS to stratify the cancer risk of such lesions.

In her forward to the textbook *Fundamentals of Radiology*, Lucy Frank Squires wrote: "It is easier to measure than to think." I think that is where we find ourselves today when we discuss cystic pancreatic neoplasms: Most of these criteria involve size, but really the issue is biology. What can we do as a group, interested in pancreatic surgery, to try to bring a newer level of clarity to this? Hopefully your prospective trial design will be part of the answer, and we would like to participate in your effort if you are looking for additional collaborators.

My first question is: do you think an 82% rate of symptomatic cystic lesions is atypical? I believe our rate is probably much lower, perhaps between 5% and 10% given the rate with which these cysts are identified incidentally. Why do you think your series is so enriched in symptomatic cysts? My own view is that that is a selection bias based on the referring physician's decision that a different, unseen population is going to be observed.

My second question is: can you explain why patients with biliary and pancreatic ductal dilation were more likely to have cancer even in the absence of a pancreatic mass or mural nodularity on their preoperative imaging? My guess is that you defined patients with pancreatic ductal dilatation more than 4 mm as being a high-risk group. That means to me that most of these patients had main-duct IPMN. As a result, what you are really saying is that main-duct IPMN is associated with cancer, and I think we would all agree with that.

My third question is: what do you do with branch-duct IPMNs? In the absence of MRCP data, we would not know which cystic lesions are branch-duct IPMN and which are not. You suggest that mucinous features should be considered high risk if they measure between 1 and 3 cm, suggesting to me that a good-risk patient with a 1- to 3-cm branch-duct IPMN is probably going to end up having surgery. We would have a little rebellion among our gastroenterologists if we tried to implement that size criterion. I just wonder how you handle such lesions?

Closing Discussant

Dr. Eugene P. Ceppa: To address your first comment in terms of further evaluation on more of a molecular level, I know there is some work in looking at cyst fluid analysis and in particular biologic markers for K-ras. Certainly, in the future this would provide a more sensitive approach in diagnosing these lesions.

Your second comment regarding the high number of symptomatic lesions—and this is something our group has discussed previously—is one of the limitations of our study.

This is solely a resection series. We looked at patients retrospectively and tried to figure out why is it that these patients were resected? Presumably, most of them in this series were symptomatic, attesting a reason why a particular surgeon decided a resection was necessary. So you make a valid point. Something we are investigating now is to assess the denominator. Trying to identify, at our institution, how many patients are actually being serially observed for these cystic lesions. I believe once we incorporate that denominator to this numerator of resections, then that number of symptomatic patients would actually decrease significantly.

Your third comment regarding the correlation of pancreatic duct dilatation in IPMN is exactly what we think. We think this is a surrogate marker for main-duct IPMNs, and it is another way of saying the same thing.

Discussant

Dr. James Moser: Does that mean that we are using a new definition for main-duct IPMN then? Should we be using 4 mm or more to define main-duct IPMN?

Closing Discussant

Dr. Eugene P. Ceppa (Duke University Medical Center, Durham, NC): I would not go as far as saying that but this was a particular finding that we saw after reviewing EUS findings by gastroenterologists.

You have identified the weakness in our algorithm how to address the branch-duct IPMNs appropriately. Certainly the size criteria are data specific in that other series have shown the size criteria for IPMNs. But more specifically, the rate of malignancy is much lower in branch-duct IPMNs as compared to main duct and that puts a hole in the algorithm that we have presented here today.

Discussant

Dr. Carlos Fernandez-del Castillo (Boston, MA): I just want to clarify something. Those consensus guidelines that you presented are intended for branch-duct IPMNs. If I take all cystic tumors, the largest cystic tumors are the serous cystadenomas, and even a 20-cm serous cystadenoma is going to be benign.

So, I do not think you can really conclude what you are saying because you have mixed many different types of tumors. Furthermore, the vast majority of your patients were symptomatic. Those were patients that, according to those guidelines, even if they were all branch-duct IPMNs, should be operated upon.

Regarding the second conclusion that endoscopic ultrasound is useful, I do not think you have showed us data indicating so. Solid components by endoscopic ultrasound

can be unreliable, and you have not shown us that it is more specific, or more sensitive, than MRI or CT scan.

What kind of cyst fluid analysis, precisely, is the one that is going to get us out of the ditch in determining the high-risk or the low-risk ones?

Discussant

Dr. Eugene P. Ceppa: Thank you for your comments, Dr. Castillo. Regarding the slide that I used in this presentation from the International Consensus Guidelines, it was more of an example of algorithms that are present from the numerous publications by many of the authors in this room, just to give a brief summary. I did recognize during the presentation that the depicted algorithm was just for branch-duct IPMNs.

Now, as to what did we show to recognize that EUS is more valuable? The anatomic findings regarding duct dilatation had never been previously reported and could be included one's decision making.

Previous publications on EUS have shown value of looking at cytology and/or CEA fluid cyst analysis. At our institution during the study's time interval, not every single person underwent EUS, specifically over the first 4 years, nor those who did undergo EUS had samples submitted for cyst fluid analysis. It would have been something that would have been helpful and may have contributed greater to our study, I agree. However, if we would have presented the last 3 years, it would have been an incomplete data set, and I do not know that I could really draw any conclusions. Thank you.

Discussant

Dr. Michael G. Sarr (Mayo Clinic, Rochester, MN): I assume you define malignancy as invasive disease and not in situ carcinoma or dysplasia?

Discussant

Dr. Eugene P. Ceppa: In our series, we defined invasive and in situ as malignant. We did not include any high-grade dysplasia.

Discussant

Dr. Michael G. Sarr: But in situ is in your category of cancer?

Closing Discussant

Dr. Eugene P. Ceppa: Correct. There were two in situ lesions and the rest invasive lesions defined as malignant.

Characteristics and Outcomes of Patients Undergoing Debridement of Pancreatic Necrosis

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Abstract

Background Pancreatic necrosis is associated with high morbidity and mortality. The Atlanta Classification underwent proposed revisions in 2007 to better categorize acute pancreatitis.

Methods From 1999 to 2008, patients with pancreatic necrosis treated with surgical debridement were analyzed. Computed tomography (CT) images were independently reviewed to classify of pancreatic collections according to the revised Atlanta classification.

Results Seventy-three patients were categorized as infected extrapancreatic necrosis (40%), sterile extrapancreatic necrosis (29%), infected pancreatic necrosis (15%), sterile pancreatic necrosis (11%), or post-necrotic collection (5%). Mortality was 14%, and morbidity was 55%. Debridement with external drainage or open packing was associated with higher mortality than cystgastrostomy ($p=0.03$). Atlanta Classification was not associated with operative procedure or mortality. Degree of chronic disease, demonstrated by albumin level, and infection were associated with longer stay ($p<0.05$).

Conclusion Type of necrosis by the revised Atlanta Classification was not associated with outcomes or type of operation. Debridement by cystgastrostomy was associated with lower mortality rates than external drainage or open packing. Length of stay was increased in patients with evidence of chronic disease, infection, and postoperative complications. Necrotizing pancreatitis continues to be associated with significant morbidity and mortality and should undergo aggressive treatment at tertiary care centers.

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Introduction

Acute pancreatitis has long been recognized as a disease with potential high rates of morbidity and mortality.

Severity of disease ranges from mild edematous pancreatitis, which may be treated conservatively, to severe acute or necrotizing pancreatitis requiring invasive monitoring, frequent imaging, and often endoscopic or operative intervention.[1] The more severe form, developing in up to 20% of patients with acute pancreatitis, is characterized by pancreatic or peripancreatic necrosis or extrapancreatic organ dysfunction.[2,3] Through the evolution of our understanding of acute pancreatitis, diagnosis and treatment strategies have greatly changed and progressed over the past few decades, with recent studies showing improved outcomes.[4,5]

Pancreatic necrosis has traditionally been defined as the presence of nonviable pancreatic parenchyma or peripancreatic fat. Currently, contrast-enhanced computed tomography (CECT) is used to evaluate those with severe acute pancreatitis and will accurately diagnose pancreatic and peripancreatic necrosis. Despite recent advancements in imaging, the diagnosis and assessment of severity of pancreatic necrosis is still challenging and continues to involve the assessment of both clinical and radiographic data. In an effort to standardize the difficult diagnosis and severity of acute pancreatitis, an international symposium was held in 1992, resulting in the Atlanta Classification.[6] As technology and techniques have improved, a revision of the Atlanta Classification was started in 2007 in order to incorporate improved imaging techniques and a more thorough understanding of the underlying pathophysiology of acute pancreatitis.

As management strategies evolve, decisions to intervene are increasingly based on radiologic extent and location of necrosis and the presence of infection, as opposed to relying solely on the clinical status of the patient. Monitoring and antibiotic improvements have been made, but appropriate timing and technique of intervention for pancreatic necrosis continues to be debated.[1,5,7–9] Surgical debridement and drainage is the gold standard treatment for pancreatic necrosis; however, several groups have recently advocated a more conservative strategy. This less invasive approach aims to decrease morbidity through focused efforts with interventional endoscopy.[1,10–12]

Again, operative debridement is the definitive treatment for necrotizing pancreatitis. A shift has occurred from debridement of all patients with necrosis to only operating when infection is identified or clinical deterioration occurs. Debridement with open packing and marsupialization of the necrotic cavity was the first described surgical treatment. However, this technique was associated with relatively high morbidity and mortality.[13,14] Pancreatic debridement gradually evolved and incorporated closed packing, resulting in lower mortality, and Fernandez-del Castillo et al. made further modifications to include external drainage of the necrotic cavity.[14] Since these changes, other groups have advocated necrosectomy with continuous lavage in order to decrease the number of operations,[5] while laparoscopic approaches via the retroperitoneum have begun to show success in select series.[15] Our group at the University of Alabama at Birmingham (UAB) has opted to perform the majority of debridements through a cystgastrostomy approach, allowing for internal enteric drainage.[16]

Keeping in mind the wide range of diagnostic modalities, management options, and difficulty in accurately categorizing pancreatic necrosis, we reviewed our experience in the treatment of pancreatic necrosis with surgical debridement. The 2007 proposed revision of the Atlanta Classification (personal conversation with Dr. Michael Sarr, Mayo Clinic, Rochester, Minnesota) was used to categorize the necrosis, infection, and standardize our outcome data.

Materials and Methods

Patients with pancreatic necrosis undergoing operative debridement at UAB between January 1, 1999 and December 31, 2008 were included. Operations were open debridement with abdominal packing, open debridement with external drainage, and debridement via cystgastrostomy with internal drainage.

Independent blinded review of CECT was conducted by a faculty of the UAB Department of Radiology to categorize pancreatic necrosis according to the 2007 revision of the Atlanta Classification. Walled-off necrosis (WON) was further categorized as primarily pancreatic or extra-pancreatic and as sterile or infected. Presence of infection was presumed preoperatively by suggestive radiographic findings, specifically the presence of extraluminal gas in areas of necrosis on CECT. We did not perform fine needle aspiration in order to detect infection in the preoperative setting. Clinical data was reviewed and categorized to assess operative and hospital course as well as morbidity and mortality rates. Mortality was defined as in-hospital death, regardless of postoperative date. Morbidity was assessed in several categories: infectious, renal, pulmonary, gastrointestinal, coagulopathic, and neurologic (see Table 1).

Table 1 Morbidity after Debridement of Pancreatic Necrosis

Morbidity	Percent
Infectious	25
Pulmonary	19
Renal	12
Gastrointestinal	11
Coagulopathic	9
Neurologic	2

Statistical analysis utilized was SAS version 9.1, (SAS Institute, Inc., Cary, NC) to determine the significance of each category. Normality was assessed for continuous variables of interest and accordingly median±interquartile range or means±SD were reported. Frequencies with percents were reported for the categorical variables.

We favor debridement by internal drainage and cystgastrostomy. This is conducted by making a limited midline incision carried into the peritoneal cavity. An upper hand, fixed retractor is placed for exposure. The anterior stomach is visualized, and the necrotic cavity is usually easily palpable. A longitudinal gastrotomy of about 7 cm is made along the greater curvature and entry into the lesser sac through the omentum is avoided. Hand-held retractors are used to expose the posterior gastric wall, and an 18-gauge needle is used to sound the cavity. Once purulence or fluid is extracted, cautery enters the cystic necrotic cavity. An endovascular 45 mm stapling device with 2.5 mm staples is used several times to create at least an 8-cm cystgastrostomy. If hemostasis is not adequate, the staple line is cauterized or oversewn. Blunt and thorough debridement is undertaken with ring forceps, suckers, and forceful irrigation. Necrotic debris is sent for tissue culture and pathology. Access to the left colonic gutter to the iliac fossa is usually possible. Access to the right gutter past the pancreatic head may be more difficult due to the duodenum. If a large right-sided collection is present, a counter incision along the right side, just superior to the iliac wing, may be necessary. Once hemostasis is achieved, the nasogastric tube is passed into the cavity to allow irrigation. Our practice is to flush the nasogastric tube with 60 to 100 cc every 4 h for 2–3 days. A gastrostomy with a 22-gauge Foley catheter and a jejunstomy with a 14-French red rubber tube are performed. The gastrotomy is closed with single layer running 3-0 monofilament absorbable suture. As preoperative sampling of the necrotic cavity by fine-needle aspiration is not routinely done at our institution, definitive presence of infection was determined by intraoperative fluid and tissue samples with gram stain, aerobic, anaerobic, and fungal culturettes.

Results

Seventy-three patients (mean age 51 years, 74% male) were included in the analysis. Necrosis was classified as predominantly extrapancreatic (69%), pancreatic (26%), or, when occurring in less than 4 weeks from the original episode of pancreatitis, a postnecrotic fluid collection (5%). These three categories were then further characterized as infected (58%) and sterile (42%) based on operative culture data (see Table 2). Presumed infection based on preoperative CECT was confirmed by operative culture data in 89.5% of patients.

Table 2 Type of Necrosis Based on Proposed 2007 Atlanta Classification Revision

Primarily extrapancreatic	50 (69%)
Sterile	21 (29%)
Infected	29 (40%)
Primarily pancreatic	19 (26%)
Sterile	8 (11%)
Infected	11 (15%)
Post-necrotic pancreatic collection	4 (5%)
Sterile	2 (2.5%)
Infected	2 (2.5%)
Total sterile	31 (42%)
Total infected	42 (58%)

Operations included cystgastrostomy with debridement and internal drainage[16] (46 patients, 63%), open debridement with external drainage (22 patients, 30%), and open debridement with packing (five patients, 7%). Overall mortality was 14% (range, 5–107 days postoperative), and the complication rate was 35%. Mean hospital stay was 34 days (range, 1–104 days). There was no difference in complication rate between the types of operation ($p>0.05$). Those undergoing open debridement with external drainage (24%) or packing (67%) had a significantly higher mortality than those undergoing cystgastrostomy with debridement and internal drainage (6.5%) ($p=0.03$). Those undergoing open debridement with external drainage (22 patients) or open packing (five patients) had a significantly longer length of hospital stay than those undergoing cystgastrostomy with internal drainage ($p<0.01$; see Table 3).

The type of necrosis and the presence of infection did not correlate with the type of operation performed (peripancreatic vs. pancreatic $p>0.05$; infected vs sterile $p>0.05$). The causative organism identified from operative culture was most commonly a single bacterial organism (55%), followed by fungal (27%), and 30% of cultures

Table 3 Operative Management of Study Population

Open debridement with external drainage	22 (30%)
Morbidity	72%
Mortality	23%
Length of hospital stay (mean)	38.2 days
Open debridement with packing	5 (7%)
Morbidity	60%
Mortality	40%
Length of hospital stay (mean)	26 days
Open debridement with cystgastrostomy/ internal drainage	46 (63%)
Morbidity	28%, $p>0.05$
Mortality	7% $p=0.03$
Length of hospital stay	13.8 days, $p<0.01$

grew multiple organisms. The most common bacterial isolates were enterococcus (21%) and staphylococcus (20%) species and *Candida albicans* from fungal cultures (27%). Type of infection did not correlate with mortality (*p*>0.05), morbidity (*p*>0.05), or length of hospital stay (*p*>0.05). Resistant organisms were found in 20% of infections and did not correlate with worse outcome (*p*>0.05). However, when comparing patients with infected versus sterile necrosis, those with infection had a prolonged hospital stay, whether of bacterial or fungal origin (median, 15 days vs 11 days, *p*>0.05).

The majority of patients included in the study had some degree of debilitation. We used albumin levels, as it was collected routinely throughout the duration of the study period, in order to assess the severity of debilitation and somewhat quantify degree of chronic disease. However, we were unable to assess length of hospitalization prior to operation due to the high percentage of patients who were transferred for tertiary care. Degree of chronic disease and debilitation was assessed as none (albumin >3.5 gm/dL), mild (2.5–3.5 g/dL), moderate (1.5–2.5 g/dL), or severe (<1.5 gm/dL). No significant association was observed between preoperative albumin level and morbidity or mortality (p>0.05). Severe debilitation, found in 13 patients, was associated with an increased length of hospital stay (mean stay, 47 vs 17 days, *p*<0.01). Prior to operation 38% received enteral nutrition and 30% parenteral nutrition. There was no significant difference in morbidity or mortality between the enteral and parenteral feeding groups (*p*>0.05). However, patients receiving no preoperative nutrition had a significantly longer length of stay compared to those receiving nutrition (median, 17.5 days vs 11 days, *p*=0.02).

Discussion

Since the time of the Atlanta symposium in 1992,[6] many advances have been made in the study of acute pancreatitis; specifically, advancements in understanding pathophysiology, imaging techniques, and refining the different options for treatment. Given the progress that has occurred in medical and surgical specialties, a further revision of the Classification was undertaken. The goal was to improve patient care and physician communication and to standardize further research efforts.

Two distinct phases of acute pancreatitis, based on pathophysiology, are now described. The first phase, usually lasting 1–2 weeks, is mainly classified clinically. Multiple scoring systems, such as the Marshall Scoring System, have been proposed to standardize the clinical status of patients during this very early stage of acute pancreatitis. It has been noted that these scoring systems are

most helpful when categorizing the severity of acute pancreatitis within the initial 2 weeks from onset of systems. Only five patients in our study had surgical evaluation during this early period. Furthermore, over half of our patients had been transferred from another hospital for tertiary care, and their initial clinical status was unknown. For these reasons, we chose not to stratify our patients according to an early clinical scoring system.

The second phase is classified morphologically as local complications seen on CECT. New entities now recognized by the revision include acute interstitial edematous pancreatitis, acute necrotizing pancreatitis, acute peripancreatic fluid collection, postnecrotic pancreatic/peripancreatic collection (PNPC), pancreatic pseudocyst, and WON, further defined as pancreatic or extrapancreatic; pancreatic abscess, infected pseudocyst, pancreatic phlegmon, hemorrhagic pancreatitis, and persistent acute pancreatitis have been abandoned (per personal conversation with Dr. Michael Sarr, Mayo Clinic, Rochester, MN; Table 4). Recent observations have suggested that extrapancreatic necrosis may be less aggressive than the primarily pancreatic form.[17] Although our experience showed the majority of patients as categorized with predominantly extrapancreatic necrosis on CECT, our data did not show a significant difference between type of necrosis and outcome. However, the current proposed revision to the Atlanta Classification does not differentiate between the extent or amount of necrosis. It simply classifies necrosis as *predominantly* pancreatic or extrapancreatic. Therefore, the extent or amount of necrosis was not taken into account in our study.

Surgical therapy evolved significantly over the study period. In the initial years of our study, debridement with either open packing or external drainage was more frequently undertaken. Correlating with other published data,[13] debridement with open packing in our study was associated with a higher mortality. However, in the more

Table 4 Key Differences between 1992 and 2007 Atlanta Classification

1992 Atlanta classification	2007 proposed revision
<4 weeks from onset of pancreatitis	<4 weeks from onset of pancreatitis
Acute fluid collection	Acute post-necrotic collection
	Acute peri-pancreatic fluid collection
	Sterile vs. infected
>4 weeks from onset of pancreatitis	>4 weeks from onset of pancreatitis
Pancreatic abscess Infected pseudocyst	Walled-off necrosis
Hemorrhagic pancreatitis	
Pancreatic phlegmon	
	Pancreatic vs. peripancreatic

recent years, we have chosen to perform open debridement and internal drainage via cystgastrostomy when feasible. This procedure requires a well-formed necrotic wall to create the cystgastrostomy. For this reason, we advocate reserving surgical therapy for several weeks to allow for cyst maturation. Early necrotic collections, occurring within 4 weeks of onset of symptoms, which lack this well-formed wall, are classified as postnecrotic fluid collections by the revised Atlanta Classification. Five patients in our study had these postnecrotic collections (see Table 2). Interestingly, we were able to perform cystgastrostomy on four of these five patients. However, the average length of time from CT scan to operation in these patients was 2 weeks. This delay could have allowed for cyst maturation. Although many factors play a role in deciding which operation is most appropriate, there was no significant association between the type of necrosis (whether pancreatic or extra-pancreatic) and surgical procedure performed.

The overall mortality for our series was 14%, and the cystgastrostomy group had a mortality of 7% (see Table 3). Mortality was highest in the open packing group, and this predominantly occurred in the early years of the study. One factor to account for this is the improvement in perioperative care, especially in the arena of critical care. However, the cystgastrostomy group still had lower mortality than the external drainage group even in the most recent years of study (7% vs. 20%, respectively, years 2004–2008). Open packing was not performed during these years. This suggests that improvements in perioperative care cannot solely account for lower mortality rates among patients undergoing debridement by cystgastrostomy.

Regardless of the surgical treatment, the presence of infection is one of the clear indications for operation. While presence of infection of any type has been associated with worse outcome,[18] as supported by our series, some authors have observed increased complications and length of hospitalization with specific types of infections, such as resistant organisms and fungal infection.[19] Based on increasing incidence of resistant infections in some series, several centers do not use prophylactic antibiotics for necrotizing pancreatitis. Our own practice is similar, and we reserve the use of antibiotics for necrotizing pancreatitis until the presence of infection has been confirmed. However, we were unable to show a significant difference in outcomes based on type or susceptibility of infection.

While infection is an indication for surgery, not all of our patients had infected necrosis. Other indications for surgical debridement include patient demise and persistent inability to tolerate oral intake. While we have no set period of time at which a patient is considered to have failed nonoperative management, the average number of days from admission to surgery for patients who had no preoperative evidence of infected necrosis was 20 days.

Maximizing nutritional support is important in the treatment of necrotizing pancreatitis. Enteral nutrition is considered to carry a lower risk of complications when compared to the parenteral route, and it has been shown to improve outcomes[20,21]. Although we attempted enteral supplementation whenever possible, we found no differences in outcomes between those receiving enteral or parenteral nutrition. Due to disparity in the size of these groups, we most certainly lacked sufficient power to comment further on this topic.

There is a large body of recent literature that addresses improved surrogate markers, such as C-reactive protein (CRP) and pre-albumin, for nutritional status. One of the criticisms of more traditional nutritional markers, such as albumin, is that it is affected by chronic illness; therefore, it may be more useful as a measure of patient debilitation, rather than nutritional status. Because albumin levels have been consistently measured for our patients throughout the past decade, unlike CRP and pre-albumin, which have only recently been followed, we chose to comment on patient debilitation, rather than nutritional status, using these albumin levels.

We defined debilitation as hypoalbuminemia less than 3.5 g/dL. The severely debilitated group, not surprisingly, had longer hospital stays but did not experience higher complications rates. Furthermore, those receiving no preoperative nutrition had worse outcomes.

Pancreatic debridement of any type still carries a significant complication rate. We have shown, although through a retrospective review, that the type of necrosis or the presence of infection did not correlate with the type of procedure performed. Based on this, we continue to advocate debridement via cystgastrostomy when appropriate.

At some centers, interventional endoscopy is being used much more frequently in the management of necrotizing pancreatitis. However, a major limitation of endoscopy remains that the procedure is relatively "blind" with potential for complications such as bleeding and perforation. With the advent of endoscopic ultrasound (EUS), drainage of pancreatic fluid collections can be undertaken real-time with complication rates less than 1%.[11] While recent studies suggest that clinical outcomes of EUS-guided drainage of pancreatic pseudocysts were comparable to surgery,[11] the success rate for endoscopic treatment of necrotic fluid collections is less than 50%.[12]

Conclusion

Acute necrotizing pancreatitis with or without infection is a disease that carries high rates of morbidity and mortality. At our center, surgical technique has shifted to incorporate internal drainage after debridement and outcomes appear to be improving, whether the necrosis is mainly pancreatic or

peripancreatic, infected or sterile. However, those with infected necrosis and preoperative debilitation had worse outcomes, and we stress the importance of preoperative nutrition and early intervention.

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Discussion

Dr. Charles Vollmer (Boston): It is a fine work, Sebron, and I really appreciate the well-written manuscript as well as your very crisp presentation. It is a real nice takeoff from this morning's great session on pancreatitis with all the giants in pancreatitis there discussing some similar topics. I think it is sort of underappreciated that this could be a nice technical maneuver for, in particular, the problem of the disconnected pancreatic segment. I have become much more of a fan of this sort of approach for infected peripancreatic collections, thinking that this is no different than many other surgical diseases where the principle of incision and drainage alone is applied to closed-space sepsis. I have a couple questions about your experience a UAB.

First, what factors distinguish your decisions to choose any given type of operation in this series, (technical, morphological, patient acuity factors, etc). Because you did not really tell us about the demographic characteristics of this cohort, is it just good luck that certain patients can have this cystogastrostomy approach based on the topography of the cyst layout?

The second thing is, how many times when you do such a supposedly definitive approach like this does it fail? How many times have you had to use counter drainage or even something more subtle like prolonged antibiotics for a festering "unwellness" after one of these cyst-enterostomies?

The third thing I would like to know is, among the three cohorts that you showed us, what is the average length of time for each in terms of when you acted for the operative intervention?

And the last question is maybe for the esteemed audience as well. Do we actually need to, as you describe, do aggressive debridement in these cystogastrostomy cases? Is it okay to leave the necrotum behind, relying on the body's ultimate reabsorption/remodeling powers, perhaps, and just relieve the forceful turgor of the cyst itself which is most likely the reason for symptoms?

Dr. Sebron W. Harrison: Thank you, Dr. Vollmer. Regarding the first question, what is our basis for choosing a particular type of surgery? The important thing is that there has to be a fairly significant degree of central necrosis.

This means that if there is no area of central necrosis, then a cystogastrostomy is probably not going to result in any amount of success.

Therefore, for the cases without a fairly large amount of central necrosis, would be instances in which we would look more towards external drainage. We do not routinely perform open packing.

Regarding our failure rate, this is a very good question. There were two patients in our series who, after cystgastrostomy with internal drainage, had to go back to the operating room. This was not as extensive as the original surgery but rather due to failure to thrive postoperatively back for a counterincision. This is usually made just above the iliac crest on the right because there is often a component from the duodenum that we were not able to adequately drain through our cystogastrostomy or with blunt dissection.

The average preoperative time is definitely a potential shortcoming of our study because a large number of our patients were transferred from outside the hospital; we simply do not have the data.

However, the average time—I do not have the individual data, but the average time—overall, for our three procedures was 7 days. This does not take into account the number of days that they spent at another hospital before we had surgical consultation at our own hospital.

Dr. Sebron W. Harrison: Absolutely. Like Dr. Fernandez addressed, one of the great pearls is when talking to these patients or when talking to people who are asking for advice, the best advice you can, perhaps, give is to sit tight. And that is exactly what we do. We usually wait at least 3–4 weeks to give time for this necrosis to wall-off in order to allow cystogastrostomy to be effective.

Dr. Kevin E. Behrns (Gainesville, FL): Why operate on these folks? Why not do this endoscopically or laparoscopically?

Dr. Sebron W. Harrison: Our gastroenterology group has commented on that very thing in two recent publications. One is that endoscopic drainage for pancreatic pseudocysts is successful as long as it does not contain necrotic debris.

However, when there is a component of necrotic debris, they have been as successful. The success rate was much less than 50%, and as a gastroenterologist, he admits that endoscopic treatment is inferior to surgical management.

These patients often have failure to thrive, are unable to tolerate PO, and that is why we take them to operation, regardless of whether they already had an attempt at endoscopic drainage.

Dr. Henry Pitt (Indianapolis): Your overall mortality was 14%. My question is whether this rate is acceptable?

During the same time period, at Indiana University, Tom Howard reported a 5% mortality in a similar group of patients.

In looking at the NSQIP national data for 2007 from 200 hospitals for pancreatic debridement, the mortality was 6.7%. Therefore, the bar is getting higher, and the mortality that we should be shooting for is lower than 14%.

Dr. Sebron W. Harrison: I would absolutely agree with that statement and that is why our cystgastrostomy mortality rate was only 7%. Fourteen percent was the entire mortality rate over the procedure and that included 60% mortality for those who underwent open packing.

I would absolutely agree that this is unacceptably high and that is why we are advocating this particular type of procedure.

2009 SSAT PLENARY PRESENTATION

TRAIL and Triptolide: An Effective Combination that Induces Apoptosis in Pancreatic Cancer Cells

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Abstract

Introduction An emerging therapy in oncology is the induction of apoptotic cell death through anti-death receptor therapy. However, pancreatic cancer is resistant to apoptosis including anti-death receptor therapy. We have previously described how triptolide decreases resistance to apoptosis in pancreatic cancer cells in vitro and in vivo. We hypothesized that triptolide decreases tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in pancreatic cancer cells. The aim of this study was to evaluate the effects that combined therapy with TRAIL and triptolide have on different parameters of apoptosis.

Methods Four different pancreatic cancer cell lines were exposed to triptolide, TRAIL, or a combination of both drugs. We assessed the effects that combined therapy with TRAIL and triptolide has on cell viability, apoptosis, caspase-3 and caspase-9 activities, and poly(ADP)-ribose polymerase cleavage.

Results Pancreatic cancer cells were resistant to TRAIL therapy; however, combined therapy with triptolide and TRAIL significantly decreased the cell viability in all the cell lines and increased apoptotic cell death as a result of caspase-3 and caspase-9 activation.

Conclusions Pancreatic cancer is highly resistant to anti-death receptor therapy, but combined therapy with TRAIL and triptolide is an effective therapy that induces apoptotic cell death in pancreatic cancer cells.

Introduction

Pancreatic cancer is associated with a poor prognosis that has not significantly changed over the past 30 years. Major pancreatectomies are now considered a safe procedure with a low mortality; however, less than 10–15% of the patients with pancreatic cancer are candidates for surgery because most patients present with locally advanced tumors or systemic disease. In addition, most patients will develop a locoregional or distant recurrence within the next 2 years after surgery.[1] Given the high recurrence rate, adjuvant chemotherapy with or without radiotherapy is an important component in the treatment of pancreatic cancer. Multiple drugs have been approved as a standard of care (i.e., gemcitabine, 5-fluorouracil, and erlotinib). Despite the use of these drugs, the long-term outcome for pancreatic cancer remains very poor, with current 5-year survival rates of less than 5%.[2–4]

The poor response associated with conventional chemotherapy has created a shift in pancreatic cancer research in order to identify mutations that are responsible for the aggressive biologic behavior or confer resistance to treatment. At the same time, this trend has led to the development of multiple targeted drugs that induce apoptosis, restore the cell cycle in cancer cells, or restore or block

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the deleterious effects of the mutations that give resistance to apoptosis.

One of the most promising approaches to targeted therapies in oncology is the induction of apoptosis in cancer cells. Apoptosis or programmed cell death can be induced by two different mechanisms. The first pathway is triggered by different stimuli such as DNA damage, radiotherapy, or chemotherapy that induce the mitochondrial release of cytochrome c and apoptosis-inducing factor. Once cytochrome c is present in the cytoplasm, it binds to APAF-1 and procaspase-9 to form the apoptosome complex, which in turn activates caspase-9. Caspase-9 is an initiator caspase that amplifies the signal by activating effector procaspase-3, procaspase-7 and procaspase-6.[5] In contrast, the extrinsic or death receptor pathway is mediated by different ligands and their receptors. Upon binding to their ligands, these receptors recruit both Fas-associated death domain and procaspase-8 to form the death-inducing signaling complex (DISC). After the DISC is formed, procaspase-8 gets activated; active caspase-8 can directly activate the effector caspases or, through BID cleavage, induce the activation of the mitochondrial pathway.[6]

As previously mentioned, the tumor necrosis factor (TNF) superfamily has different ligands that induce apoptosis. The first two members described were TNF-α and Fas ligand. Upon ligation to their receptors, these two peptides trigger apoptosis in normal and cancer cells; therefore, systemic therapy with these peptides induces a systemic shock response manifested with hypotension, severe liver failure, and death. TNF-related apoptosis-inducing ligand (TRAIL) was more recently discovered.[7] The main difference between this relatively new member and TNF-α or Fas ligand is that TRAIL is more selective to cancer cells; it only induces apoptosis in tumor cells. Multiple preclinical and clinical studies have shown that TRAIL is a safe therapy associated with minimal toxicity to normal cells.[8]

Pancreatic cancer cells are known to be highly resistant to apoptosis, including TRAIL therapy. We have previously demonstrated that triptolide, a diterpene triepoxide extracted from the Chinese plant *Tripterygium wilfordii*, induces apoptosis in pancreatic cancer cells, both in vitro and in vivo.[9] Consequently, we hypothesized that triptolide therapy decreases resistance to TRAIL therapy in human pancreatic cancer cells. The aim of this study was to assess the effects that combined therapy with TRAIL and triptolide has on different markers of apoptosis in pancreatic cancer cells.

Materials and Methods

Cells Culture and Drugs MIA-PaCa2 and PANC-1 cells were obtained from the American Type Culture Collection.

Both cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PS). S2-013 and S2-VP10 cells were kindly provided by Dr. Buchsbaum (University of Alabama at Birmingham). S2-013 and S2-VP10 cells were grown in RPMI supplemented with 10% FBS and 1% PS. All cell lines were grown under standard conditions at 37°C in a humidified atmosphere containing 5% CO_2. DMEM, RPMI, PS, and FBS were obtained from Invitrogen Corporation. Triptolide (Calbiochem) was diluted in dimethyl sulfoxide. Recombinant human TRAIL (also known as Apo2 ligand; Invitrogen Corporation) was diluted in sterile water. All drugs were stored in aliquots according to manufacturer's recommendations.

Determination of Cell Viability Four different pancreatic cancer cell lines were seeded into 96-well plates (5×10^3/well) and allowed to adhere for 24 h. Cells were treated with vehicle (control), increasing concentrations of TRAIL (0–20 ng/ml) alone, or in the presence of a low dose of triptolide (50 nM). Cell viability was measured using Dojindo Cell Counting Kit-8 according to manufacturer's protocol. After 48 h of treatment, 10 μl of tetrazolium substrate were added into each well. The plates were protected from light and incubated for 1 h in a humidified atmosphere at 37°C containing 5% CO_2. Absorbance was measured in a plate reader (BioTek) at an absorbance of 450 nm. Cell viability was measured in triplicates; each experiment was repeated four times.

Determination of Apoptosis Pancreatic cancer cells (2.5×10^5 cells/well) were seeded into six-well plates. After a 24-h incubation, cells were treated with vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of TRAIL plus triptolide (using the same doses). After 24 h of treatment, the externalization of phosphatidylserine was measured by flow cytometry using the Guava Nexin Kit as previously described.[9] Apoptosis was measured in duplicates; each experiment was repeated four times.

Quantification of Caspase-3 and Caspase-9 Activities Caspase-3 and caspase-9 activities were analyzed using the Caspase-Glo luminescent-based assays (Promega) according to the manufacturer's instructions. Cells (1×10^4 cells/well) were seeded in 96-well white opaque plates and a corresponding optically clear 96-well plate. Cells were allowed to adhere for 24 h and treated with vehicle, TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs. After 4, 8, 12, and 18 h of treatment, 100 μl of Caspase-Glo-3 and Caspase-Glo-9 were added into each well. Plates were gently mixed for 1 min, and after 30 min of incubation, plates were read using a luminometer (BMG

Labtech). The corresponding 96-well clear well plate was used to measure the number of viable cells with CCK-8 reagent. Caspase activity was normalized to the number of viable cells. Caspase activity was measured in triplicates and repeated four times.

Determination of PARP Cleavage by Western Blotting Cells (8×10^5) were plated in 10-cm dishes. Once cells were 70% confluent, they were treated with vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs. After 24 h of treatment, cells were harvested and washed with 1× phosphate-buffered saline. Cells were resuspended in lysis buffer (Boston bioproducts Inc.; 65 mmol/L Tris–HCL (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Nonidet P40, 1% sodium deoxycholate, 1 μg/ml aprotinin, and 100 μg/mL phenylmethylsulfonyl fluoride) with freshly added protease inhibitor cocktail (Roche) for 30 min at 4°C and stored at −80°C. The next day, cell lysates were cleared by centrifugation for 20 min at 13,000×g. Total protein concentration was measured using the bicinchoninic acid protein assay according to manufacturer's protocol (Pierce). Equal amount of protein (10 μg) were resolved over 10% Tris–HCl polyacrylamide gels and transferred onto nitrocellulose membranes (Bio-Rad laboratories). Membranes were incubated in blocking buffer (5% bovine serum albumin) for 1 h. The blot was subsequently incubated with polyclonal rabbit anti-human poly(ADP)-ribose polymerase (PARP; Santa Cruz Biotechnology) or polyclonal rabbit anti-human Actin (Santa Cruz Technology). After three washes, membranes were incubated for 1 h with the appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Technology). Blots were detected with chemiluminescence. Actin expression was used as an internal control.

Statistical Analysis Values are expressed as mean ± SE. The significance of the difference between control and each experimental test condition was analyzed by one-way ANOVA using GraphPad InStat Software. The difference was considered statistically significant if $p < 0.05$.

Results

Effect of Triptolide and TRAIL on Cell Viability We selected four different pancreatic cancer cell lines based on the level of aggressiveness and resistance to TRAIL therapy. We used two metastatic cancer cell lines (S2-013 and S2-VP10), which are known to be TRAIL resistant, one non-metastatic with intermediate sensitivity to TRAIL (PANC-1), and finally, one non-metastatic TRAIL-sensitive cell line (MIA-PaCa2). These cell lines were exposed to increasing concentrations of TRAIL (0–20 ng/ml). After 48 h of treatment, most pancreatic cancer cells were resistant to TRAIL therapy; only MIA-PaCa2 cell line exhibit a significant decrease in the cell viability (Fig. 1a). However, when all cell lines were co-incubated with TRAIL and triptolide (50 nM), the cell viability in all the cell lines tested significantly decreased. This effect was seen in MIA-PaCa2 (Fig. 1a), Panc-1 (Fig. 1b), S2-VP10 (Fig. 1c), and S2-013 (Fig. 1d).

Combined Therapy with Triptolide and TRAIL Increases Apoptosis Apoptosis is the main mechanism by which TRAIL induces cell death.[6,7,10] We have previously demonstrated that triptolide enhances apoptotic cell death in pancreatic cancer cells.[9] Therefore, we decided to assess the effect that combined therapy with TRAIL and triptolide has on apoptosis. We used Annexin V staining to measure the externalization of phosphatidylserine as a marker of apoptosis. Four different pancreatic cancer cell lines were exposed to vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both (TRAIL 1.25 ng/ml+ Triptolide 50 nM). After 24 h of treatment, single therapy with TRAIL or triptolide had a minimal effect on apoptosis; however, if pancreatic cancer cells are exposed to combined therapy with TRAIL and triptolide, the number of cells undergoing apoptosis significantly increases. This effect was statistically significant as compared to single therapy with each drug alone (Fig. 2).

Combined Therapy with Triptolide and TRAIL Increases Caspase-3 Activity Caspases are the main enzymes that mediate apoptosis.[8,11] Any stimuli that triggers apoptosis eventually leads to the activation of the effector (also known as executioner) caspases, which include caspase-3, caspase-6, and caspase-7. In order to corroborate that apoptosis is the main type of cell death that occurs when pancreatic cancer cells are exposed to combined therapy with TRAIL and triptolide, we measured the activation of caspase-3 and caspase-7 using a luminescence assay. For this purpose, we exposed our four pancreatic cancer cell lines to vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs for 18 h. Figure 3 illustrates how a low dose of TRAIL or triptolide induces minimal activation of caspase-3 and caspase-7; however, if both drugs are combined using the same low doses, the activation of caspase-3 and caspase-7 considerably increases. This effect can be seen in all the cell lines tested: MIA-PaCa2, PANC-1, S2-VP10, and S2-013.

In order to validate the previous result, we evaluated the effect that combined therapy with TRAIL and triptolide have on PARP cleavage. PARP is one of the caspase-3

Figure 1 Effect of TRAIL and triptolide on cell viability and apoptosis in pancreatic cancer cells. After 48 h of treatment, most pancreatic cancer cell lines are TRAIL-resistant. TRAIL therapy alone only decreases the cell viability in MIA-PaCa2 cells (**a**). However, combined therapy with increasing concentrations of TRAIL in the presence of triptolide (50 nM) significantly increases the number of cells dying. This effect is also present in TRAIL-resistant cell lines, as is shown in **b**–**d** (PANC-1, S2-VP10, and S2-013, respectively). *Points* means, *bars* SE (*n*=4, run in triplicates).
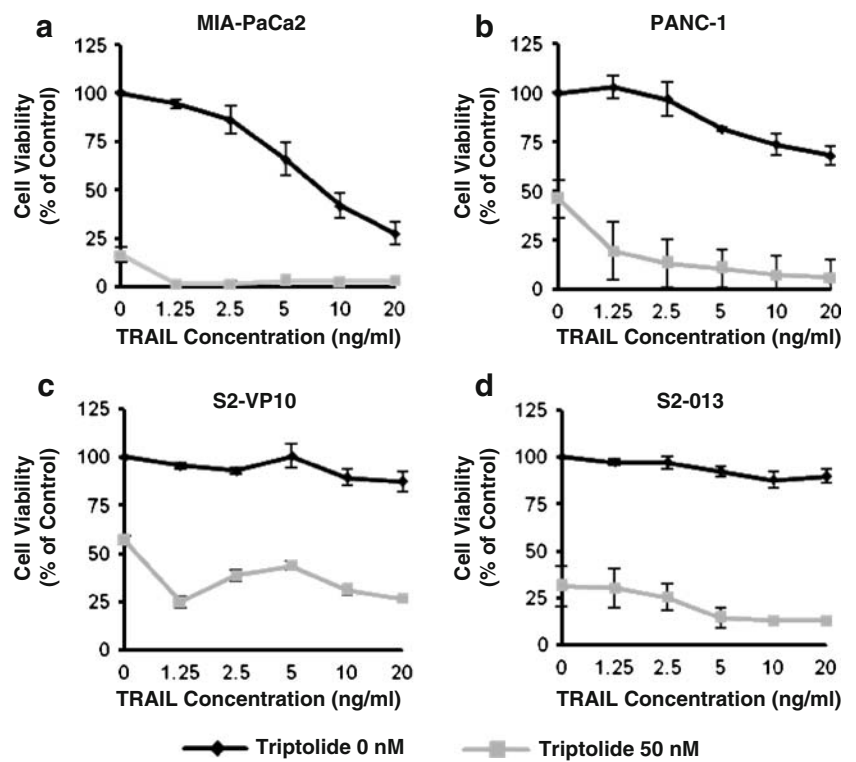
substrates that mediate apoptosis; once caspase-3 gets activated, it cleaves PARP. We exposed MIA-PaCa2, S2-013, and S2-VP10 cells to vehicle, TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs. As indicated in Fig. 4, PARP cleavage occurs in all cell lines only when both drugs are co-administered. The results of this set of experiments validate that combined therapy with TRAIL and triptolide increases the activity of caspase-3 and

Figure 2 Effect of TRAIL and triptolide on annexin V. After 24 h of single therapy with a low dose of TRAIL or triptolide, there was no significant externalization of phosphatidylserine; however, combined therapy using a low dose of both drugs increased the number of cells that stained positive for annexin V, indicating that apoptosis is occurring in the cells. This effect is seen in TRAIL-sensitive (MIA-PaCa2, **a**) and TRAIL-resistant cell lines (**b** PANC-1, **c** S2-VP10, and **d** S2–013). *Column* mean, *bar* SE, *$p<0.01$ and **$p<0.001$ as compared to control, TRAIL, or triptolide.
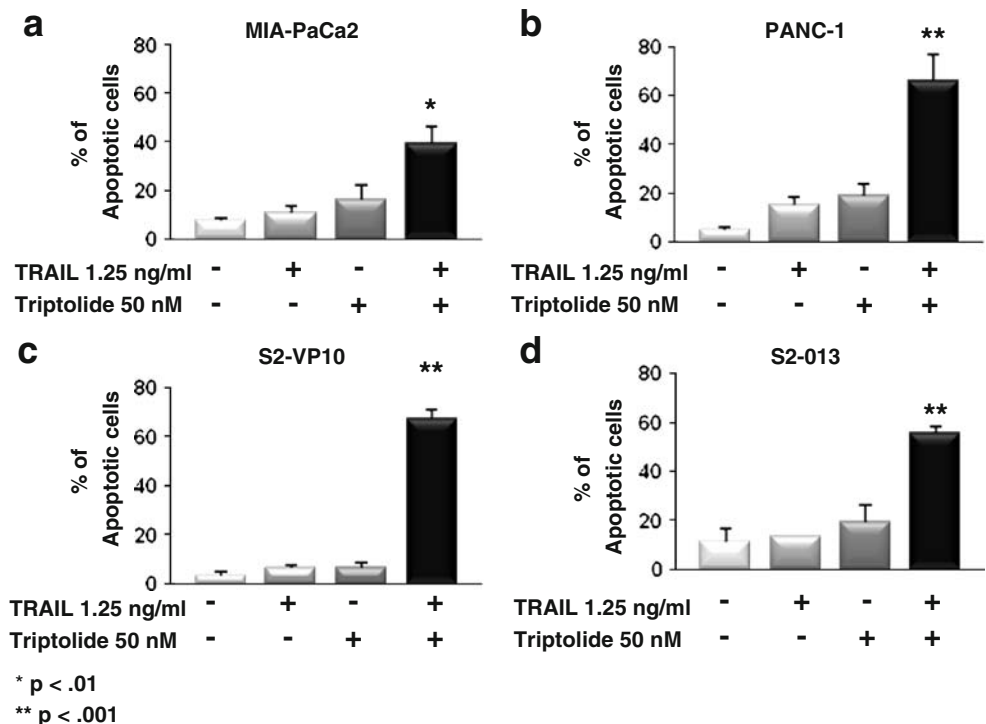
Figure 3 Effect of TRAIL and triptolide on caspase-3 activity. A low dose of TRAIL (1.25 ng/ml) or triptolide (50 nM) has minimal effect on the activation of caspase-3 and caspase-7, but if both drugs are combined, the activities of caspase-3 and caspase-7 significantly increase after 18 h of exposure. This effect is seen in MIA-PaCa2 (**a**), PANC-1 (**b**), S2-VP10 (**c**), and S2-013 (**d**). *Column* mean, *bar* SE (*n*=4 run in triplicates) *p<0.0001 or **p<0.0009 as compared to control, triptolide, or TRAIL.
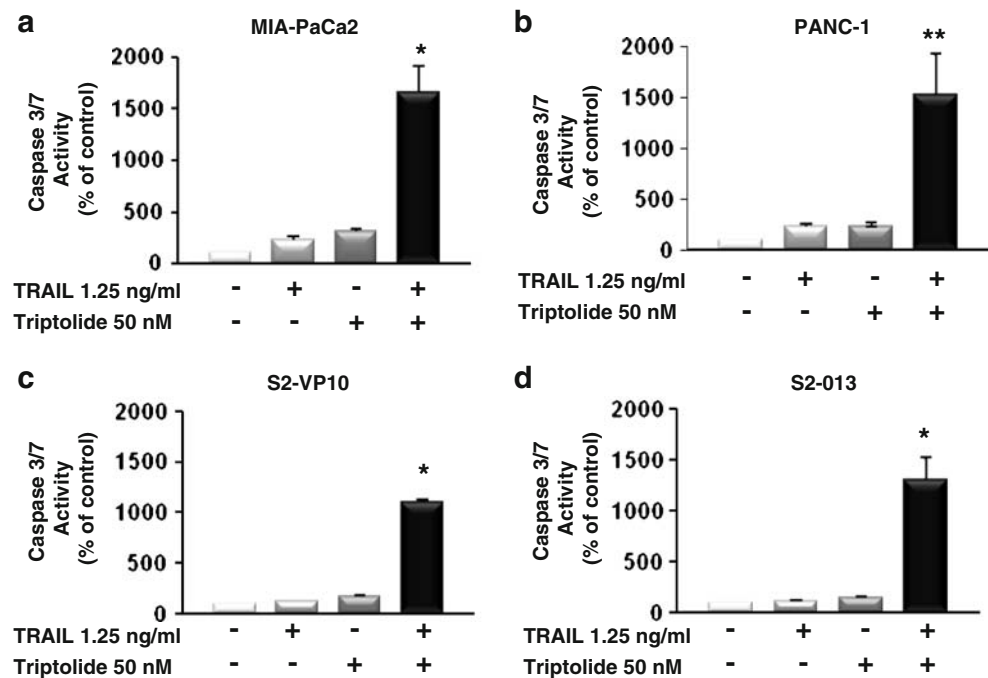
caspase-7. This effect is present in all cell lines regardless of TRAIL sensitivity.

Effect of TRAIL and Triptolide on the Mitochondrial Apoptotic Pathway Effector caspases (3 and 7) are activated directly by caspase-9 (intrinsic pathway). We decided to measure caspase-3 and caspase-9 at different time points to assess if the mitochondrial pathway is being activated by TRAIL and triptolide. As seen in Fig. 5, combined therapy with both drugs induced an increase in the activity of caspase-9 and caspase-3. This result was seen in the four cell lines evaluated, which suggests that when pancreatic cancer cells are incubated with both drugs, there is a time-dependent activation of the mitochondrial apoptotic pathway. It is also evident in Fig. 5 that the decrease in cell viability occurs at the same time that caspase-9 and

caspase-3 activation is occurring. Finally, we exposed our cell lines to vehicle (control), TRAIL 1.25 ng/ml, triptolide (50 nM), and combined therapy with both drugs. After 18 h of exposure, we measured caspase-9 activity. As seen in Fig. 6, the activity of caspase-9 was increased significantly when MIA-PaCa2 and S2-VP10 cells were treated with both drugs as compared to each drug alone.

Discussion

Pancreatic adenocarcinoma remains a devastating tumor with a poor prognosis because it has an aggressive biological behavior. As a result, up to 80% of patients with pancreatic cancer will not be able to undergo resection and
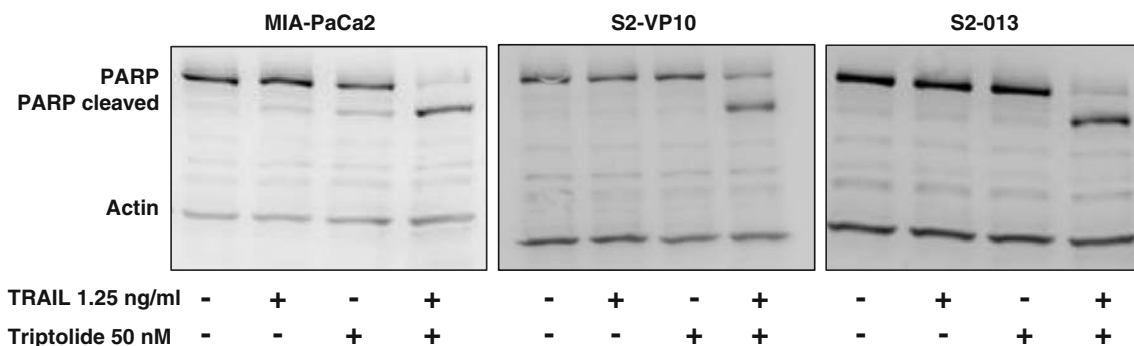
Figure 4 Effect of TRAIL and triptolide on PARP cleavage. Single therapy with low doses of either TRAIL or triptolide did not induce any cleavage of PARP but combined therapy with both drugs increased PARP cleavage. The results of this experiments correlate with the increase activity of caspase-3 and caspase-7 in the previous figure. This experiment was repeated at least three times with each cell line, all with similar results.
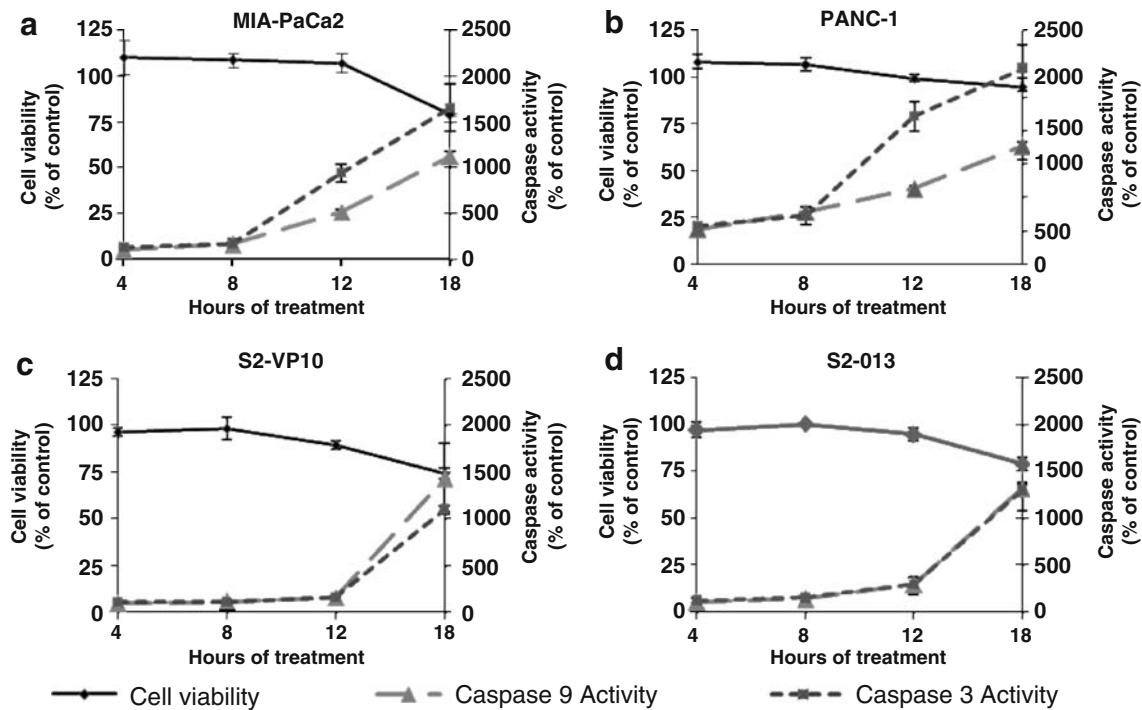
Figure 5 Effect of combined therapy with TRAIL and triptolide on the activity of caspases (3 and 9). Combined therapy with TRAIL (1.25 ng/ml) and triptolide (50 nM) induces an increase in the activity of procaspase-3 and procaspase-9. This increase is time-dependent.

The increase seen in the activity of caspase-3 and caspase-9 occurs when the cell viability starts to decrease. This effect was seen in all the cell lines tested (**a** MIA-PaCa2, **b** PANC-1, **c** S2-VP10, **d** S2-013). *Points*, mean; *Bar*, SE ($n=4$, run in triplicates for each time point).

require the administration of chemotherapy. The current drugs considered as the standard of care for pancreatic cancer have a minimal impact in the long-term survival of patients with pancreatic cancer, which is reflected by the

pronounced lethality of this tumor.[12] Since the chemotherapy that is considered as the standard of care for pancreatic cancer has not been able to induce a significant impact in the overall survival of patients with pancreatic cancer, new

Figure 6 Effect of TRAIL and triptolide on caspase-9 activity. Monotherapy with TRAIL or triptolide induces minimal caspase-9 activation; however, combined therapy with both drugs induces a significant increase in the activation of caspase-9. This effect is seen in all cell lines. *Column* mean, *bar* SEM; *$p<.001$ ($n=4$, run in triplicates) as compared to control, TRAIL, and triptolide.
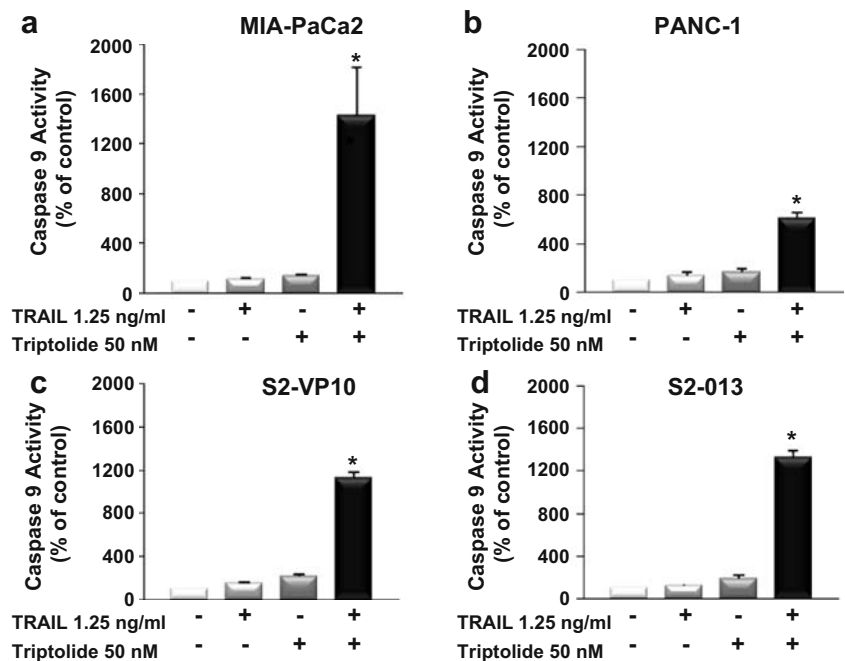
forms of therapies that specifically target pancreatic cancer are required.

Our group has previously described that triptolide, a diterpenoid triepoxide present in a Chinese herb, induces the release of cytochrome c from the mitochondria, which in turn induces sequential activation of procaspase-9 and procaspase-3. Once caspase-9 and caspase-3 are activated, they induce apoptotic cell death in pancreatic cancer cells in vitro. We have also shown that triptolide decreases the tumor growth and locoregional invasion in an orthotopic model of pancreatic cancer in vivo, which suggests that triptolide is a good candidate for pancreatic cancer therapy.[9] Nevertheless, the clinical experience and treatment of other solid tumors tell us that only a few solid tumors respond to single agent-based chemotherapy. Chronic exposure to chemotherapeutic agents can induce the selection of clones that are resistant to that particular agent; therefore, overtime tumor resistance can occur. Additionally, solid organ tumors sometimes have intrinsic resistance to the drug before any treatment has started. It cannot be overemphasized that the probability that drug resistance develops over the course of the disease decreases if different agents are combined. Combined therapy also allows decreasing drug doses, decreasing the likelihood of toxicity.

Anti-death receptor therapy is a relatively new form of cancer treatment; this type of therapy has proven to induce apoptosis of multiple cancer cell lines in vitro and tumor regression in some xenograft models without affecting normal cells.[6,10,13] Since the preclinical evidence has been promising, this therapy is now being evaluated in phase I and II clinical trials. Results from these trials suggest that anti-death receptor therapy is safe in humans because it is not associated with significant toxicity.[14] While death receptor therapy has been promising in solid tumors, the majority of human pancreatic cancer cell lines are known to be highly resistant to drugs that induce apoptosis, including anti-death receptor therapy.[15,16] Our initial experiments also validate that most pancreatic cancer cells are resistant to TRAIL therapy.

Since both TRAIL and triptolide induce apoptosis in pancreatic cancer cells, we formulated the hypothesis that combined therapy with these two compounds increases the effectiveness as compared to single therapy. Our results prove the fact that low doses of TRAIL and triptolide induce a significant increase in apoptosis as compared to single therapy either with TRAIL and triptolide. It is important to mention that the doses of both drugs are considerably lower than the doses used in single therapy. All our results showed the same trend: Combined therapy with both drugs increases the externalization of phosphatidylserine, procaspase-3 and procaspase-9 activation, and PARP cleavage. Taken together, these results suggest that combined therapy with TRAIL (death receptor therapy) and triptolide is a promising therapy that requires further investigation. Cells can be classified into two types according to the main pathway that induces apoptosis. If cancer cells do not require activation of the mitochondrial pathway, they are considered type I cells, but if tumor cells require activation of the intrinsic or mitochondrial pathway, cells are classified as type II. In a similar way to what has been described, we found that pancreatic cancer cells are type II because they require activation of the intrinsic pathway to undergo apoptosis after TRAIL therapy.

Conclusions

Combined therapy with TRAIL and triptolide is a new promising therapy for pancreatic cancer that increases the activation of caspase-3 and caspase-9. As a result, this therapy increases the number of cells undergoing apoptotic cell death as compared to monotherapy with TRAIL or triptolide. This effect is not exclusive of TRAIL-sensitive cell lines because it is also seen in cell lines that are known to be highly aggressive and resistant to TRAIL. Combined therapy with TRAIL and triptolide is a novel therapy in pancreatic cancer that requires further investigation.

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Dr. Daniel Borja-Cacho, Presenter (University of Minnesota, Minneapolis, MN)

Discussant

Dr. Jeffrey Matthews (University of Chicago Medical Center, Chicago, IL): Thank you for that presentation.

Just a few questions about triptolide. Do you know if the doses that you are using are similar to the kinds of levels that you would get from eating a Chinese herb? Or at what level are you starting to see the effects of the triptolide, and do you know of its toxicity profile when given in vivo?

Secondly, do you have any insight into what the actual target of triptolide is that might be upstream from some of these changes in gene expression?

Finally, I am wondering if you have had the chance to go back and look at archived surgical specimens or specimens that you obtain fresh in your pancreatic surgical program to know whether there is a difference in the pattern of XIAP expression in pancreatic cancer cells versus other cancer cell types that are perhaps less resistant to this therapy? This might help understand whether that accounts for the unusual resistance of pancreatic cancer to standard therapies.

Discussion of Paper #19

Title of Paper: Triptolide and TRAIL: An Effective Combination that Activates Both the Intrinsic and Extrinsic Apoptotic Pathways in Pancreatic Cancer Cells

Closing Discussant

Dr. Daniel Borja-Cacho: The first question: in vitro, the optimal dose that we have used to study the mechanism of action of triptolide is 200 nM. The equivalent dose that we have used for in vivo with mice is 0.2 mg kg^{-1} day^{-1}. The toxic dose in vivo that we found so far is 0.8. Therefore, we have a therapeutic window.

Since triptolide is very efficacious in treating pancreatic tumors in mice, the University of Minnesota is planning to undertake a phase I trial for Triptolide in the near future.

About this work, the interesting finding is that a lower dose of Triptolide, 50 nM, is working with the combination. This is very encouraging because it seems that, for synergistic studies, a lower dose is enough. We still need to find the optimum dose in vivo for this combination.

Most of the toxicity that has been reported regarding triptolide is confined to the liver. Hepatotoxicity is the main concern. This is present when doses higher than 0.8 mg/kg are used. However, the doses we are using are much lower than this and do not appear to have any toxic effects.

Regarding your second question, we are definitely very interested to see why XIAP expression is decreased and why the expression of other antiapoptotic proteins is coming down. We are currently studying other transcription factors such as heat shock factor 1, which gives resistance to cells and regulate different antiapoptotic proteins such as heat shock protein 70 and gives resistance to chemotherapeutic agents. That is the main reason why we are not emphasizing that triptolide inhibits XIAP expression. We are also looking for other transcription factors.

Finally, we know that XIAP is overexpressed in pancreatic cancer cells. However, we have not so far evaluated it in our patients. It will be an interesting study to do to try to predict which patients are going to respond to the treatment, similar to patients with breast cancer.

Discussion of Paper #19

Title of Paper: Triptolide and TRAIL: An Effective Combination that Activates Both the Intrinsic and Extrinsic Apoptotic Pathways in Pancreatic Cancer Cells

Discussant

Dr. Daniela Basso (Padua, Italy): A quick question about toxicity. For the combination of this therapy, did you test whether in vitro is safe for normal cells? How do normal cells respond to this treatment?

Discussion of Paper #19

Title of Paper: Triptolide and TRAIL: An Effective Combination that Activates Both the Intrinsic and Extrinsic Apoptotic Pathways in Pancreatic Cancer Cells

Closing Discussant

Daniel Borja-Cacho: Anti-death receptor therapy including recombinant TRAIL is not toxic to normal cells because these receptors are only expressed in cancer cells. In general, normal cells do not express them. For example, normal pancreatic duct cells are not known to express these receptors.

Multiple phases 1 and 2 trials have shown the safety of this therapy with minimal toxicity. Similarly, our studies indicate that triptolide is also safe both in vivo and in vitro at doses, which are efficacious.

Does the Mechanism of Lymph Node Invasion Affect Survival in Patients with Pancreatic Ductal Adenocarcinoma?

Ioannis T. Konstantinidis · Vikram Deshpande · Hui Zheng · Jennifer A. Wargo · Carlos Fernandez-del Castillo · Sarah P. Thayer · Vasiliki Androutsopoulos · Gregory Y. Lauwers · Andrew L. Warshaw · Cristina R. Ferrone

Abstract

Background Lymph node metastases are prognostically significant in pancreatic ductal adenocarcinoma. Little is known about the significance of direct lymph node invasion.

Aim The aim of this study is to find out whether direct lymph node invasion has the same prognostic significance as regional nodal metastases.

Methods Retrospective review of patients resected between 1/1/1993 and 7/31/2008. "Direct" was defined as tumor extension into adjacent nodes, and "regional" was defined as metastases to peripancreatic nodes.

Results Overall, 517 patients underwent pancreatic resection for adenocarcinoma, of whom 89 had one positive node (direct 26, regional 63), and 79 had two positive nodes (direct 6, regional 68, both 5). Overall, survival of node-negative patients was improved compared to patients with positive nodes (N0 30.8 months vs. N1 16.4 months; $p<0.001$). There was no survival difference for patients with direct vs. regional lymph node invasion ($p=0.67$). Patients with one positive node had a better overall survival compared to patients with ≥2 positive nodes (22.3 and 15 months, respectively; $p<0.001$). The lymph node ratio (+LN/total LN) was prognostically significant after Cox regression ($p<0.001$).

Conclusions Isolated direct invasion occurs in 20% of patients with one to two positive nodes. Node involvement by metastasis or by direct invasion are equally significant predictors of reduced survival. Both the number of positive nodes and the lymph node ratio are significant prognostic factors.

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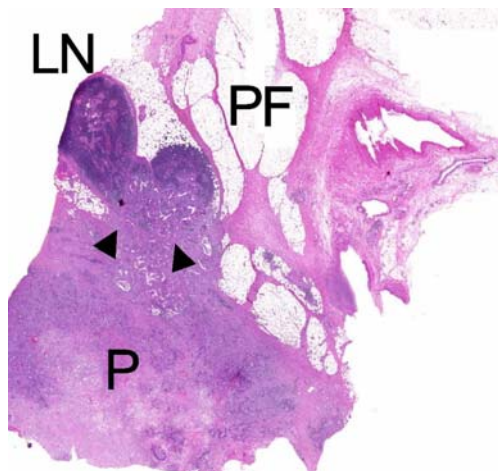e-mail: cferrone@partners.org

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the USA. The American Cancer Society estimates that 42,470 people will be diagnosed with pancreatic cancer and 35,240 will die of the disease in 2009.[1] Approximately 10–20% of patients diagnosed with pancreatic ductal adenocarcinoma harbor resectable tumors. However, overall survival continues to be poor even for resected patients, with a median survival of 17 to 18 months and a 5-year survival of 12–18%.[2–6]

Many studies have documented the prognostic significance of positive lymph nodes. Patients with lymph node metastases have a significantly lower 5-year survival rate than patients with node negative disease.[3–5,7] The number of positive lymph nodes also appears to influence patient survival with two or more positive nodes associated with a worse outcome.[6,8] Prospective randomized trials have evaluated the role of extended lymph node dissections. Despite the increased number of lymph nodes resected, there was no survival benefit but an increased morbidity.[9–11]

There are no studies addressing the significance of direct lymph node invasion by pancreatic adenocarcinoma (Fig. 1). Our first aim was to determine the frequency and prognostic impact of direct lymph node invasion. Our second aim was to determine the impact of the lymph node ratio (LNR, ratio of positive nodes to total nodes) on overall survival.

Materials and Methods

Study Design Review of a retrospectively created database (1/1/1993–1/1/2001) and a prospectively main-tained database (1/1/2001–7/31/2008) was performed to identify patients who underwent surgical resection of pancreatic ductal adenocarcinoma. Patients with pancreatic adenocarcinoma arising within intraductal papillary mucinous neoplasms were excluded. Clinical data evaluated included gender, age, race, family history, presenting symptoms (presence of abdominal pain and jaundice), operative procedures, neoadjuvant and adjuvant therapy, and disease-specific survival. Pathological data evaluated included TNM stage, size of the tumor, histological type, degree of differentiation, perineural, lymphatic, perivascular invasion, and surgical margin status. Tumors were graded as poorly, moderately, or well differentiated. Operative mortality was defined as death within 30 days of the operation. Overall survival was measured from the date of surgery until the time of death or last follow-up. Patients were staged according to the AJCC 6th edition.

Patients with positive lymph nodes were divided into two groups, direct and regional. Direct invasion of a node by tumor was defined by the presence of a continuous column of tumor cells extending from the intra- or extrapancreatic portion of the primary lesion to the involved lymph node. Regional nodal metastasis lacked this continuity between the primary pancreatic lesion and the lymph node. For lymph nodes directly invaded by the tumor, the available pathology slides were reviewed by a single GI pathologist (V.D.). For node-positive patients, the ratio of the number of positive nodes to the total number of nodes resected was calculated (LNR). The period from 1/1/1993 to 7/31/2008 was divided into three time periods (A: 1993–1997, B: 1998–2002, and C: 2003–2008) in order to assess changes in the number of assessed lymph nodes.

This study was approved by the Institutional Review Board of the Massachusetts General Hospital.

Statistical Analysis Statistical analysis of the data was done utilizing SPSS 11.0 for windows (Statistical Package for the Social Sciences, Inc., Chicago, IL). Continuous variables including age, tumor size, and LNR were dichotomized at their median values for the purpose of statistical analysis. Comparisons for continuous variables with normal distributions were conducted with the *t* test and for continuous variables without normal distributions by the Mann–Whitney test or Kruskal–Wallis test. Categorical variables were analyzed using the chi-square test. Survival curves were constructed with the Kaplan–Meier method. Univariate comparisons were performed with the log-rank method. Cox proportional hazards model was used for those factors found to be significant in the univariate analysis. Level of statistical significance was set at $p=0.05$.

Figure 1 In direct invasion (*arrowheads*) the tumor is directly invading lymph nodes situated in the peripancreatic fat (*P* pancreas, *PF* peripancreatic fat, *LN* lymph node).

Results

Clinicopathologic Characteristics

A total of 517 patients underwent resection for a pancreatic ductal adenocarcinoma, of whom 52.8% were females. The clinicopathologic characteristics of the patients and the operations performed are listed in Table 1. The median age of the cohort was 67 years old. Pancreaticoduodenectomy was the most frequent operation performed (84.3%). The majority of patients (67.5%) had Stage IIB disease with a median tumor size of 3 cm (range 0.3–12.5 cm).

The postoperative mortality was 0.8%. Median and mean follow-up were 16 and 24.9 months, respectively (range 0–166 months). The median survival for the entire cohort (n=517) was 19.7 months, and the 5-year actuarial survival was 17.3%. Patients with node-positive disease (n=349) had a statistically significant decrease in median and 5-year survival compared to patients with node-negative disease (n=168) (16.4 versus 30.8 months and 5-year actuarial survival of 9% versus 31%, respectively; $p<0.001$).

Patterns of Lymph Node Involvement: Direct Invasion versus Regional

The clinicopathologic characteristics of patients with direct lymph node invasion and positive regional lymph nodes were similar (Table 1). A single positive lymph node was identified in 89 patients (17.2%). Direct node invasion was present in 26 patients (29.2%), and a positive regional node was present in 63 patients (70.8%). Two positive lymph nodes were identified in 79 patients (15.3%). Direct invasion of both nodes occurred in six patients (7.6%), two positive regional nodes were identified in 68 patients (86%), and five patients had both direct and regional nodes (6.3%). No patients with three or more positive lymph nodes had all their nodes directly invaded by the tumor. Therefore, we limited our analysis to patients with one or two positive nodes. Patients who had both direct and regional lymph node involvement were also excluded from further analysis. The location of the tumor (head versus body/tail) did not differ significantly between patients with one or two directly invaded nodes (p=0.43). Overall survival for patients with one or two directly invaded nodes was not

Table 1 Clinicopathologic Characteristics of 517 Patients with Resected Pancreatic Adenocarcinoma

	All Patients	Direct	Regional	P value
Number of patients (%)	517	32	131	
Age median (range)	67 (33–90)	69.5 (47–82)	68 (43–90)	0.85
Female gender	273 (52.8)	21 (65.6)	66 (50.3)	0.12
Abdominal pain	225 (43.5)	13 (40.6)	54 (41.2)	0.94
Jaundice	345 (66.7)	21 (65.6)	83 (63.3)	0.64
Operations				
Pancreaticoduodenectomy	436 (84.3)	25 (78.1)	109 (83.2)	0.5
Distal pancreatectomy	73 (14.1)	7 (21.9)	22 (16.8)	
Total pancreatectomy	8 (1.5)	0	0	
Surgical margins R0	360 (69.6)	24 (75)	97 (74)	0.91
Median tumor size, cm (range)	3 (0.3–12.5)	2.6 (1–7)	3 (0–7)	0.43
T stage				
T1	19 (3.7)	0	1 (0.8)	0.23
T2	40 (7.7)	0	10 (7.6)	
T3	458 (88.6)	32 (100)	120 (91.6)	
Grade				
Well	18 (3.5)	1 (3.1)	6 (4.6)	0.75
Mod	282 (54.5)	19 (59.4)	67 (51.1)	
Poor	205 (39.7)	12 (37.5)	55 (42)	
Other (not assessed, undifferentiated, mixed types)	12 (2.3)	0	3 (2.3)	
Perineural invasion	407 (78.7)	30 (93.7)	104 (79.4)	0.05
Lymphatic invasion	220 (42.6)	17 (53.1)	55 (42)	0.25
Vascular invasion	222 (42.9)	13 (40.6)	52 (39.7)	0.92

Patients with one or two positive nodes are divided into direct and regional (p values reflect the comparison between direct and regional LN groups with one or two positive nodes)

significantly different from patients with one or two positive regional nodes ($p=0.67$; Fig. 2).

Number of Lymph Nodes and Survival

The median number of pathologically examined lymph nodes for all patients was 13 (range 1–49). The number of nodes evaluated increased over time (Table 2).

Node-negative patients had a median survival of 30.8 months, a 5-year survival of 31% and a median number of 10 lymph nodes assessed. In node-negative patients, overall survival did not differ between those who had ≥10 nodes evaluated ($n=85$) and patients with <10 nodes evaluated ($n=83$; $p=0.69$). However, patients who were node-negative with <10 nodes had a survival approaching that of patients with one positive node ($p=0.11$).

Node-positive patients had a median survival of 16.4 months and a 5-year survival of 9%. For patients with node-positive disease, the median number of assessed lymph nodes was 15. The overall median survival for these patients was 16.4 months whether or not they had <15 nodes assessed ($p=0.5$, Fig. 3).

The median number of positive lymph nodes was 3. Patients with a single positive node had a significantly better survival than patients with two or more positive nodes (22.3 months for one positive node vs. 16 months for two positive nodes vs. 15 months for >2 positive nodes; log rank, $p<0.001$, Fig. 4). The median lymph node ratio was 0.2. The survival of patients with a LNR of ≥0.2 ($n=181$) was significantly worse than patients with a LNR<

Table 2 Factors Influencing the Number of Assessed Lymph Nodes

(Parameter)	Median Number of LNs	P value
Time period		
1993–1997 (105)	9	<0.001
1998–2002 (161)	13	
2003–2008 (251)	15	
Operation		
Whipple (436)	14	0.1
Distal pancreatectomy (73)	12	
Total pancreatectomy (8)	19	
Tumor stage		
Intrapancreatic (T1,T2) (59)	13	0.26
Extrapancreatic (T3) (458)	14	
Nodal status		
N0 (168)	10	<0.001
N1 (349)	15	

0.2 ($n=168$; 14 vs. 22 months, respectively, $p<0.001$; Fig. 5).

Multivariate Survival Analysis

For the entire cohort of 517 patients, the median survival was 19.7 months, and the 5-year actuarial survival was 17.3%. On univariate analysis, predictors of survival were: size and differentiation of the tumor, presence of lymphatic and perivascular invasion, negative surgical margins (R0), and LNR.
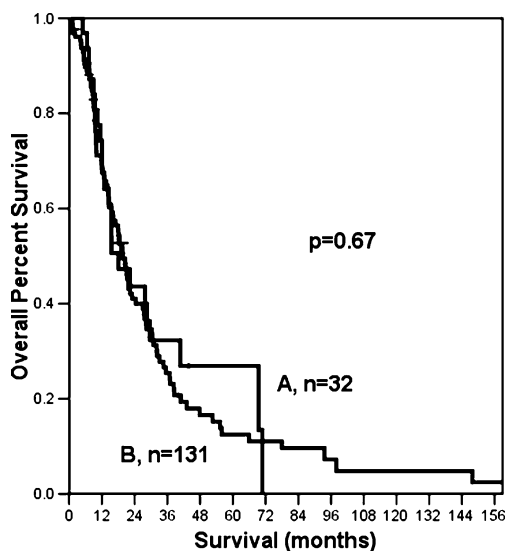
Figure 2 Patients with one or two positive nodes have similar survival whether the node is directly invaded by the tumor (*A*), or is a regional node (*B*).
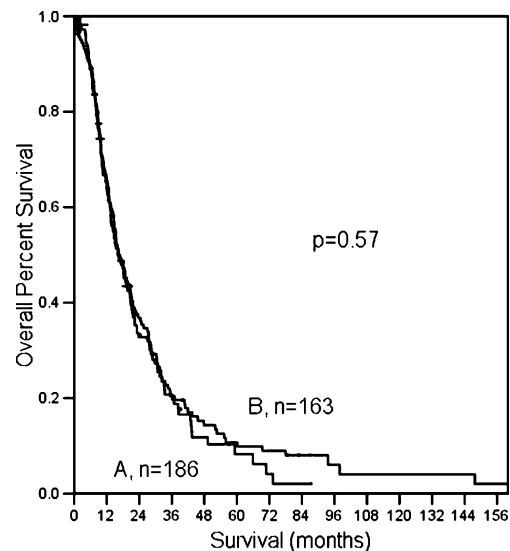
Figure 3 Median number of nodes evaluated in patients with positive nodes does not affect survival. *A* Total number of resected nodes ≥15; *B* N1 patients with resected nodes <15.
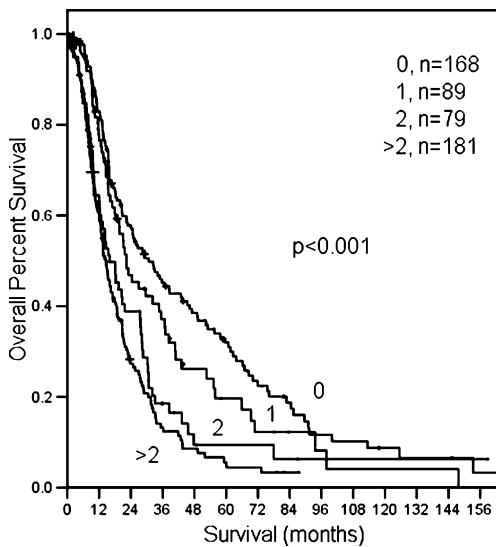
Figure 4 Patients with negative nodes have a better prognosis compared to patients with one, two, or more than two positive nodes.

Patients harboring well-differentiated tumors less than 3 cm in size, with no evidence of perivascular or lymphatic invasion and a LNR less than 0.2 that were resected with microscopically negative surgical margins had the most favorable outcome. After Cox proportional hazards multivariate analysis, LNR remained the most significant prognostic factor for survival (Table 3).

Discussion

Lymph node involvement by cancer is consistently a significant prognostic factor for overall survival in
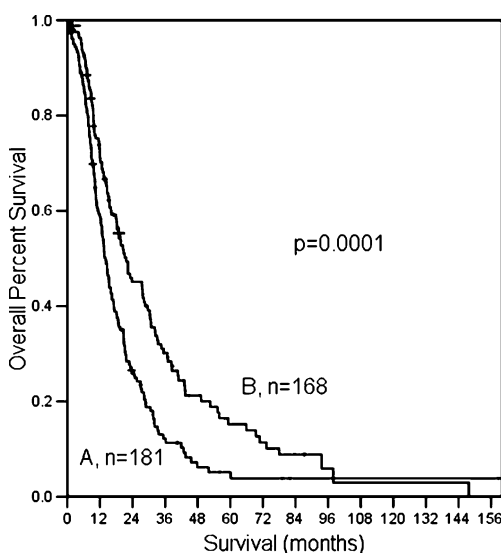
Figure 5 The influence of LNR on overall survival; *A* LNR≥0.2; *B* LNR<0.2.

Table 3 Univariate and Multivariate Analysis of Prognostic Factors

Factor	Univariate P value	Multivariate P value
Size≥3 cm	<0.0001	0.003
Differentiation	0.044	0.003
LNR≥0.2	<0.0001	<0.0001
Perivascular Invasion	0.0002	0.06
Lymphatic Invasion	0.0047	0.19
R0	<0.0001	0.005

patients with resected pancreatic adenocarcinoma.[3–7] In this report, node-negative patients experienced a 5-year actuarial survival of 31%, whereas node-positive patients had a 5-year actuarial survival of 9%. While many studies outline the importance of lymph node involvement, no prior studies address the impact of direct tumor extension into lymph nodes.

Direct lymph node invasion was documented in 29.2% of patients with a single positive node and in 7.6% of patients with two positive nodes. The probability of identifying direct lymph node invasion by the tumor was similar for resected cancers located in the head, body, and tail of the pancreas. Patients with positive regional nodes can harbor earlier stage tumors (T1/T2) than patients with extrapancreatic extension of the tumor into lymph nodes (T3). However, there was no survival difference between patients with positive direct or regional nodes.

Increased awareness of the prognostic significance of lymph node positivity has led to improved lymph node retrieval. In our cohort, the median number of examined nodes increased progressively from 1993 to 2008. A significantly larger number of nodes were retrieved in node-positive patients, which has been described in other surgical series as well.[6,12]

In our study, evaluating more lymph nodes than the median number of nodes was not associated with improved survival in either the N0 or N1 groups. Node-negative patients had a survival of 30.8 months, similar to the 25.3 months in the series by Pawlik et al.[13] and 27 months in the series by House et al.[6] The survival benefit of node-negative disease seems to be lost when the patient is characterized as node-negative based on a small number of assessed lymph nodes. House et al.[6] reported that patients characterized as N0 based on less than 12 nodes had a similar survival to patients with a single positive node and more than 12 nodes assessed. We similarly found that patients characterized as node-negative based on less than 10 nodes assessed had a similar survival to patients with one positive node. The effect of the total number of assessed lymph nodes on survival has been examined in multiple studies using the SEER database.[12,14,15] These

studies suggest that patients should have at least 15 nodes assessed to be adequately staged which emphasizes the need for both careful surgical dissection and pathologic assessment.

The median survival of N1 patients was 16.4 months, similar to the 16 months reported by House et al.[6] and 16.5 by Pawlik et al.[13] A single positive lymph node was identified in 25.6% of patients, similar to the 28% reported by House et al.[6] Tomlinson et al.[14] identified a single positive node in 60% of the patients in the SEER database. However, the median number of assessed lymph nodes in the SEER database was only 7, a factor which could contribute to the high number of patients with a single positive node among the N1 group. In our series, patients with one positive node had a better survival when compared to patients with two positive nodes. However, the presence of more than two positive nodes was not associated with a further decrease in survival.

Recent series have emphasized the importance of the ratio of positive to total lymph nodes (LNR) as a prognostic tool in many GI cancers, including the esophagus,[16] stomach,[17,18] colon,[19] and pancreatic adenocarcinoma.[13,20,21] In our study, the median lymph node ratio was 0.2, and patients with a LNR higher than 0.2 had a significantly worse prognosis. The LNR remained highly significant on multivariate analysis. The cutoff values associated with the greatest differences in survival were 0.15 and 0.16, similar to the 0.18 value reported by House et al.[6]

Potential weaknesses of this study are related to its retrospective nature. Although the pathologic description of the gross specimen at our institution includes the location of the positive lymph nodes, it is possible that the rate of direct invasion is underreported. Prospectively performed studies in pancreatic ductal adenocarcinoma are needed to address the true rate and prognostic significance of direct lymph node invasion.

Conclusion

Isolated direct lymph node invasion by pancreatic ductal adenocarcinoma occurs in at least 20% of patients with one or two positive lymph nodes. The number of positive lymph nodes, not the mechanism of lymph node involvement, is a significant predictor of overall survival. Patients with a single positive lymph node have an improved survival compared to patients with two or more positive nodes. The LNR remains a powerful prognostic tool after adjusting for other prognostic factors.

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ORIGINAL ARTICLE

Esophagogastric Junction Distensibility After Fundoplication Assessed with a Novel Functional Luminal Imaging Probe

Monika A. Kwiatek · Peter J. Kahrilas · Nathaniel J. Soper · William J. Bulsiewicz · Barry P. McMahon · Hans Gregersen · John E. Pandolfino

Abstract

Objective The aim of the study was to compare the esophagogastric junction (EGJ) compliance in response to controlled distension in fundoplication (FP) patients and controls using the functional luminal imaging probe (FLIP).

Background FP aims to replicate normal EGJ distensibility. FLIP is a new technology that uses impedance planimetry to measure intraluminal cross-sectional area (CSA) during controlled distension.

Methods Ten controls and ten FP patients were studied with high-resolution esophageal pressure topography (HREPT) and then the FLIP placed across the EGJ. Deglutitive and interdeglutitive EGJ distensibility was assessed with volume-controlled distension. The FLIP measured eight CSAs spaced 4 mm apart within a cylindrical saline-filled bag along with the corresponding intrabag pressure.

Results The EGJ formed an hourglass shape during distensions with the central constriction at the diaphragmatic hiatus. The distensibility of the hiatus was significantly greater during deglutitive relaxation in both subject groups, but FP patients exhibited reduced EGJ distensibility and compliance compared to controls. During the interglutitive period, the corresponding increase in intrabag pressures at larger volumes were also greater in FP patients implying a longer segment of EGJ constriction. The EGJ distensibility characteristics did not correlate with HREPT measures.

Conclusions FLIP technology was used to compare EGJ distensibility in FP patients and control subjects. The least distensible locus within the EGJ was always at the hiatus. EGJ distensibility was significantly reduced, and the length of constriction increased in FP patients. Future FLIP studies will compare patients with and without post-FP dysphagia and gas bloat, symptoms suggestive of an overly restrictive FP.

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Patients with gastroesophageal reflux disease have an abnormally compliant esophagogastric junction (EGJ)[1–4]

that inadequately impedes reflux of gastric contents and thus contributes to a greater likelihood of esophageal mucosa injury and reflux-related symptoms. Increased EGJ compliance is likely multifactorial with potential contributing defects of lower esophageal sphincter pressure, extrinsic compression by the crural diaphragm, and misalignment between the two manifest as a sliding hiatal hernia.[3,5,6] Potential deleterious mechanical consequences of increased EGJ compliance include increased volumes of liquid reflux,[7] a reduced threshold for eliciting transient LES relaxations,[8] and allowing gastric juice to track within the closed sphincter.[9–11] Surgical antireflux procedures aim to correct the defective EGJ by fashioning a mechanical antireflux barrier that allows adequate EGJ opening for passage of swallowed ingesta into the stomach as well as gastric venting when required.[12,13] Ideally, a normal healthy EGJ would be replicated.

Postoperative integrity of the EGJ junction is usually assessed by manometry. Such functional assessments are often provoked by persistent or recurrent gastroesophageal reflux symptoms suggestive of a defective fundoplication (FP) or because of postoperative dysphagia. Although manometric technology has evolved recently to high-resolution esophageal pressure topography (HREPT),[14–16] it still fundamentally measures intraluminal pressure. However, the surgical modification of the EGJ during fundoplication may not be best gauged by measurement of intraluminal pressure. Fundoplication entails tightening of the diaphragmatic hiatus and construction of a loose floppy fundic wrap around the distal esophagus, neither of which necessarily affects the intraluminal pressures. Alternatively, FP integrity may be better assessed when challenged with intraluminal distension.[17]

Measurement of intraluminal distensibility at the EGJ is complex. The distending pressure must be localized within the EGJ and dimensional measurements restricted to the area of interest. Although this can be achieved with a barostat (or hydrostat), this is somewhat cumbersome and requires concurrent fluoroscopic imaging.[2–4] Nonetheless, barostat assessment of compliance at the narrowest locus within the EGJ after FP suggested it to be similar in asymptomatic FP patients compared to control subjects.[2] A potentially more robust method for measuring EGJ distensibility, capable of making measurements at multiple adjacent segments without need for fluoroscopy, is by adaptation of the principle of impedance planimetry[18,19] into a functional luminal imaging probe (FLIP). FLIP recordings allow dynamic imaging of EGJ distention as a three-dimensional structure based on instantaneous measurement of multiple intraluminal cross-sectional areas with concurrent pressure measurements, thereby facilitating measurement of EGJ distensibility.[20,21] Hence, the aim of the current study was to compare the EGJ distensibility in FP patients during the interdeglutitive period and during deglutitive EGJ relaxation to that of asymptomatic control subjects using the FLIP.

Materials and Methods

Subjects

Ten asymptomatic control subjects (2M, 23–50 years) and ten patients who have had laparoscopic Nissen FP surgery (2M, 42–68 years) were studied. The control subjects were recruited from a pool of volunteers who had neither gastrointestinal symptoms, any prior gastrointestinal surgery, nor were taking medications known to affect gastrointestinal function. FP patients were recruited successively from referrals to the Gastroenterology Outpatient Clinic and Gastrointestinal Diagnostic Laboratory for follow-up assessment of mild to moderate postoperative symptoms. All subjects gave written informed consent. The study protocol was approved by the Northwestern University Institutional Review Board.

Functional Luminal Imaging Probe

Esophagogastric junction distensibility was measured using a custom-made FLIP designed to measure intraluminal cross-sectional areas (CSAs) as a function of distention pressure as previously described.[21] In brief, the probe assembly was 80 cm long, with the proximal 68 cm constructed from a 4.5-mm outer diameter nine-lumen polyurethane tube and the distal 12 cm constructed of a 1.6-mm outer diameter double-lumen polyethylene tube (Fig. 1; GMC Medical,

Figure 1 Distal end of FLIP showing the electrode and pressure measurement loci within the bag (modified from McMahon et al.[20]).
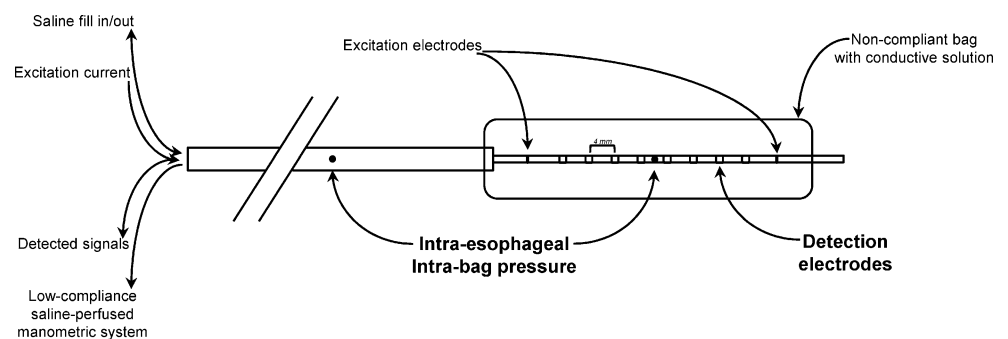
Hornslet, Denmark). A noncompliant 35-μm-thick polyest-herurethane bag was mounted on the distal end. Within the bag was a 3.2-cm segment comprised of nine-ring electrodes spaced 4 mm apart for impedance planimetry measurement. Excitation electrodes at either end emitted a constant low current of 100 μA at a frequency of 5 kHz making the voltage measured across each of the eight adjacent pairs of ring electrodes proportional to the impedance between them. As the bag was filled with 0.2% saline, the impedance across each segment was thus inversely proportional to the CSA of the bag at that locus. Maximal bag diameter was 3.2 cm. The probe also contained two low compliance saline perfused channels (1 mm ID), connected to external pressure trans-ducers (Edwards TruWave, Edwards Lifesciences, Irvine, CA, USA), providing pressure measurements within and 2.5 cm proximal to the bag.

Measurements from the eight electrode pairs and pressure transducers were sampled at 10 Hz with the data acquisition system, transmitted serially to a personal computer, and displayed in real-time using custom-made software programmed in Labview® version 6.1 (National Instruments, Austin, TX, USA). The probe was calibrated at body temperature prior to each study by filling the bag with 0.2% saline within a calibration block containing a set of cylindrical cutouts with CSAs ranging from 50 to 616 mm^2. The pressure transducers were calibrated at 0 and 75 mmHg.

High-Resolution Manometry

HREPT data were obtained using a solid-state manometric assembly (4.2 mm outer diameter) with 36 circumferential sensors spaced at 1-cm intervals (Sierra Scientific Instruments, Los Angeles, CA, USA), the recording character-istics of which have been previously described.[22,23] Pressure transducers were calibrated at 0 and 100 mmHg using externally applied pressure prior to the study.

Experimental Protocol

Studies were performed in a supine position after at least a 6-h fast. Patients underwent transnasal placement of the manometry assembly, which was positioned to record from the hypopharynx to the stomach with about five intragastric sensors. The assembly was fixed in place by taping it to the nose. The manometric protocol included at least a 30-s period of baseline recording in a supine position followed by a series of ten 5-ml and two 10-ml test water swallows. Once the manometric assembly was removed, the FLIP was placed transnasally into the stomach and withdrawn until the bag was centered at the EGJ based on HREPT measurements.[23,24] Bag position was also confirmed fluo-roscopically by partially filling the FLIP bag (20–30 ml) and observing transit of swallowed barium into the stomach

(Fig. 2). The probe was then fixed in place by taping it to the nose. Interdeglutitive (30 s) and deglutitive (dry swallow) FLIP measures of CSA and distention pressure were made with the bag filled to 30, 40, 50, and 60 ml. Each volume was tested in triplicate and repeated if the subject inadvertently swallowed. Swallows were evident by a peristaltic contraction at the perfused channel 2.5 cm proximal to the bag. EGJ geometry was monitored in real time to assure proper bag placement, and instances of suspected migration were confirmed fluoroscopically before repositioning and repeating the measurement.[20]

Data Analysis

High-Resolution Manometry

The HREPT plots were analyzed to characterize EGJ morphology and deglutitive function in terms of end-expiratory EGJ pressure, inspiratory augmentation of EGJ pressure, length of the EGJ high-pressure zone (HPZ), abdominal length of the EGJ HPZ, and integrat-ed relaxation pressure (IRP) during deglutitive relaxation as previously described.[22–27] Distal esophageal peristalsis was considered normal when the peristaltic amplitude and velocity were ≥30 mmHg and <10 cm/s. Failed or hypotensive peristalsis with 50–60% of test swallows constituted intermittent hypotensive peristalsis, 70–90% frequent hypotensive peristalsis, and 100% absent peri-stalsis. Distal esophageal contractile vigor was measured by the distal contractile integral (DCI). Peristalsis-related intrabolus pressure (IBP) was measured 1 cm proximal to the EGJ and summarized as an average pressure during the 3 s of maximal IBP during esophageal emptying (IBP$_{esoph}$).[28]
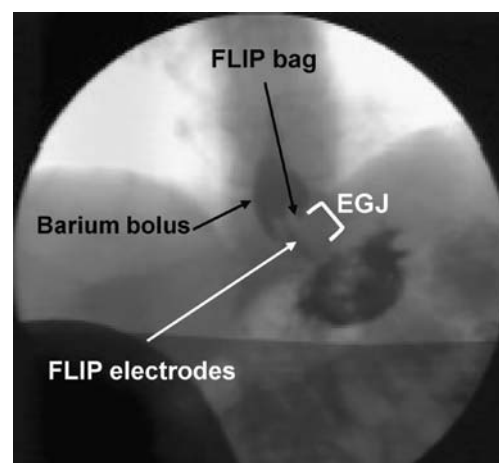
Figure 2 A fluoroscopic image with a distended FLIP bag in situ straddling the EGJ following a 5-ml barium swallow.

Functional Luminal Imaging Probe

Interdeglutitive EGJ CSAs and intrabag pressure were assessed at each FLIP bag volume by quantifying the 50th percentile of each measure during each test 30-s recording. The corresponding deglutitive EGJ measures were assessed during the period between a dry swallow and the distal esophageal peristaltic or postdeglutitive EGJ contraction. The deglutitive EGJ response was quantified by the 1-s nadir in the intrabag pressure and the corresponding CSAs. Measurements of CSA were made at each of the eight electrode pairs covering a span of 3.2 cm.

EGJ compliance (volume vs. pressure) was calculated based on the intrabag pressure and an approximation of EGJ volume across the range of FLIP bag volumes associated with measureable distention. EGJ volume was estimated by identifying the narrowest CSA (invariably at the diaphragmatic hiatus), extending distally for three additional CSAs and applying the formula $(CSA_x + CSA_{x+4\,mm} + CSA_{x+8\,mm} + CSA_{x+12\,mm}) \times 0.004$ to convert the 4-mm segment CSAs (square millimeters) to milliliters. A linear regression analysis was then applied with the slope of the line representing EGJ compliance (milliliters per millimeters of mercury).

Statistical Analysis

The data from triplicate trials were averaged to describe the EGJ response at each FLIP bag volume for each subject. Data from all the subjects was then expressed as median (5th–95th percentile). Statistical comparisons were performed using Wilcoxon matched pairs test and Kruskal–Wallis test. The relationships between measures provided by the FLIP and HREPT were assessed with Spearman's rank correlation coefficient (r_s). A p value<0.05 was considered significant.

Results

Demographic and HREPT Data

The FP patients were assessed with FLIP at 4 months to 7 years postoperative with eight of the ten having had their surgery at Northwestern Memorial Hospital (NMH). Those eight operative reports uniformly described a laparoscopic "short floppy" Nissen fundoplication, 2.0–2.5 cm in length, constructed with a 51–60-Fr Maloney dilator placed within the esophagus and mobilization of the fundus by dividing the short gastric vessels. Operative reports were not available for the two patients who had their surgery elsewhere but who were certain that they had complete 360° fundoplication on the basis of preoperative consultation with their surgeons.

The symptoms prior to and following the surgery were recorded in seven of the eight FP patients who had their surgery at NMH. Symptoms of heartburn and regurgitation were consistently present in six of the seven patients prior to the surgery. The three other patients reported having severe heartburn and regurgitation before the surgery. At the time of the study, four of the ten patients reported mild dysphagia, three of the ten patients reported bloating, three of the ten patients reported chest pain, one of the ten patients reported nausea, one of the ten patients reported abdominal pain, two of the ten patients reported heartburn, and one of the ten patients reported heartburn and regurgitation. None of these problems were sufficiently severe for any of these patients to undergo revision surgery.

HREPT data on EGJ parameters showing similar contractile function between control subjects and FP patients are summarized in Table 1. One significant difference between groups was that the length of the EGJ HPZ, both total and intra-abdominal, was slightly shorter in FP patients (p<0.02). None of the subjects had a HREPT signature of hiatal hernia defined as a separation greater than 2 cm between the components of the EGJ HPZ (LES and crural diaphragm).[24] The barium swallow used to confirm the position of the FLIP bag across the EGJ, verified the absence of hiatal hernia. With respect to peristaltic function, one of the normal controls had frequent hypotensive peristalsis while the remainder were normal. Among the FP patients, two had frequent and one had intermittent hypotensive peristalsis. However, the distal esophageal contractile vigor, summarized as the DCI of the normal and hypotensive peristaltic contractions, was comparable between groups (control, 2,640 (1,297–3,429); FP, 2,193 (418–6,578) mmHg s cm, p=0.55). An abnormally high deglutitive IRP (>15 mmHg) was detected in one control and two FP patients.

Table 1 Esophagogastric Junction Pressure Morphology and Deglutitive Function Reported as Median (5th–95th Percentile)

	Controls	Fundoplication
Expiratory EGJ pressure (mmHg)	18 (10–36)	15 (6–22)
Inspiratory EGJ augmentation (mmHg)	17 (8–37)	13 (7–26)
Length of EGJ HPZ (mm)	45 (42–57)	37 (29–45)*
Abdominal length of EGJ HPZ (mm)	28 (24–44)	23 (19–30)*
Deglutitive IRP (mmHg)	12 (5–17)	13 (6–17)
IBP$_{esoph}$	17 (12–21)	18 (11–24)

Deglutitive IRP was abnormal (>15 mmHg) in one control and two patients. Median (5th–95th percentile)

HPZ high-pressure zone, *IRP* integrated relaxation pressure

*p<0.05 vs. controls

EGJ Distensibility

When straddling the EGJ, the FLIP bag assumed an hourglass shape with the central constriction at the diaphragmatic hiatus in both control subjects and FP patients. The hourglass shape was present during both the interdeglutitive period and deglutitive relaxation at all FLIP bag volumes (Fig. 3). In fact, evident in Table 2, many subjects in both groups had CSA measurements at the hiatus that were the minimum detectable (50 mm^2) implying that all of the saline within the FLIP bag displaced proximal or distal to it. This suggested that the hiatus was uniformly the least distensible locus within the EGJ. Only with the FLIP bag volume of 60 ml was there nearly consistent hiatal distention above the minimum, at which point the hourglass opened and closed with respiration confirming this to be the diaphragmatic hiatus. Of note, the 60-ml bag volume resulted in pronounced hiatal opening in controls during deglutitive relaxation to a CSA significantly greater than that observed in FP patients (Table 2; $p<0.001$).

In the EGJ dynamic described above, distensile pressure within the FLIP bag increased with increasing bag volume in both subject groups and in both test conditions (Table 3; $p<0.0001$). Furthermore, the distending pressure within the FLIP bag was consistently greater in FP patients than in control subjects particularly with FLIP bag volumes of 40, 50, and 60 ml ($p<0.05$; Table 3). Conceptually, pressure within the FLIP bag increased when the increased volume of saline within it could no longer disperse to highly compliant regions proximal or distal to the EGJ presumably because the bag was filled to capacity in those regions. Hence, the observed difference in pressure between the control subjects and FP patients implies that there was a longer zone of measured constriction in the FP patients. This difference was further brought out by the estimated EGJ volume, a measure that utilized the CSA of the hiatus and three distal adjacent FLIP segments. Examining Fig. 4, both during the interdeglutitive period and during deglutitive relaxation, the EGJ of control subjects was widely distended at distensile pressures insufficient to achieve any measureable opening during the same conditions in the FP patients.

The data in Fig. 4 can also be utilized to estimate EGJ compliance, defined as the slope of the EGJ volume vs. intrabag pressure relationship. Since the data points associated with the 40-ml FLIP bag volume did not achieve measureable EGJ distention, this could only be done with the 50- and 60-ml data points. As evident in Fig. 5a, interdeglutitive EGJ compliance was comparable between control subjects and FP patients ($p=0.13$). As expected, deglutition tended to increase EGJ compliance in controls ($p=0.08$); the same change in compliance was not seen in FP patients ($p=0.92$; Fig. 5b).

HREPT vs. FLIP EGJ Measures

The data from controls and FP patients were pooled to test hypotheses on association between FLIP vs. HREPT measures of distal esophageal function. Hypothetically, a less compliant EGJ might exert greater closing pressure and distal resistance for the bolus traversing the esophagus resulting in greater intrabolus pressure. However, there were no significant correlations between FLIP measure of interdeglutitive EGJ compliance and HREPT measures of expiratory EGJ pressure ($r_s=-0.12$, $p=0.63$) or inspiratory EGJ augmentation ($r_s=0.25$, $p=0.29$). Likewise, deglutitive EGJ compliance did not correlate with IBP_{esoph} ($r_s=0.14$, $p=0.56$). However, IBP_{esoph} was related to DCI ($r_s=0.56$, $p=0.01$) suggesting that the contractile vigor of the distal esophagus increased with outflow resistance. An interesting contrast between the two technologies was that while HREPT estimates of sphincter length found the FP patients
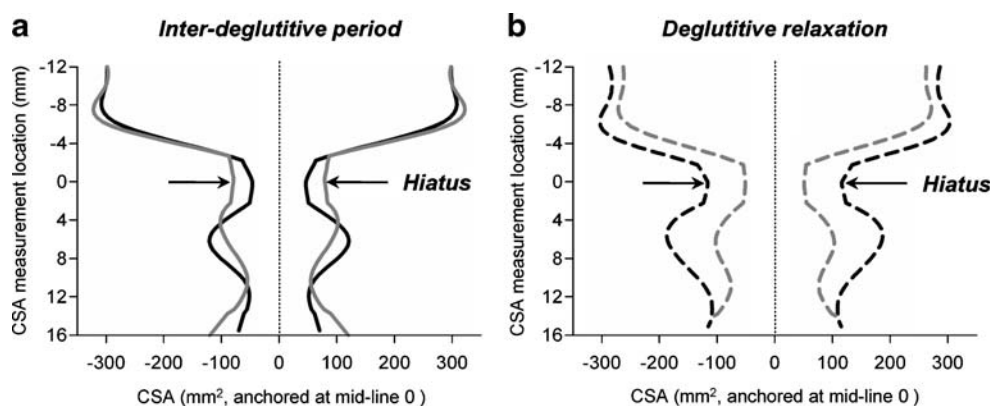
Figure 3 Esophagogastric junction geometry as depicted by the FLIP. The hourglass shape of the EGJ narrowed at the hiatus (y-axis=0 cm) in both control subjects (*black*) and fundoplication patients (*gray*).

The *panels* show the EGJ measurements with a 60-ml FLIP bag volume during the interdeglutitive period (**a**) and deglutitive relaxation (**b**).

Table 2 CSA (Square Millimeters) of the Diaphragmatic Hiatus (Narrowest EGJ CSA Measured by the FLIP) During Volume Distensions Reported as Median (5th–95th Percentile)

FLIP bag volume (ml)	Control subjects		Fundoplication patients	
	Interdeglutitive	Deglutitive	Interdeglutitive	Deglutitive
30	50 (50–68)	52 (50–166)	51 (50–60)	52 (50–70)
40	50 (50–50)	51 (50–55)	51 (50–56)	50 (50–54)
50	50 (50–52)	54 (50–106)	61 (52–88)	60 (50–109)
60	93 (50–182)	233 (55–429)**	159 (68–245)	102 (68–272)*

The minimal detectable CSA was 50 mm^2

*$p<0.001$ vs. controls; **$p<0.05$ vs. interdeglutitive period

Discussion

The EGJ has two distinct dimensions of function—that during nondeglutitive periods to prevent reflux by maintaining closure and that during periods of opening to facilitate trans-EGJ flow, be it esophagogastric or gastroesophageal. Manometry, or more recently HREPT, directly measures closure forces. This investigation tested the ability of the FLIP, a novel device based on impedance planimetry technology, to quantify EGJ opening CSA in response to controlled intraluminal distension variables. Studies were done on control subjects and patients with satisfactory to good functional outcome from laparoscopic Nissen fundoplication. The major findings of the study were that (1) the FLIP isolated the hiatus as the least distensible locus within the EGJ in both subject groups, (2) the distensibility of the hiatus was significantly greater during deglutitive relaxation in both subject groups, (3) fundoplication patients exhibiting reduced EGJ distensibility and reduced EGJ compliance during deglutitive relaxation compared to control subjects, (4) fundoplication patients exhibited a longer segment of

to have a significantly shorter HPZ than control subjects, FLIP measures of EGJ volume, by inference length, found the FP patients to have a significantly longer zone of constriction.

reduced distensibility than did controls, and (5) EGJ attributes demonstrated with FLIP measurements were not mirrored by HREPT findings.

The finding that the least distensible locus within the EGJ is at the hiatus supports similar findings made using barostat[2,4] or hydrostat[3] technology. This was found to be true irrespective of the presence of hiatus hernia or fundoplication. The significance of quantifying this measurement is that this variable dominates the equation for trans-EGJ flow (Flow rate $= dP \times D^4/CVL$) in which dP is the trans-EGJ pressure gradient, D is the opening diameter, C is a constant, V is viscosity, and L is the length of constriction.[29] Although the length of constriction also figures into the equation, note that D, the diameter of maximal constriction, is raised to the fourth power causing it to be the dominant variable. It follows that this variable is a key determinant of both the efficacy of swallow-related esophageal emptying and the volume of refluxate during periods of sphincter relaxation.[2] In postfundoplication patients, distensibility within the hiatus is a direct consequence of the details of operative hiatal repair. Quite possibly, this variable, a generally underappreciated source of technical variability in fundoplication surgery, is a major determinant of postoperative outcome in terms of dysphagia and gas bloat.

The FLIP findings of a less distensible hiatus and a longer length of constriction post-FP relative to control

Table 3 Pressure (Millimeters of Mercury) Within the FLIP Bag During Distension Reported as Median (5th–95th Percentile)

FLIP bag volume (ml)	Control subjects		Fundoplication patients	
	Interdeglutitive	Deglutitive	Interdeglutitive	Deglutitive
30	16 (13–19)	13 (10–16)**	19 (13–21)	13 (8–18)**
40	17 (11–21)	14 (8–18)**	22 (17–27)*	17 (12–20)**
50	20 (13–26)	15 (10–20)**	26 (19–31)*	21 (14–26)*·**
60	23 (15–30)	17 (11–25)**	30 (21–34)*	23 (16–28)*·**

*$p<0.05$ vs. controls; **$p<0.05$ vs. interdeglutitive period
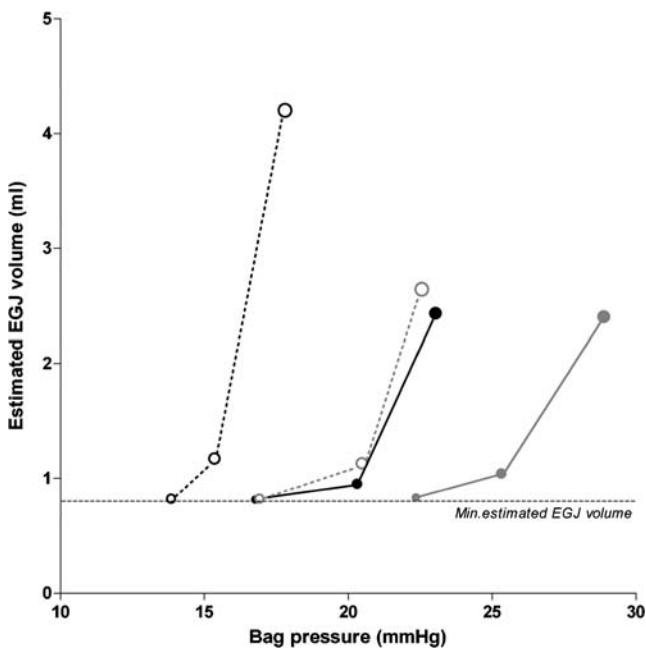
Figure 4 Measured FLIP bag distensile pressure and estimated EGJ volume with the FLIP bag filled to 40 ml (*lower dots*), 50 ml (*middle dots*), and 60 ml (*upper dots*). Both control subjects (*black*) and fundoplication patients (*gray*) exhibited measureable EGJ distention during the interdeglutitive period (*solid lines*) and deglutitive relaxation (*dashed lines*) only with 50- and 60-ml FLIP bag volumes. Both groups exhibited increased EGJ volume during deglutitive relaxation. However, the distensile pressures associated with EGJ distention were consistently 8–10 mmHg greater in the FP patients compared to the control subjects (see also Table 3).

subjects, despite somewhat conflicting conclusions based on HREPT measures (Table 1), highlight the distinction between measuring resistance to physically opening the EGJ lumen (FLIP) and measuring contraction within a closed lumen (HREPT). To assume that these techniques are equivalent, assumes that a decrease in contractile pressure, mainly attributable to LES and crural diaphragm

contraction, parallels luminal opening dimensions in the absence of that contractile activity. In fact, these two properties have no necessary relationship to each other as the latter is instead related to wall properties of the EGJ and the external constraint on the EGJ imposed by the diaphragmatic hiatus and fundoplication, if present. Fundoplication surgery is clearly designed to modify these latter variables and for that reason, the outcome is better measured with a technique such as FLIP. A "short floppy" fundoplication constructed with a larger caliber dilator within the esophageal lumen should have no obvious effect on the contractility of the LES or crural diaphragm but should limit EGJ distensibility. In fact, evident in Fig. 4, this is what was observed. EGJ distensibility during the interdeglutitive period (with both the LES and crural diaphragm contracting) was similar between subject groups but distensibility during deglutitive relaxation was significantly greater in the control subjects. Although beyond the scope of the current work, it would be of great interest to examine the profile of EGJ distensibility in postfundoplication patients with bothersome dysphagia or gas bloat to see if they are quantifiably different.

Although findings from the current study generally corroborate those obtained from a barostat distention study of a similar population of fundoplication patients,[2] there is an important difference. Both studies demonstrated increased length of the constricted segment after fundoplication but only the current FLIP study demonstrated reduced compliance during deglutitive relaxation; the barostat study suggested distensibility similar to that of control subjects.[2] The explanation for this discrepancy is likely methodological. In the barostat study, only a single two-dimensional plane was imaged leaving it vulnerable to error related to asymmetry of the EGJ. FLIP, on the other hand, calculates CSA bases on impedance characteristics irrespective of luminal shape and, thus, is inherently more accurate. FLIP also has the advantage of utilizing data from several
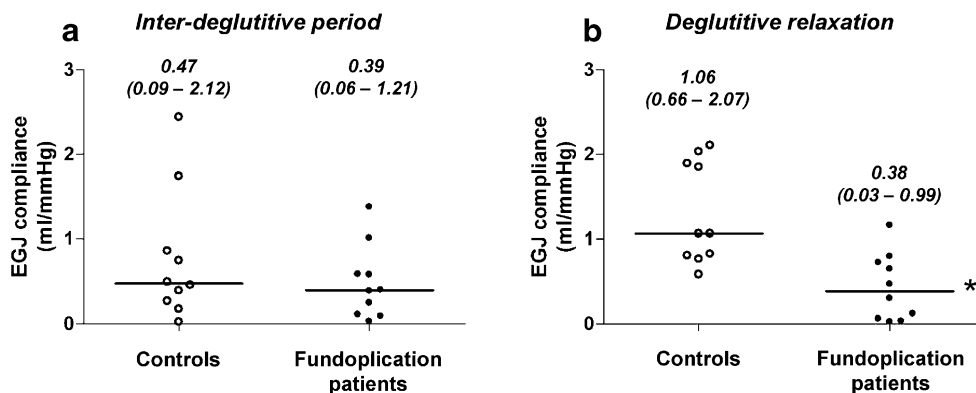
Figure 5 Esophagogastric junction compliance in controls and postfundoplication patients during the interdeglutitive period (**a**) and deglutitive relaxation (**b**). Median (5th–95th percentile); *$p < 0.05$ vs. controls.

adjacent segments within the EGJ, whereas the barostat study analyzed only the single locus of greatest constriction. Together, these advantages, as well as the rapid sampling of the FLIP device, argue that the FLIP is likely the more accurate method for ascertaining intraluminal CSA.

The key data related to EGJ distensibility and compliance gleaned from the FLIP measurements (summarized in Figs. 3 and 4) depend on measurement of the pressure within the FLIP bag rather than the volume within the bag. Although the design of the device does allow for measurement of intrabag pressure, the initial concept of its design was for volumetric distension, which is less relevant when assessing the EGJ. The problem with volumetric distension is that a substantial portion of the measurement length of the FLIP resides outside of the zone of interest (the EGJ and hiatus), instead residing in the far more compliant distal esophagus or the nearly infinitely compliant proximal stomach. Hence, the initial saline volume instilled into the FLIP bag disperses to these more compliant ends before challenging the area of interest. EGJ distension occurs only when the more compliant ends are filled to capacity and intrabag pressure increases with added volume. In the current study, this occurred only with bag volumes of 50 and 60 ml (Fig. 3) making the data obtained with lesser distention volumes irrelevant to the EGJ. Given these considerations, improvements in FLIP design making it more applicable to the EGJ would reduce the overall bag capacity so that lesser volumes are required to achieve EGJ distension, make the pressure sensor more robust by incorporating solid state technology, and, hopefully, introduce an easier method to achieve pressure controlled distension, akin to hydrostat technology.[3]

Conclusion

This experiment evaluated the utility of FLIP technology in a comparison of EGJ distensibility in FP patients and control subjects. The FLIP found the least distensible locus to be at the hiatus in both subject groups. The other major finding was that EGJ distensibility was reduced and the length of constriction increased post-FP. These features were not paralleled by manometric findings emphasizing the difference between assessing contractility in a closed lumen and distensibility (opening dimensions) in the setting of EGJ relaxation. Further study will be needed to ascertain whether or not differences in FLIP measures of EGJ distensibility correlate with significant postoperative symptoms of dysphagia or gas bloat.

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ORIGINAL ARTICLE

Clinicopathological Characteristics of Remnant Gastric Cancer After a Distal Gastrectomy

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Abstract

Introduction The survival rate of patients with remnant gastric cancer (RGC) is unfavorable in comparison to that of cancer in the nonresected stomach. However, when RGC is curatively resected, no significant differences have been reported between both groups in regard to survival. The aim of this study is to analyze the clinicopathological factors influencing a curative resection of RGC.

Methods Thirty-eight consecutive patients with RGC from January 1, 1994 through March 31, 2009 were enrolled in this retrospective study.

Results Their primary diseases were gastric cancers (21; 55.3%) and benign diseases (17; 44.7%). The type of the reconstruction methods of first gastrectomy were Billroth I (28; 73.7%) and Billroth II (10; 26.3%). A total of 31 patients underwent a laparotomy. Twenty patients underwent a curative resection, four patients underwent a palliative resection, and seven underwent a nonresective operation. A total of seven patients underwent an endoscopic resection for early gastric cancer, and all patients received a curative resection. Univariate and multivariate logistic regression analyses were performed to identify the clinicopathological and background factors influencing a curative resection of RGC. A multivariate analysis revealed only an annual follow-up endoscopic examination after the initial gastrectomy to be an independent factor for a curative resection ($p=0.016$; odds ratio, 35.3).

Conclusions An annual follow-up endoscopic examination an after initial gastrectomy may be related to improving the prognosis of patients with RGC.

Introduction

Gastric cancer remains the second leading cause of death worldwide, and it is the most common malignancy in Japan, Asia, South America, and Eastern Europe.[1] A distal gastrec-

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tomy is a very common treatment for patients presenting with low one-third gastric cancer. Remnant gastric cancer (RGC) after a distal gastrectomy is a unique clinical entity with relatively fewer cases. The incidence of RGC has been reported to account for 1–2% of all gastric cancers in Japan.[2] Many reported features of RGC such as the frequency, tumor location, interval following gastrectomy, type of tumor, optimal treatment, and prognosis demonstrated some differences.[3–6] The survival rate of patients with RGC is unfavorable in comparison to that of cancer in the non-resected stomach. In comparison with primary gastric cancers, RGC are commonly detected at advanced stages with extended lymph node metastases or the infiltration of adjacent organs.[3,5,7] In addition, the symptoms of RGC are similar to those of post-gastrectomy syndrome.[5] However, when RGC is resected curatively, there have been reported to be no significant differences between both groups in

survival.[5] Therefore, the timely and accurate diagnosis of early RGC may be important for improving its prognosis.

This study analyzed the clinicopathological characteristics of patients with RGC. Furthermore, the clinicopathological and background factors influencing a curative resection of RGC were examined.

Materials and Methods

Patients

From January 1, 1994, through March 31, 2009, 38 patients were admitted to Wakayama Medical University Hospital with RGC after a distal gastrectomy. In our institute, 1,984 consecutive patients underwent surgery for histologically confirmed gastric cancer during the same period. Of these patients, RGC after a distal gastrectomy was identified in 31 patients (1.6%). RGC was defined as all cancers arising from the remnant stomach after a distal gastrectomy and includes local recurrence in the gastric stump after distal gastrectomy for gastric cancer.[2,8,9] The medical records of 38 patients were obtained from the hospital database, which includes the patients' background, time intervals between the initial operation and diagnosis of RGC, surgical data, tumor characteristics, the follow-up methods, and survival time. Tumors in the remnant stomachs were observed by endoscopy, and cancers were verified in all patients after a histopathological examination. Tumor locations were classified as anastomotic and non-anastomotic. Tumor invasion (T) and lymph node status (N) were classified by International Union against Cancer criteria.[10]

Surgical Treatment and Endoscopic Resection

The general indication for surgical treatment of RGC is the complete resection of the carcinoma combined with a radical lymph node dissection. When the pancreas, esophagus, or liver is directly infiltrated by the tumor, then a gastrectomy is performed with an additional pancreaticoduodenectomy, pancreaticosplenectomy, hepatic resection, or thoracotomy. Lesions with a preoperative endoscopic diagnosis of differentiated type intramucosal cancer without ulcer findings, differentiated type intramucosal cancer no larger than 3 cm in diameter with ulcer findings, and differentiated type minute invasive submucosal (less than 500 μm below muscularis mucosa) cancer no larger than 3 cm in diameter were considered for an endoscopic resection.[11]

Statistical Analysis

The StatView 5.0 software package (Abacus Concepts, Inc, Berkeley, CA) was used for all statistical analyses. The quantitative results are expressed as the mean ± standard deviation. Statistical comparisons between the two groups were performed with the χ^2 test. A value of $P < .05$ was considered to be significant. Univariate and multivariate logistic regression analyses were performed to identify the factors affecting curative resections. The factors with univariate $P < .05$ were included in a multivariate analysis. The factors with multivariate $P < .05$ were defined as independent factors.

Results

Clinicopathological Features of Remnant Gastric Cancer

The detailed characteristics of the 38 patients are listed in Table 1 consisting of 30 males and eight females; mean age was 66±10 years. Their primary diseases were gastric cancers (21; 55.3%) and benign diseases (17; 44.7%). The types of the reconstruction methods for a first gastrectomy were Billroth I (28; 73.7%) and Billroth II (10; 26.3%). The mean interval between the initial operation and the diagnosis of RGC was 15±11 years. In 21 patients (55.3%), symptoms such as obstructions, pain, nausea, and bleeding were present when the RGC was diagnosed. The tumor was located at the anastomotic site in 10 patients (26.3%) and at the non-anastomotic site in 28 (73.7%). The mean tumor size was 37±25 mm. Twenty-five patients (65.8%) had a macroscopically localized tumor, while 13 (34.2%) had an infiltrative type. The histological diagnosis of RGC was made for all 38 patients. The tumor was differentiated (papillary, moderately, or well-differentiated adenocarcinoma) in 23 patients (60.5%), and the tumor was

Table 1 Clinicopathological Features of the 38 Patients with Remnant Gastric Cancer

	$n=38$
Age (years)	66±10
Sex (male/female)	30/8
Initial gastric disease (cancer/benign)	21/17
Reconstruction of first operation (B-I/B-II)	28/10
Interval (years)	15±11
Symptom at diagnosis (yes/no)	21/17
Location (anastomotic/non-anastomotic)	10/28
Macroscopic type (localized/infiltrative)	25/13
Histology (differentiated/undifferentiated)	23/15
Tumor size (mm)	37±25
TNM stage (I/II/III/IV)	19/4/3/12
Curative resection (yes/no)	26/12

B-I/B-II Billroth I reconstructions

undifferentiated (poorly or undifferentiated adenocarcinoma, signet-ring cell carcinoma, or mucinous adenocarcinoma) in 15 patients (39.5%). Nineteen (50.0%), four (10.5%), three (7.9%), and 12 (31.6%) patients had TNM Stages I, II, III, and IV, respectively. A total of 26 patients (68.4%) underwent a curative resection.

Surgical Methods for Remnant Gastric Cancer

A total of 31 patients underwent a laparotomy. Twenty patients received curative resection, four patients underwent palliative resection, and seven underwent nonresective operation (bypass surgery, one patient; diagnostic laparotomy, six patients). Twenty patients received total gastrectomy, one patient received total gastrectomy with additional hepatic resection, and one patient received total gastrectomy with additional pancreaticoduodenectomy (Table 2). A total of seven patients underwent endoscopic resection for early gastric cancer. Five of these seven patients received endoscopic submucosal dissection, and two patients received endoscopic mucosal resection (Table 2). All seven patients underwent a curative resection.[9,11]

Clinicopathological Characteristics of Remnant Gastric Cancer According to Initial Gastric Disease

The clinicopathological characteristics were examined according to initial gastric disease (Table 3). Twenty-one patients underwent the initial gastrectomy for gastric cancer (Gastric cancer group); the remaining 17 for benign disease (Benign disease group). The two groups were similar in age, sex, symptoms at diagnosis, macroscopic type of tumor, histological differentiation of tumor, and the curative resection rate. The mean interval between the

Table 2 Surgical Methods Used for the 38 Patients with Remnant Gastric Cancer

Operation	n=31
Resection (*n*=24)	
Total gastrectomy	22
Total gastrectomy + PD	1
Total gastrectomy + H	1
Nonresection (*n*=7)	
Bypass surgery	1
Diagnostic laparotomy	6
Endoscopic resection (*n*=7)	
ESD	5
EMR	2

PD pancreaticoduodenectomy; *H* hepatic resection; *ESD* endoscopic submucosal dissection; *EMR* endoscopic mucosal resection

initial operation and diagnosis of RGC for the Gastric cancer group was shorter than that for the Benign disease group (9±10 vs. 22±9 years, *p*<.001). The RGC in the Gastric cancer group were located more frequently at non-anastomotic sites than in the benign disease group (*p*<.05). In addition, there was a higher incidence of large tumors in the benign disease group (*p*<.05). The distribution of TNM stage was significantly different between these two groups (*p*<.05).

Clinicopathological Factors Influencing a Curative Resection

Univariate and multivariate analyses were performed to identify the clinicopathological and background factors influencing a curative resection of RGC. Table 4 shows the results of 13 parameters uni- and multivariately examined as potential factors for the 26 patients with curative resections versus the 12 patients without curative resections. Patients who received a follow-up endoscopic examination at least once a year in the interval between the initial gastrectomy and diagnosis of RGC were defined as a follow-up endoscopic examination. Two patient factors (symptom at diagnosis of RGC, follow-up endoscopic examination after the initial gastrectomy) and two tumor factors (macroscopically localized tumor, differentiated type of tumor) differed significantly between these two groups (*p*<.05). A multivariate logistic regression analysis revealed only the follow-up endoscopic examination after the initial gastrectomy to be an independent factor for curative resection (*p*=.016).

Discussion

This study compared the clinicopathological characteristics of RGC according to the initial gastric disease. The examination comparing RGC after malignant and benign disease has great importance in order to understand the long-term effects of different carcinogenic processes. Cancer development after a distal gastrectomy for benign disease is attributed to environmental changes affecting remnant mucosa which were created surgery. The main factor responsible for these changes is duodenogastric reflux of bile and pancreatic juice.[7,12,13] On the other hand, RGC after malignant disease presumably originates from some precancerous conditions which had already existed before the initial operation. These cancers are most likely metachronous lesions.[14] Therefore, it is generally consistent that the RGCs after benign diseases were frequently located at anastomotic sites, and the mean interval between the initial operation and the diagnosis of RGC for the benign disease group was longer than that for the malignant disease

Table 3 Clinicopathological Characteristics in 38 Patients with Remnant Gastric Cancer According to the Initial Gastric Disease

	Gastric cancer (n=21)	Benign disease (n=17)	P value
Age (years)	67±10	64±9	NS
Sex (male/female)	15/6	15/2	NS
Reconstruction of first operation (B-I/B-II)	16/5	12/5	NS
Interval (years)	9±10	22±9	0.0004
Symptom at diagnosis of second lesion (yes/no)	10/11	11/6	NS
Location (anastomotic/non-anastomotic)	2/19	8/9	0.0232
Macroscopic type (localized/infiltrative)	16/5	9/8	NS
Histology (differentiated/undifferentiated)	14/7	9/8	NS
Tumor size (mm)	29±19	46±28	0.0413
TNM stage (I/II/III/IV)	13/0/0/8	6/4/3/4	0.0140
Curative resection (yes/no)	14/7	12/5	NS

NS not significant; *B-I/B-II* Billroth I reconstructions

group. These results were consistent with the results of previous studies.[2–4,6,7]

Long-term exposure of the gastric mucosa to duodenal contents is thought to be one of the major causes of RGC after a distal gastrectomy. Chronic duodenogastric reflux causes different histological changes in the gastric stump such as intestinal metaplasia, dysplasia, and adenoma.[3,13] Most carcinomas derive from dysplasia; thus, a dysplasia–carcinoma sequence can be assumed to be pathogenetic for RGC. In fact, RGC after a distal gastrectomy with Roux en Y reconstructions were not found in this institute. The others also reported the lowest risk with Roux en Y reconstruction.[7,15]

The rate of a curative resection of the RGC was 68.4% in this study. These values were similar to those reported elsewhere, e.g., where the curative resection rate was 65–85%.[2,4,16,17] Multivariate logistic regression analyses were used to identify factors affecting curative resections demonstrated that an annual follow-up endoscopic examination after the initial gastrectomy was the only independent factor. It is generally thought that it is difficult to detect early RGC by endoscopic examination since the inner space is narrow and the surface of the mucosa is often reddish and uneven due to postoperative gastritis after the gastrectomy. In particular, it is difficult to detect depressed or flat-type cancers in the remnant stomach.[5] However, recent progress in endoscopic examinations has made it possible to detect early RGC. Greene described clearly that a surveillance program with periodic endoscopy has contributed to the discovery of early-stage cancers in the remnant stomach.[18] The important prognostic factors in RGC patients are the TNM classification (depth of tumor

Table 4 Univariate and Multivariate Analysis of the Factors Affecting a Curative Resection

Factors	Univariate analysis P value	Multivariate analysis	
		P value	Odds ratio (95% CI)
Age, year (<67 or >67)	0.2762		
Sex (female or male)	0.1290		
Symptom at diagnosis of second lesion (no or yes)	0.0154	0.5349	2.990 (0.094–95.108)
Initial gastric disease (cancer or benign)	0.8692		
Reconstruction of first operation (B-I or B-II)	0.8499		
Interval, year (<15 or >15)	0.7142		
Location (non-anastomotic or anastomotic)	0.8969		
Macroscopic type (localized or infiltrative)	0.0017	0.8799	1.272 (0.056–28.847)
Histology (differentiated or undifferentiated)	0.1118		
Tumor size, mm (<40 or >40)	0.0014	0.2698	9.688 (0.172–547.109)
CEA, ng/dl (<5 or >5)	0.3179		
CA19–9, U/ml (<37 or >37)	0.4312		
Follow-up endoscopic examination (yes or no)	0.0021	0.0160	35.275 (1.941–641.111)

CI confidence interval; *B-I/B-II* Billroth I reconstructions

invasion, nodal state, and distant metastasis) and an R0 resection.[2,4,16,17] Therefore, an annual follow-up endoscopic examination is recommended for all patients with a previous distal gastrectomy in order to increase the early detection of RGC. In addition, annual screening will also reduce the number of overlooked cases. The current institutional policy is to perform annual surveillance endoscopy commencing 1 year after the gastrectomy for at least 10 years. This surveillance program is similar to that reported by Ohashi et al.[2]

A total of seven patients were able to undergo a curative endoscopic resection for early gastric cancer arising from remnant stomach, and all of seven patients received annual follow-up endoscopic examinations after the initial gastrectomy. Endoscopic therapy such as EMR or ESD is applicable for treatment of early-stage RGC.[19,20] These endoscopic therapies are considered to be minimally invasive in comparison to a surgical resection, and they are also expected to result in a high quality of life. These findings emphasize the importance of early detection of RGC following gastric cancer.

In the future, the number of patients with RGC who have previously undergone a gastrectomy for gastric cancer is, therefore, expected to increase because patients with gastric or duodenal ulcer are not treated by a gastrectomy due to the increased use of anti-ulcer drugs. The interval between the initial distal gastrectomy and the diagnosis of RGC for a malignant disease was short because of these precancerous conditions. Therefore, annual surveillance endoscopy should be performed, commencing 1 year after the gastrectomy, and special attention is also required during endoscopic examinations.

In conclusion, the performance of lifelong annual follow-up endoscopic examinations after the initial gastrectomy may, therefore, improve the prognosis of patients with RGC.

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ORIGINAL ARTICLE

Survival Benefit of Non-curative Gastrectomy for Gastric Cancer Patients with Synchronous Distant Metastasis

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Abstract

Background The prognosis for gastric cancer patients with distant metastasis is very poor. The purpose of this study was to evaluate the survival benefit of non-curative gastrectomy for gastric cancer patients with synchronous distant metastasis.

Methods From 1992 to 2002, 253 gastric cancer patients with synchronous distant metastasis underwent surgery at the Department of Surgery, Ruijin Hospital, China. The clinicopathological characteristics and survival were compared between resection and non-resection groups.

Results The 5-year survival rate was 6.5% for patients in resection group and 0% for patients in non-resection group ($P<0.001$). Multivariate analysis showed that liver metastasis, peritoneal dissemination, and non-resection were significantly associated with poor prognosis in gastric cancer patients with distant metastasis. The survival difference between resection and non-resection groups was only observed in patients with single peritoneal dissemination ($P<0.001$), but were not in patients with single liver metastasis ($P=0.428$), distant nodes involvement ($P=0.490$) and multiple metastatic sites ($P=0.192$), respectively.

Conclusions Our results suggests that there were no survival benefit from non-curative gastrectomy for patients with single liver, distant nodes, or multiple sites metastasis. However, only patients with single peritoneal dissemination had survival benefit from non-curative resection. The value of non-curative resection should be evaluated by well-designed clinical trials.

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Introduction

Although the incidence of gastric cancer has decreased in China and worldwide, gastric cancer is still one of the leading causes of death by malignancy.[1–3] Treatment outcome for patients with gastric cancer has been improved because the proportion of patients with early gastric cancer has increased and gastrectomy with D2 lymphadenectomy is recommended as a standard surgery.[4,5] However, there is a great number of patients diagnosed at late stage with synchronous distant metastasis, and the prognosis for those patients is still very poor, even after surgical treatment and anticancer therapy. When dealing with such patients, the therapeutic strategy is in controversy. Several studies reported that non-curative resection might provide some survival advantage. However, other studies showed there was no role for non-curative gastrectomy but increasing postoperative morbidity and prolonging hospital stay in patients with distant metastasis.[6–10]

Table 1 Clinicopathological Characteristics Comparison of Gastric Cancer Patients with Distant Metastasis Between the Resection and Non-resection Groups

Variables	Resection (n=137, %)	Non-resection (n=116, %)	P-value
Age (mean, years)	57.6±13.6	60.7±13.0	0.07
Gender			0.573
Male	76(55.5)	70(60.3)	
Female	61(44.5)	46(39.7)	
Size(mean, cm)	6.7±2.9	9.5±3.7	<0.001
Tumor location			<0.001
Lower	82(59.9)	42(36.2)	
Middle	38(27.7)	31(26.7)	
Upper	9(6.6)	25(21.6)	
Whole	8(5.8)	18(15.5)	
Macroscopic type			0.103
Borrmann 1	6(4.4)	2(1.7)	
Borrmann 2	28(20.4)	20(17.2)	
Borrmann 3	61(44.5)	59(50.9)	
Borrmann 4	27(19.7)	19(16.4)	
Unknown	15(10.9)	16(13.8)	
Depth of invasion			0.001
T1–3	86(62.8)	48(41.4)	
T4	51(37.2)	68(58.6)	
LN metastasis			
N0	10(7.3)		
N1	72(52.6)		
N2	39(28.5)		
N3	16(11.7)		
Site of distant metastasis[a]			0.001
Liver	49(35.8)	52(44.8)	
Peritoneum	65(47.4)	85(73.3)	
Distant nodes	29(21.2)	4(3.4)	
Ovary	8(5.8)	2(1.7)	
Lung	0	2(1.7)	
Number of metastatic sites			0.06
Multiple	14(10.2)	24(20.7)	
Single	123(89.8)	92(79.3)	
Histologic classification[b]			0.394
Differentiated	46(33.6)	20(17.2)	
Undifferentiated	82(59.9)	47(40.5)	
Unclassified	9(6.6)	49(42.2)	

[a] Some patients had multiple metastatic sites

[b] The unclassified patients were excluded when comparing

The purpose of this study was to evaluate the survival benefit from non-curative gastrectomy for gastric cancer patients with synchronous distant metastasis.

Materials and Methods

From January 1992 to December 2002, 1,390 patients with histologically proven gastric cancer underwent surgery at the Department of Surgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. According to the fifth edition of the UICC TNM classification,[11] the distant metastasis sites include liver, lung, brain, ovary, bone, bone marrow, skin, peritoneum, and distant lymph node metastasis including involvement of extra-abdominal and nos. 13–16 lymph nodes. Finally, 253 patients diagnosed of gastric cancer with synchronous distant metastases were enrolled in this study. Among them, 101 had liver metastasis, two had lung metastasis, ten had ovary metastasis, 33 had distant nodes metastasis, and 150 had peritoneal dissemination (38 had multiple metastatic sites).

The non-curative gastrectomy was defined as gastrectomy with a postoperative residual[12] and was decided by surgeons based on patients' general health, symptom,

extent of tumor, and feasibility of resection. All clinico-pathological variables including age, gender, tumor size, tumor location, macroscopic type, the depth of invasion, status of lymph node metastasis, histological differentiation, number and site of distant metastasis, surgical type, and survival status were defined according to Japanese Classi-fication of Gastric Cancer and collected from the gastric cancer database of our center. Then, we compared the clinicopathological characteristics and survival for gastric cancer patients with synchronous distant metastasis be-tween the resection and non-resection group by using univariate and multivariate survival analysis. Special attention was paid to the survival benefit of non-curative gastrectomy.

Follow-up

Evaluation of patient survival was by follow-up contact using telephone, the outpatient records, or mails. Patient follow-up lasted until death or the cut-off date of December 31, 2007. Finally, 22 patients (8.7%) had been lost to follow-up. The median follow-up period was 21.8 months (range, 1–81 months).

Statistical Analysis

The statistical analysis was performed with Statistical Package for Social Science (SPSS) version 13.0 for Windows (SPSS, Inc, Chicago, Illinois). Data were ana-lyzed statistically using student t test and chi-square tests. Survival rate was analyzed using the Life table method, and the difference between the curves was assessed using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model for survival analysis. A P-value of <0.05 was considered statistically significant.

Results

Surgical Types

Of the 253 patients, 137 (54.2%) underwent gastrectomy, the other 116 (45.8%) underwent non-resection surgery. In the resection group, the types of resection and reconstruc-tion were selected by the location of the lesions. Among them, 112 received distal subtotal gastrectomy with Billoth I in 76 patients and Billoth II in 36 patients. Four patients received proximal subtotal gastrectomy with esophagogas-trostomy. The remaining 21 patients received total gastrec-tomy with Roux-en-Y reconstruction. In the non-resection group, by-pass surgery and laparotomy were performed for 52 and 64 patients, respectively.

Clinicopathological Characteristics

The comparison of clinicopathological features between the resection and non-resection groups was shown in Table 1. The mean tumor size of the non-resection group was significantly larger than that of resection group (9.5 vs. 6.7 cm, $P<0.001$). There were larger proportions of the upper 1/3 tumor location (21.6% versus 6.6%) and whole gastric involvement (15.5% versus 5.8%) in the non-resection group than the resection group. Adjacent organ invasion (T4) was found more frequently in the non-resection group (58.6%) than the resection group (37.2%). The proportion of distant nodes involvement was larger in the resection group (21.2%) than that in the non-resection group (3.4%). However, peritoneal dissemination was found less frequently in the resection group (47.4%) than that in the non-resection group (73.3%). The differences in gender, age, macroscopic type, liver metastasis, histological classification, and number of metastatic sites between the two groups were not significant.

Survival Analysis

The median survival in patients who had non-curative gastrectomy was longer than that in patients who had non-resection surgery (12.1 months vs. 6.8 months). The 5-year survival rate was 6.5% for patients in the resection group and 0% for patients in the non-resection group. There was a significant difference in survival between the two groups ($P<0.001$, Fig. 1).
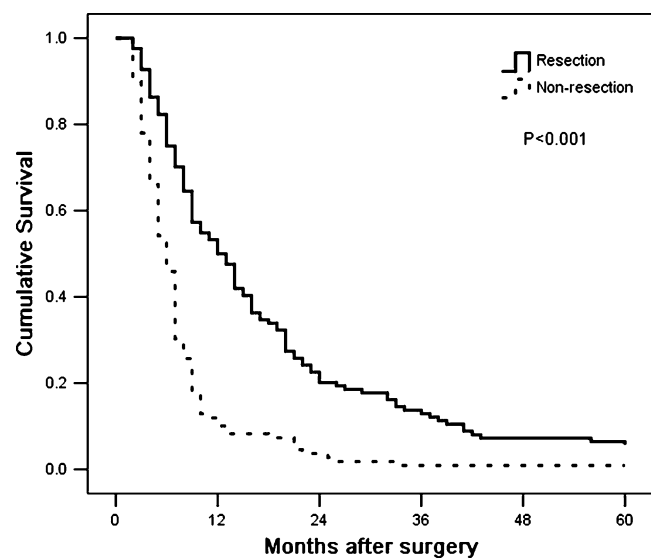
Figure 1 Survival curves for patients with non-curative resection versus non-resection. The 5-year survival rate was 6.5% for patients in the resection group and 0% for patients in the non-resection group. There was a significant difference in survival between the two groups ($P<0.001$).

The univariate survival analysis showed that tumor size (P=0.038), number of distant metastatic sites (P=0.005), liver metastasis (P=0.03), peritoneal dissemination (P=0.004), and non-curative resection (P<0.001) were factors influencing the long-term survival in gastric cancer patients with distant metastasis (Table 2). Multivariate analysis of survival, including factors selected from univariate analysis, showed that liver metastasis (RR= 1.618, P=0.01), peritoneal dissemination (RR=1.474, P=

0.03), and non-resection (RR=2.239, P=0.001) were significantly associated with poor prognosis in gastric cancer patients with distant metastasis (Table 3).

To minimize the selected bias between the two arms, we compared the survival curves between the resection group and non-resection group stratified by number and sites of distant metastasis. The 5-year survival rate of the resection group was significantly higher than that of the non-resection group in patients with single metastatic site (P<0.001), while it was

Table 2 Univariate Survival Analysis for Gastric Cancer Patients with Distant Metastasis

Variables	Patients' number	Mean survival time (months)	5-year survival rate	P-value
Age (years)				0.551
<65	153	7.1	4.3	
≥65	100	6.5	4.1	
Gender				0.674
Male	146	7.5	4.6	
Female	107	8.2	4.3	
Size (cm)				0.038
<5	71	12.2	4.2	
5–10	154	8.5	6.3	
≥10	28	6.0	0	
Tumor location				0.057
Lower	124	9.1	3.8	
Middle	69	7.6	3.9	
Upper	27	6.9	2.8	
Whole	33	5.0	1.8	
Macroscopic type				0.235
Borrmann 1	7	12.4	0	
Borrmann 2	54	9.0	4.3	
Borrmann 3	130	8.7	4.2	
Borrmann 4	62	7.2	2.0	
Depth of invasion				0.132
T1–3	134	12.1	4.0	
T4	119	10.2	3.2	
Number of distant metastatic sites				0.005
Single	215	9.1	3.4	
Multiple	38	6.0	2.8	
Liver metastasis				0.030
Absent	159	8.8	5.3	
Present	94	7.2	1.5	
Peritoneal dissemination				0.004
Absent	99	10.4	4.5	
Present	154	8.1	4.3	
Histology				0.189
Differentiated	66	8.4	4.6	
Undifferentiated	129	9.3	3.8	
Resection				<0.001
Yes	137	12.1	6.5	
No	116	6.8	0	

Table 3 Multivariate Survival Analysis for Gastric Cancer Patients with Distant Metastasis

| Variables	Risk ratio	95% CI	P-value
Size			
5–10 cm vs. <5 cm	0.897	0.622–1.293	0.559
>10 cm vs. <5 cm	0.966	0.549–0.776	0.987
Liver metastasis			
Yes vs. no	1.618	1.120–2.335	0.01
Peritoneal dissemination			
Yes vs. no	1.474	1.039–2.090	0.03
No. of metastatic sites			
≥2 vs single	1.030	0.512–1.793	0.917
Resection			
No vs Yes	2.239	1.572–3.189	0.001

not in patients with multiple metastatic sites ($P=0.192$, Fig. 2). Furthermore, in patients with single metastatic site, we compared the survival rates between the resection group and non-resection group stratified by sites of metastasis. The significant survival difference of survival between the resection and non-resection groups was only observed in patients with peritoneal dissemination ($P<0.001$), but not in patients with liver metastasis ($P=0.428$) and distant nodes involvement ($P=0.490$), respectively (Fig. 3).

Discussion

Early detection of gastric cancer is of importance for a good prognosis. However, screening for gastric cancer is not routinely performed because of large population and economic problems in China. So, some patients with gastric cancer have their diseases diagnosed at far advanced stage with synchronous distant metastasis and these patients' prognosis is very poor. The 5-year survival rate is less than 5% in such cases.[13–15]

Curative gastrectomy is still the primary treatment for advanced gastric cancer, but for patients where synchronous distant metastasis had already been found preoperatively and intraoperatively, the benefit of survival from non-curative gastrectomy is controversial.[6–10] The rationale for non-curative gastrectomy to gastric cancer patients with distant metastasis are (1) some patients need primary tumor resection to relieve potential life-threatening symptoms, such as obstruction, perforation, bleeding, or debilitating ascites, to improve function and quality of life. (2) If a significant proportion of the tumor load is removed, the residual tumor may be more responsive to adjuvant treatment. (3) Reducing the tumor burden may also have some immunologic benefits because the tumor itself can produce immunosuppressive cytokine.[8,9,16]

In this study, patients who underwent resection had significantly longer survival than those who did not. However, this result must be interpreted cautiously because of the retrospective nature of this study and some different characteristics of patients in the two arms. A surgeon's decision to resect is strongly influenced by the status of neighboring organ invasion, the number of metastatic sites, and patient's performance status. In this study, more patients had no neighboring organ invasion and lower tumor load; accounting for the tumor size and number of distant metastatic sites, underwent non-curative resection. This selection bias has been suggested to be the most important contributor to survival difference. Although the tumor size, depth of invasion, and number of metastatic sites were not independent prognostic factors in multivariate analysis for survival, the survival benefit from non-
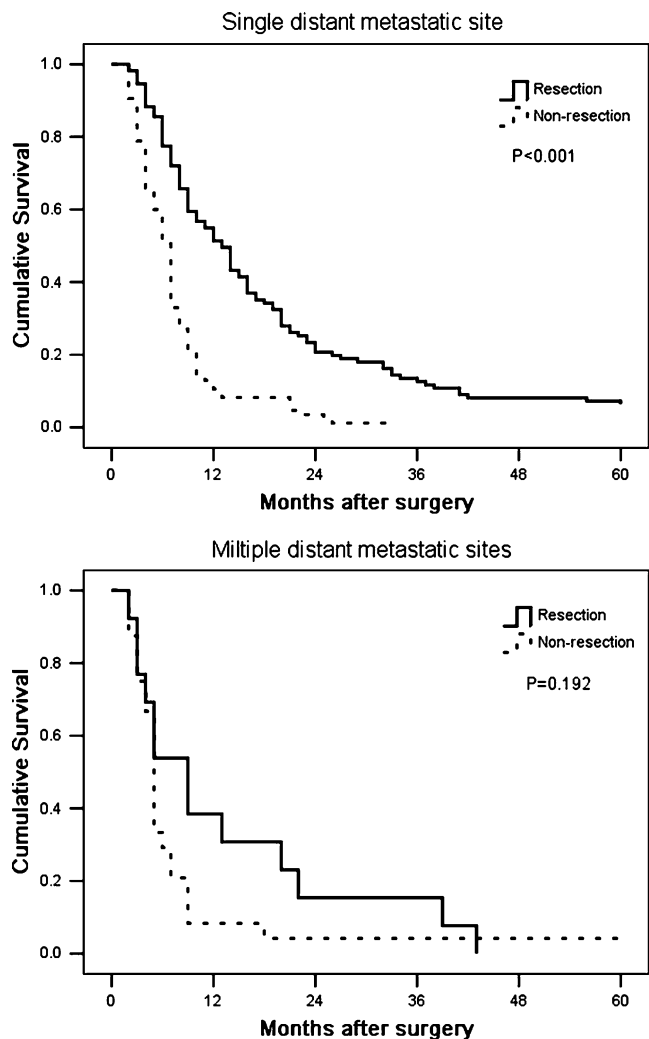
Figure 2 Survival curves between the resection group and non-resection group stratified by number of metastatic sites. The 5-year survival rate of the resection group was significantly higher than that of the non-resection group in patients with single metastatic site ($P<0.001$), while it was not in patients with multiple metastatic sites ($P=0.192$).

Figure 3 Survival rates between the resection group and non-resection group stratified by sites of metastasis. The survival difference between resection and non-resection groups was only observed in patients with peritoneal dissemination (P<0.001), but were not in patients with liver metastasis (P=0.428) and distant nodes involvement (P=0.490), respectively.

curative gastrectomy should be evaluated by further stratified analysis.

It is necessary that the number of metastatic sites should be taken into consideration before a non-curative gastrectomy is performed.[17,18] In our series, for patients with multiple metastatic sites, there was no significant difference between the resection group and non-resection group, even more patients had smaller tumor size and no adjacent organ invasion in resection group. It implies that non-curative gastrectomy has no survival benefit for patients with multiple metastatic sites. This result is consistent with the conclusion from the study by Hartgrink et al.[10] They analyzed the value of non-curative gastrectomy in patients with metastatic gastric cancer in the Dutch Gastric Cancer Trial, and found that patients with one metastatic site had a significant survival advantage over those with more metastatic sites after resection.

Furthermore, we focused on the survival benefit in patients with a single metastatic site. In the current study, the survival of patients with resection was not significantly longer than that of patients without resection in patients with single hepatic and distant nodes metastasis, respectively, even though a smaller tumor load tempted surgeons to perform gastrectomy in the resection group. It suggests that there were still no survival benefit of non-curative gastrectomy for patients with single liver or distant nodes metastasis. Another interesting finding of this study was that patients with peritoneal metastasis had better prognosis after resection. In ten of 51 patients with single peritoneal metastasis, intraoperative peritoneal hyperthermic chemotherapy (IPHC), 5 to 6 l of perfusate containing cisplatin (50 μg/ml) and mitomycin (5 μg/ml) at 43.0°C for 60 min, were performed after resection. In our previous study, we found that cytoreductive surgery combined with IPHC could prolong postoperative survival by synergistic effect between hyperthermia and antitumor drugs.[19] This may explain why the non-curative resection had survival benefit for those patients. Therefore, gastrectomy with IPHC could be recommended to the patients with single peritoneal dissemination.

The current study was not designed to compare the complications related to non-curative gastrectomy than non-resection. Although experienced surgeons in a large-volume gastric cancer center can safely perform gastrectomy with acceptable operative risks,[20,21] many studies reported that non-curative gastrectomy was significantly associated with higher postoperative mortality and morbidity rates, and
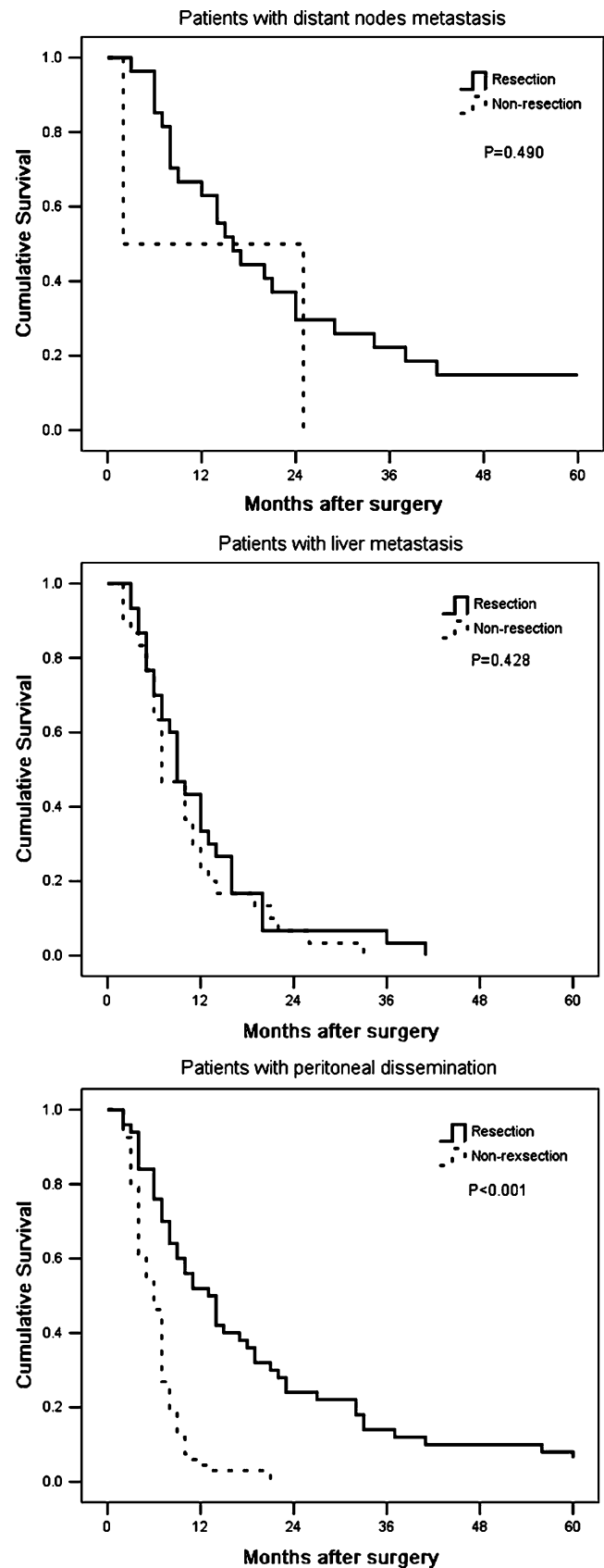
longer hospital stay.[6–9] Thus, it is important to balance the survival and complications from the non-curative gastrectomy for gastric cancer patients with distant metastasis.

The benefit of chemotherapy for patients with distant metastasis is also still controversial.[22] In our department, almost all the patients with distant metastasis were treated with chemotherapy after surgery. Because the regimen of chemotherapy varied during the period of a dozen years in this study, we did not evaluate survival benefit related to non-curative chemotherapy. In future studies, the survival benefit of chemotherapy for far advanced gastric cancer patients should be evaluated by prospective clinical trials.

In conclusion, although our findings showed that the patients who underwent non-curative resection had longer survival than those without resection, there was no survival benefit from non-curative gastrectomy for patients with single hepatic, distant nodes metastasis or multiple sites metastases. Only patients with single peritoneal dissemination had survival benefit from non-curative resection. The value of non-curative resection for distant metastatic gastric cancer should be evaluated by well-designed prospective clinical trials.

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ORIGINAL ARTICLE

Short-term Outcomes of Roux-en-Y Stapled Anastomosis after Distal Gastrectomy for Gastric Adenocarcinoma

Takeshi Fujita · Hitoshi Katai · Shinji Morita · Makoto Saka · Takeo Fukagawa · Takeshi Sano

Abstract

Background Since 2003, we have begun to perform gastrojejunostomy by mechanical stapling for Roux-en-Y reconstruction in distal gastrectomy. We performed a retrospective study to compare the short-term outcomes of anastomosis by mechanical stapling and hand suturing.

Methods We evaluated the data of 701 consecutive patients of gastric adenocarcinoma who underwent conventional open distal gastrectomy with Roux-en-Y reconstruction. The data collected included details on the method used for the Roux-en-Y reconstruction, the disease stage, extent of lymph node dissection, performance rate of truncal vagotomy, operation time, operative blood loss, length of hospital stay, and postoperative complications.

Results The operation time was significantly shorter in the group in which mechanical stapling was used for the anastomosis (MS group) than in the group in which anastomosis was performed by hand suturing (HS group; 241.1 ± 56.8 vs. 166.4 ± 48.3 min; $p<0.05$). Postoperatively, delayed gastric emptying occurred in 14 (1.9%) patients, including seven (4.2%) from the MS group and seven (1.3%) from the HS group ($p=0.038$).

Conclusion There were no significant disadvantages of employing mechanical stapling for anastomosis, except for the high rate of delayed gastric emptying. More consideration therefore needs to be given to decreasing the frequency of gastric emptying disturbance post surgery using mechanical staples.

Introduction

In digestive surgery, the use of automatic staplers for gastrointestinal anastomoses has been widely accepted and become a standard technique. In the 1980s and early 1990s, to confirm the feasibility of mechanical stapling for anastomosis, many studies have compared the outcomes of anastomosis by mechanical stapling and hand suturing.[1–8] In regard to distal gastrectomy, there have been some studies for Billroth 1 gastroduodenostomy, which is the most commonly used in Japan, and these studies have demonstrated almost equivalent outcomes between the two methods of anastomosis.[9,10]

Recently, to decrease the leakage rate and prevent bile juice reflux, Roux-en-Y reconstruction has come to be widely used after distal gastrectomy in Japan. We have previously reported the superiority of Roux-en-Y reconstruction over Billroth 1 anastomosis.[11] Since this is a relatively new surgical technique in Japan, there have not yet been sufficient comparative studies between mechanically stapled and hand-sutured anastomosis for Roux-en-Y gastrojejunostomy in this country.

The aim of this retrospective study was to assess the short-term outcomes of the use of mechanical stapling vs. hand suturing for anastomosis in Roux-en-Y gastrojejunostomy after distal gastrectomy for primary gastric cancer.

Material and Methods

We retrospectively analyzed 701 consecutive patients (475 men and 226 women) of gastric adenocarcinoma who

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underwent conventional open distal gastrectomy with Roux-en-Y reconstruction at the National Cancer Center Hospital, Tokyo, between 2004 and 2007. The perioperative clinical outcome of each patient was evaluated retrospectively by collecting data on the operative details, method used for the Roux-en-Y reconstruction after gastrectomy, the disease stage, extent of lymph node dissection, performance rate of truncal vagotomy, operation time, operative blood loss, length of hospital stay, and postoperative complications. Student's t test and the χ^2 test were used, as appropriate, for the analyses, to assess the differences in the outcomes between the two groups. p values<0.05 were considered to indicate statistical significance. All data were expressed as means±standard error of the mean.

Surgical Procedures

Distal gastrectomy was performed in the same manner in both the MS and HS groups, and the residual stomach and jejunum were reconstructed by Roux-en-Y anastomosis.

For hand-suturing, after gastrectomy, the mesentery and jejunum was divided at a portion approximately 20 cm distal to the ligament of Treitz, the first jejunal loop ascended through mainly the retrocolic route, and occasionally the antecolic route, and gastrojejunostomy was performed by end-to-side anastomosis using Gambee method (Fig. 1).
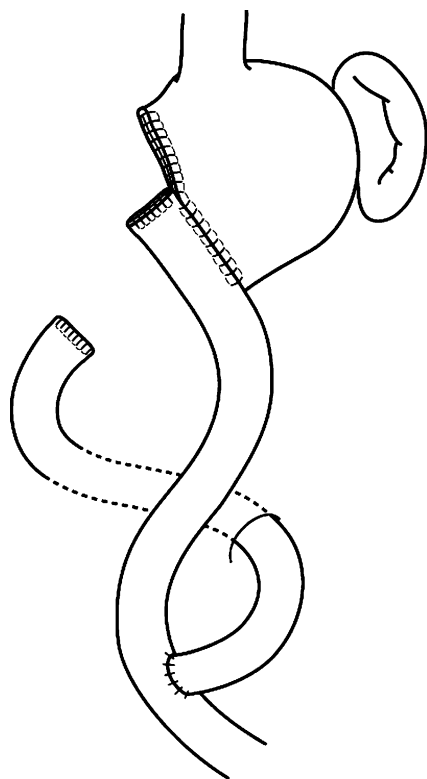
Figure 1 Gastrojejunostomy performed by hand-suturing.

For mechanical stapling, after the first jejunal loop was ascended, a six-row endo-linear stapler was positioned through the end of the jejunal Roux limb and the greater curvature of the remnant stomach to create a side-to-side gastrojejunostomy. The staple size was 3.5 mm and staple line length was 60 mm to make the size of anastomosis the same as the hand-sewn technique (Fig. 2).

The common entry hole was closed using a one-layer running suture.

In either procedure, the jejunal anastomosis was placed 30–40 cm distal to the gastrojejunal anastomosis and end-to-side or side-to-side was performed by the Gambee method or one-layer running suture. We have not changed the anastomotic technique over the study period. Selection of stapled or hand-sewn technique depends on surgeon preference.

Results

The mean age was 63±11.6 years (range, 27–90). The resected specimens were classified according to the 13th edition of the Japanese classification of gastric carcinoma: 416 patients (59.3%) had stage I disease, 125 (17.8%) had stage II disease, 118 (16.8%) had stage III disease, and 42 (6%) had stage IV disease.[12] D0 or D1 lymph node dissection was performed in 204 patients (29.1%), D2 or more extended dissection was performed in 493 patients (70.9%).

Among the 701 patients, mechanical stapling was used for the anastomosis in 163 (23%) patients (MS group) and hand suturing was used in 538 (77%) patients (HS group). The characteristics of the patients in each group are shown in Table 1.

There were no significant differences in the sex, age, stage, performance rate of truncal vagotomy, or extent of lymph node dissection between the two groups. The perioperative outcomes are summarized in Table 2. The total operation time was significantly shorter in the MS group than in the HS group (241.1±56.8 vs. 166.4±48.3 min; $p<0.05$).

In regard to the postoperative complications, there were no significant differences between the two groups except for the higher incidence of delayed gastric emptying in the MS group.

We defined "delayed gastric emptying" as patients who required starvation for gastric emptying disturbance and stayed admitted at the hospital for over 28 days (4 weeks) after the operation. Upper GI series or endoscopic examinations were performed to rule out other causes that might produce similar clinical symptoms, such as remnant gastritis, intestinal obstruction, and Roux limb stasis.

Among the 701 patients, the patients who were not tolerating oral nutrition and hospitalized over 14 days of

Figure 2 Gastrojejunostomy performed by mechanical stapling. **a** Endo-linear stapler was positioned through the end of the jejunal Roux limb and the greater curvature of the remnant stomach. **b** The common entry hole was closed using a one-layer running suture.
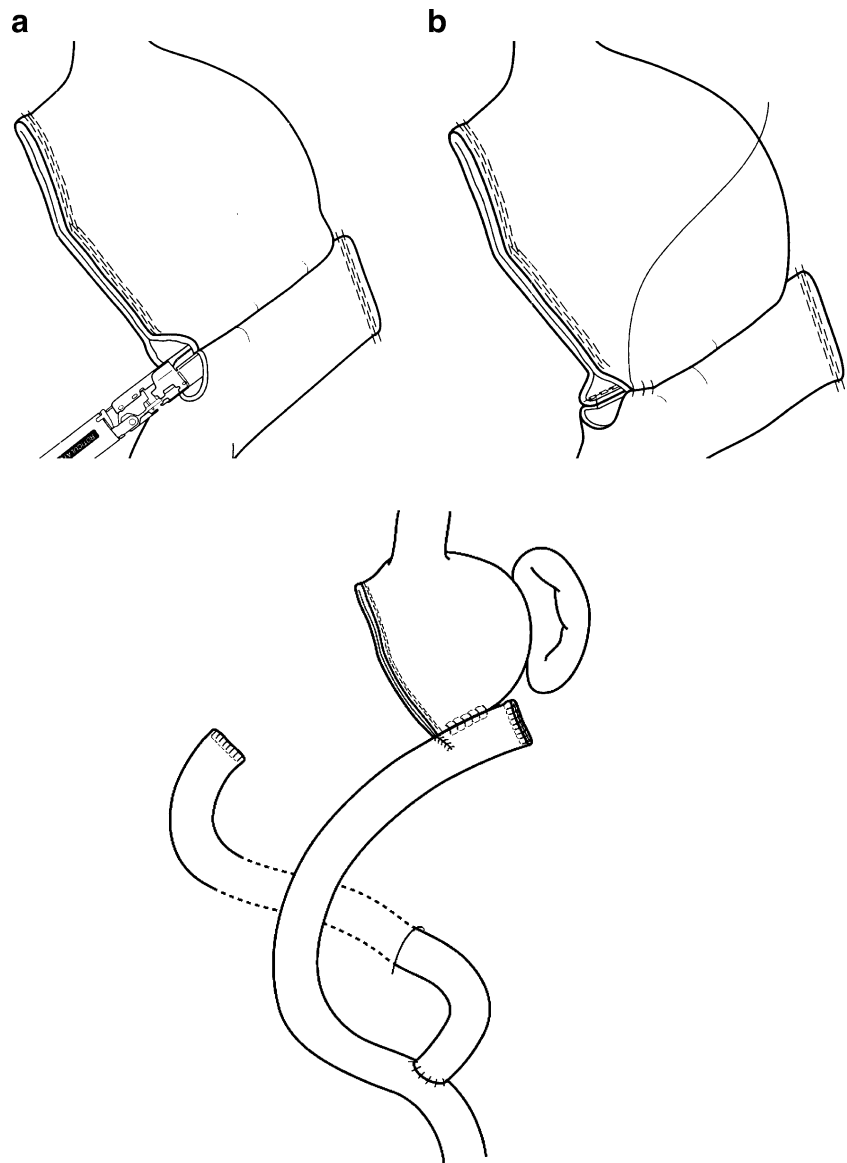
operation were 11 (2%) patients in the HS group and 11 (6%) patients in MS group ($p=0.006$), respectively. The patients who were hospitalized over 21 days of operation were ten (1.8%) patients in the HS group and ten (6.1%) patients in the MS group ($p=0.009$), respectively.

The number of patients who persisted severe postoperative delay of gastric emptying over 28 days was 14 (1.9%) overall, seven (4.2%) in the MS group and seven (1.3%) in the HS group ($p=0.038$), respectively. The postoperative mortality rate was zero.

Upper gastrointestinal series was performed in 13 of 14 patients with delayed gastric emptying and congestion at residual stomach was observed in 12 patients.

Upper gastrointestinal endoscopy was performed in ten of the 14 patients with delayed gastric emptying. Six patients from the MS group with delayed gastric emptying had mucosal edema around the anastomosis, while only two of the patients from the HS group showed this finding.

Discussion

Since the first report of a mechanical anastomosis was published by Fain et al.[13] in 1975, mechanical staplers have been used widely for establishing anastomoses in the field of digestive surgery.

Mechanical stapling was immediately introduced for colorectal anastomoses in low anterior resections and esophagojejunostomy after total gastrectomy.

These procedures were technically difficult to perform with hand suturing because of the poor surgical field of view and the difficulties associated with operating in deep

Table 1 Characteristics of Patients

	Hand-sutured group ($n=538$)	Mechanical-stapled group ($n=163$)
Sex(male/female)	359/179	116/47
Age(years)	62.9±11.7	63.5±11.2
Stage		
I	316	100
II	100	25
III	96	22
IV	26	16
Vagal nerve sparing	291	46
Lymph node dissection (D0, D1/≥D2)	148/390	56/107

and narrow spaces. Mechanical stapling has now become a standard technique in these procedures.

In recent years, with the spread of laparoscopic surgery, mechanical staplers have come to be used more and more frequently even in open surgery.

In Japan, some studies have been reported comparing the use of mechanical stapling and hand suturing for Billroth 1 anastomoses.

Although Takahashi et al.[10] noted that mechanical stapling has the disadvantage of causing more stasis of food in the remnant stomach than hand-suturing for Billroth 1, they concluded that stapled-anastomoses are almost equivalent in quality to hand-sutured anastomoses.[9–10]

Prior to 1990, at our institution, gastrointestinal continuity following distal gastrectomy was commonly achieved with Billroth 1 anastomosis.

However in the early 1990s, increase in hospital mortality from anastomotic leakage was noted in Billroth

1 anastomoses, following the introduction of para-aortic lymph node dissection.

Since then, we have started performing Roux-en-Y reconstruction after distal gastrectomy to more safe anastomoses.

Roux-en-Y gastrojejunostomy is also known to prevent reflux into the remnant stomach.[14] We have encountered no case of anastomotic leakage at our institution since we introduced this procedure after distal gastrectomy.

Nowadays, we perform Roux-en-Y reconstruction for all cases after distal gastrectomy, irrespective of the extent of lymph node dissection.

Since 2003, we have begun to perform gastrojejunostomy by mechanical stapling in some cases, to shorten operation time.

In this study, we evaluated the short-term outcomes of mechanical stapling for gastrojejunostomy after open distal gastrectomy.

Operative time was significantly shorter in the MS group as expected. However, the average time difference of 74 min was too long to attribute solely to the anastomotic technique utilized. The reason was unknown. There is a possibility that surgeons preferred stapled anastomosis when they wanted to finish the operation in a shorter operative time.

In regard to the postoperative complications, no anastomotic leakage was encountered in our series of 701 patients and no significant statistical differences were found between the MS and HS groups, except for the higher rate of delayed gastric emptying in the MS group.

The prevalence of delayed gastric emptying after gastrectomy has been reported to range from 5% to 30%.[15–18]

Delayed gastric emptying is a significant postoperative complication that markedly reduces a patient's quality of life after distal gastrectomy.

Table 2 Perioperative Clinical Outcome

	Hand-sutured group ($n=538$)	Mechanical-stapled group ($n=163$)	p value
Operation time (min)*	241.1±56.8	166.4±48.3	0.000
Operative blood loss(mL)	317.1±263.6	223.8±258.3	
Postoperative hospital stay (days)	14.0±9.4	14.9±9.8	
Postoperative complication			
Delayed gastric emptying*			
Hospital stay over 14 days	11(2%)	11(6%)	0.006
Hospital stay over 21 days	10(1.8%)	10(6.1%)	0.009
Hospital stay over 28 days	7(1.3%)	7(4.2%)	0.038
Pancreas-related infection	15(2.7%)	5(3.0%)	
Bowel obstruction	4(0.7%)	3(1.8%)	
Surgical site infection	11(2.0%)	1(0.6%)	
Reoperation	7(1.3%)	1(0.6%)	
Stump leakage	4(0.7%)	0(0%)	
Anast leakage (gastrojejunostomy)	0	0	

Some have reported a close relation between the likelihood of development of delayed gastric emptying and the Roux-en-Y procedure.[18,19] In this study, the overall rate of delayed gastric emptying after the Roux-en-Y procedure was only 1.9% (14/701 cases), which we thought was comparable to that after the Billroth 1 procedure.

Delayed emptying was observed more frequently following anastomosis by mechanical stapling; however, the rate was still low, being only about 4.2%.

In regard to this finding of a higher frequency of gastric emptying disturbance in patients with mechanically stapled anastomosis, Kitajima et al. reported the results of their study of the wound healing process around anastomoses using the gut of dogs. They reported that in the case of hand suturing, the avascular area associated with anastomotic ischemia disappeared within 5 days of the operation as evaluated by histopathology, whereas it persisted for much longer in the case of mechanical stapling.[20]

Moreover, mucosal healing around anastomoses has been described to occur in a single phase following hand suturing, but is completed in two phases following mechanical stapling, namely, closing of the mucosal defect by the staples and epithelial healing over the granulation tissue. Thus, mucosal healing is thought to be quicker in cases where anastomosis is undertaken by hand suturing.

Delayed gastric emptying occurring in the early postoperative period is generally thought to resolve spontaneously within 6 weeks.[21,22]

All the patients in the present case series were supported by intravenous hyperalimentation while they are not tolerating oral nutrition. They recovered without operation in this study. Reoperation is usually unnecessary. Use of a mechanical stapler for anastomosis can shorten the operation time and provide consistently safe anastomoses in deep spaces, especially in laparoscopic distal gastrectomy.

In regard to the frequency of delayed gastric emptying following anastomosis using a mechanical stapler, the rate would still appear to be acceptable even though it is higher than that following anastomosis by hand suturing.

However, once this complication occurs, the patient's QOL is markedly reduced and prolonged fasting may cause severe complications such as aspiration pneumonia for high-risk patients.

More intensive effort is necessary to decrease the rate of gastric emptying disturbance following gastrojejunostomy. Also, it must be borne in mind that the cost of using mechanical staples for anastomosis is greater.

Conclusion

Mechanical stapling has become the standard method for gastrointestinal anastomoses. It can shorten the operation time and provide less stress for the surgeon as compared with hand suturing. More consideration has to be given to decrease the rate of gastric emptying disturbance following anastomosis by mechanical stapling and also the cost of this technique.

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ORIGINAL ARTICLE

Surgery for Obstructed Colorectal Malignancy in an Asian Population: Predictors of Morbidity and Comparison Between Left- and Right-Sided Cancers

Ker-Kan Tan · Richard Sim

Abstract

Introduction Surgical treatment of obstructed colorectal cancers has been associated with significant perioperative morbidity and mortality. This study was performed to review the spectrum of surgery and early outcome of patients with acutely obstructed colorectal cancers. The secondary aims were to compare right- and left-sided obstruction and to identify factors predicting morbidity and mortality in these patients.

Methods A retrospective review of all patients who underwent operative intervention for acute obstruction from colorectal malignancy from February 2003 to April 2008 was performed. Patients were identified from the hospital's operating records based on postoperative diagnosis codes of colorectal malignancy. The diagnosis of acute obstruction was confirmed through clinical assessment, radiological investigations, and surgical findings. All the complications were graded according to the classification proposed by Clavien and group.

Results Out of a total of 1,268 patients who underwent surgery for colorectal malignancy, 134 (10.6%) patients with a median age of 71 years (range, 34–97 years) were operated for acute obstruction. Left-sided malignancy accounted for 79.9% of the obstruction, with sigmoid colon being the most common site in 54 (40.3%) patients. A significant proportion (77.6%) of our patients had associated perioperative morbidity, and the mortality rate was 11.9%. Worse complications (grades of complications III to V) were more frequent in patients who had a higher American Society of Anesthesiologists score (3–4), are ≥60 years old, and had preoperative renal impairment. Stoma was created more frequently in left-sided pathology.

Conclusion In an Asian population, surgery in patients with acute colorectal malignant obstruction is associated with significant morbidity and mortality rates. Though left-sided malignant obstruction occurs more frequently and is associated with a higher incidence of stoma creation, primary resection and anastomosis is a safe option in selected patients.

Introduction

Colorectal malignancy is one of the most common cancers worldwide. The incidence of complete obstruction has been reported to be as high as 30%.[1–3] Urgent surgical treatment in obstructed colorectal cancers has been associated with

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prohibitive perioperative morbidity and mortality rates despite advances in surgical techniques and intensive care.[4,5] Some of the factors accountable for these dismal results included advanced age, American Society of Anesthesiologists (ASA) score, and site of malignancy.[4,5]

The ideal surgical option in malignant obstruction remains controversial.[6,7] Though primary anastomosis without stoma for obstructed right-sided colon malignancy has been considered safe,[7, 8] surgical options in malignant left-sided obstruction could range from defunctioning stoma, Hartmann's procedure, and primary anastomosis with or without diverting stoma.[8,9] Self-expanding metallic stenting of the malignant colorectal obstruction is another recent advance that is gaining in popularity in many institutions.[10,11]

With the majority of the current literature based on data from the Western population, a true reflection of the impact and issues surrounding obstructed colorectal malignancy in Asians is lacking. There were reports documenting lower rates of right-sided malignancy but higher incidences of distal colonic and rectal malignancies in Asians.[12,13] Furthermore, other characteristics associated with Asians with colorectal cancers would include younger age of diagnosis and less advanced malignancy. This phenomenon has been attributed to genetic risk factors, cancer biology, or other uncharacterized carcinogens.[12,13]

Hence, we undertook this study with the primary aim to review the treatment and early outcome of patients who underwent emergency surgery for acute colorectal malignant obstruction. Our secondary aims were to evaluate the various factors predicting morbidity and mortality, to determine the differences between left-sided and right-sided pathologies, and to highlight the various surgical options.

Methods

Study Population

Tan Tock Seng Hospital is a 1,300-bed hospital, the second largest in Singapore, and provides secondary and tertiary medical care for about 1.5 million people. A retrospective review of all patients who underwent operative intervention for acute obstruction from colorectal malignancy from February 2003 to April 2008 was performed. Patients were identified from the hospital's diagnostic index and operating records. Right-sided pathologies were regarded if it was located from the cecum until the transverse colon while left-sided pathologies commenced from the splenic flexure.

All our patients had evidence of acute colorectal obstruction as suggested by Fielding et al.[14] These criteria were determined by clinical assessment, radiological investigations, and surgical findings, which include the symptoms of abdominal pain and constipation, signs of abdominal distension and abnormal bowel gaseous distension on plain radiographs, and operative findings of proximal bowel distension and edema. Computed tomo-graphic (CT) scan with or without rectal contrast would be performed based on the surgeons' preference.

All patients underwent urgent surgical operation within 24 h of admission. Prior to the surgery, fluid resuscitation, parenteral antibiotics, optimization of their medical conditions, and nasogastric decompression would be administered to every patient. Resection of the tumor would be attempted in all patients except in cases of fixed and unresectable tumors or in patients who were hemodynamically unstable. All gastrointestinal anastomoses were either hand-sewn or stapled, while stoma created could be either a defunctioning or an end stoma.

The data collected included age, gender, ASA score, comorbid conditions, presenting signs and symptoms, and clinical parameters. Laboratory values, including full blood count and renal panel, were also recorded. In addition, operative findings and interventions, length of surgery, perioperative complications, mortality, and length of hospital stay were also documented.

All colorectal cancers were staged according to the guidelines of the American Joint Committee of Cancer (AJCC).[15] The grades of complications (GOC) were in concordance to the classification proposed by Clavien and group (Table 1).[16–18]

Statistical analysis was performed using both univariate and multivariate analyses. The variables were analyzed to the various outcomes using Fisher's exact test, and their odds ratio (OR) and 95% confidence interval (CI) were also reported. For the multivariate analysis, the logistic regression model was applied. All analyses were performed using the SPSS 16.0 statistical package (Chicago, IL, USA), and all p values reported are two-sided, and p values of <0.05 were considered statistically significant.

Results

During the study period, 1,268 (334 right-sided/934 left-sided) patients underwent colorectal-cancer-related surgery. Of this group, 134 (10.6%) patients presented with acute obstruction and were duly operated urgently. A total of 89 (66.4%) patients underwent preoperative CT scans while the remaining 45 (33.6%) were operated after clinical assessment and evaluation of their abdominal radiographs.

Table 1 Classification of Surgical Complications[16–18]

Grade I: any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions

Grade II: requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included

Grade III: requiring surgical, endoscopic, or radiological intervention

Grade IV: life-threatening complication(s) requiring ICU management (including organ dysfunction)

Grade V: death of a patient

Right-Sided Malignancy

There were 27 (20.1%) patients who presented with acute obstruction, which comprised 8.1% of all patients who had surgery for right-sided malignancy (Tables 2 and 3). The median age was 75 years (50–93 years). The majority ($n=22$, 81.5%) had an ASA score of 2 or 3. Nine patients (33.3%) had metastatic disease on presentation. The ileocecal valve was competent in nine cases (33.3%) while an unhealthy cecum (ischemic or perforated) was seen in five patients (18.5%). Apart from right hemicolectomy ($n=25$, 92.6%), ileo-sigmoid bypass and defunctioning loop colostomy were performed in one patient each, both with known metastatic disease. There were five (18.5%) mortalities in this group of patients with another five (18.5%) patients with grades III or IV complications. The median length of stay was 10 days (5–109 days).

Left-Sided Malignancy

A total of 107 (79.9%) patients presented with acute obstruction, which comprised 11.5% of all patients who had surgery for left-sided malignancy (Tables 2 and 3). Sigmoid colon was the most common site of involvement ($n=54$, 50.5%). The median age of this group was 70 years (34–97 years). The majority ($n=87$, 81.4%) had an ASA score of 2 or 3. Seventy-five (70.1%) patients had at least stage III or IV disease on presentation. Closed-loop obstruction due to the presence of a competent ileocecal valve was documented in 46 (43.0%) patients, and the cecum was noted to be unhealthy in 13 (12.1%) patients.

The commonest surgical procedure performed in this group of patients included anterior resection with ($n=10$, 9.4%) or without defunctioning stoma ($n=22$, 20.6%), Hartmann's procedure ($n=31$, 29.0%), and subtotal or total colectomy ($n=20$, 18.7%). A more extensive colonic resection (extended right hemicolectomy and subtotal or total colectomy) was performed in 30 (28.0%) patients. Majority of the patients ($n=56$, 52.3%) had stoma created. Eleven (10.3%) patients perished, with another 35 (32.7%) patients developing grade III or IV complications. The median length of stay was 10 days (3–99 days).

Of the 63 patients who had stoma created, only 13 patients (20.6%) had closure of their stoma. Eight had their

Table 2 Selected Characteristics of the Study Group

Characteristics	Right-sided ($n=27$) (%)	Left-sided ($n=107$) (%)	Total ($n=134$) (%)
Presentation			
Perforation of cecum from distal malignant obstruction	5 (18.5)	2 (1.9)	7 (5.2)
Malignant obstruction without perforation	22 (81.5)	105 (98.1)	127 (94.8)
Stenting			
Previous endoscopic stenting for obstructed cancer	0	2 (1.8)	2 (1.6)
Failed endoscopic stenting	0	1 (0.9)	1 (0.8)
CT scan			
Performed	23 (85.2)	66 (61.7)	89 (66.4)
Not performed	4 (14.8)	41 (38.3)	45 (33.6)
Site of malignancy			
Cecum	5 (18.5)		5 (3.7)
Ascending colon	7 (25.9)		7 (5.2)
Hepatic flexure	7 (25.9)		7 (5.2)
Transverse colon	8 (29.6)		8 (6.0)
Splenic flexure		8 (7.5)	8 (6.0)
Descending colon		19 (17.8)	19 (14.2)
Sigmoid colon		54 (50.5)	54 (40.3)
Rectosigmoid		10 (9.3)	10 (7.5)
Rectum		16 (14.9)	16 (11.9)
Staging of malignancy (AJCC classification)			
Stage I	1 (3.7)	1 (0.9)	2 (1.5)
Stage II	5 (18.5)	26 (24.3)	31 (23.1)
Stage III	12 (44.4)	41 (38.3)	53 (39.6)
Stage IV	9 (33.3)	34 (31.8)	43 (32.1)
Unknown	0	5 (4.7)	5 (3.7)

Table 3 Surgical Procedures, Techniques, and Outcome

Characteristics	Right-sided (n=27) (%)	Left-sided (n=107) (%)	Total (n=134) (%)
Surgery performed list by (R) vs (L)			
Right hemicolectomy ± stoma	25 (92.6%)	10 (9.3)	35 (26.1)
Left hemicolectomy	0	3 (2.8)	3 (2.2)
Anterior resection ± stoma	0	32 (29.9)	32 (23.9)
Hartmann's procedure	0	31 (29.0)	31 (23.1)
Subtotal/total colectomy	0	20 (18.7)	20 (14.9)
Loop colostomy	1 (3.7)	10 (9.3)	11 (8.2)
Bypass procedure	1 (3.7)	1 (0.9)	2 (1.5)
Status of cecum			
Unhealthy (Ischemic/gangrenous/perforated)	5 (18.5)	13 (12.1)	18 (13.4)
Healthy	22 (81.5)	94 (87.9)	116 (86.6)
Resection of cecum			
Yes	25 (92.6)	30 (28.0)	55 (41.0)
No	2 (7.4)	77 (72.0)	79 (59.0)
Type of anastomosis			
Hand-sewn anastomosis	3 (11.1)	11 (10.3)	14 (10.4)
Stapled anastomosis	17 (63.0)	39 (36.4)	56 (41.8)
Stoma creation	7 (25.9)	56 (52.3)	63 (47.0)
Grades of complications			
No complications	6 (22.2)	24 (22.4)	30 (22.4)
GOC I	5 (18.5)	17 (15.9)	22 (16.4)
GOC II	6 (22.2)	20 (18.7)	26 (19.4)
GOC III	2 (7.4)	11 (10.3)	13 (9.7)
GOC IV	3 (11.1)	24 (22.4)	27 (20.1)
GOC V (death)	5 (18.5)	11 (10.3)	16 (11.9)
Median length of stay (days)	10 (5–109)	10 (3–99)	10 (3–109)

ileostomy closed after an initial anterior resection, while only three patients who underwent Hartmann's procedure had their stoma reversed. The remaining two patients who had an initial defunctioning colostomy performed underwent a definitive left hemicolectomy and anterior resection 1 month after the creation of stoma. Another one patient who had an initial defunctioning sigmoid colostomy underwent an abdominoperineal resection for low rectal cancer after a period of radiotherapy and chemotherapy.

Comparison—Right-Sided Pathology vs. Left-Sided Pathology

These two groups of patients were similar in numerous aspects (Table 4). Factors such as age group, gender, ASA score, and premorbid condition were largely similar, and any differences were not statistically significant. Even the staging of the malignancy and complication rates were not vastly different. Stoma was created more frequently in left-sided pathology (OR 3.14, 95% CI 1.23–8.04, p 0.017), while surgery for left-sided pathology took longer than for right-sided lesions (OR 3.04, 95% CI 1.27–7.25, p 0.019).

Comparison—Extensive Resection vs. Limited or No Resection for Malignant Left-Sided Obstruction

The creation of stoma was much more evident in patients who had limited or no resection (OR 9.81, 95% CI 3.36–28.60, $p < 0.001$; Table 5), while more extensive resection encompassing the cecum was expectedly more frequent in patients who had an unhealthy cecum ($p < 0.001$). However, patients who had extensive resection did not have higher complication rates or longer duration of surgery. Though it would appear that patients with more advanced disease underwent a more limited resection or had no resection, the difference was not statistically significant (OR 2.03, 95% CI 0.80–5.12, $p > 0.05$).

Analysis—Predictors of Worse Complications

After multivariate analysis, the independent variables predicting a worse perioperative outcome including death (GOC III to V) would include higher ASA score (3–4), ≥60 years old, and preoperative renal impairment (Table 6).

Table 4 Comparison Between Right Versus Left-Sided Malignant Obstruction

Characteristics	Right-sided pathology ($n=27$)	Left-sided pathology ($n=107$)	OR (95% CI)	p value
>60 years old	22 (81.5%)	78 (72.9%)	0.61 (0.21–1.77)	>0.05
Male gender	15 (55.6%)	59 (55.1%)	0.98 (0.42–2.23)	>0.05
ASA score 3–4	15 (55.6%)	62 (57.9%)	1.10 (0.47–2.58)	>0.05
≥1 premorbid condition	16 (59.3%)	58 (54.2%)	0.81 (0.35–1.92)	>0.05
WBC>10.0 g/dl	12 (44.4%)	58 (54.2%)	1.48 (0.63–3.46)	>0.05
Hb≥11.0 g/dl	16 (59.3%)	80 (74.8%)	2.04 (0.84–4.93)	>0.05
Urea>9.3	10 (37.0%)	30 (28.0%)	0.66 (0.27–1.61)	>0.05
Creatinine>110	8 (29.6%)	23 (21.5%)	0.65 (0.25–1.68)	>0.05
Competent ileocecal valve	9 (33.3%)	46 (43.0%)	1.51 (0.62–3.66)	>0.05
Unhealthy cecum	5 (18.5%)	13 (12.1%)	0.61 (0.20–1.89)	>0.05
Stage III or IV disease	21/27 (77.8%)	75/102 (73.5%)	0.79 (0.29–2.18)	>0.05
Creation of stoma	7 (25.9%)	56 (52.3%)	3.14 (1.23–8.04)	0.017[a]
Duration of surgery>120 min	13 (48.1%)	79 (73.8%)	3.04 (1.27–7.25)	0.019[a]
GOC III–V	10 (37.0%)	46 (43.0%)	1.28 (0.54–3.06)	>0.05

[a] Statistically significant after multivariate analysis

Factors such as gender, site and staging of malignancy, and duration of surgery were not related. A detailed list of all the complications can be seen in Table 7.

Discussion

Despite the increased awareness of colorectal cancers, the incidence of patients presenting with complete malignant colorectal obstruction has remained alarmingly high in up to 30%.[1–3] Operative intervention in these patients has often been associated with prohibitive morbidity and mortality rates.[1–3] Some of the contributing factors would include their poor nutritional state, the direct consequences of bowel obstruction such as dehydration, electrolyte imbalance, and the high risk of postoperative septic complications from operating on feces-filled bowel.[19,20] Hence, complications such as anastomotic dehiscence, intra-abdominal abscesses, wound infection, and death were more frequently seen in these patients.[19,20]

While the patient and disease factors are unlikely to improve much preoperatively to better the eventual outcome, the most appropriate surgical procedure would then be vital to ensure the best possible conclusion. Some of the key factors that must be taken into consideration while deciding the surgical procedure would include the following issues: clinical condition of the patient, stage of disease, resectability of the malignancy, and the site and severity of obstruction.

Table 5 Comparison Between Patients Who Had Extensive Resection Against Those with Limited or No Resection for Malignant Left-Sided Obstruction

Characteristics	Extensive resection ($n=30$)	Limited or no resection ($n=77$)	OR (95% CI)	p value
>60 years old	19 (63.3%)	59 (76.6%)	1.90 (0.76–4.72)	>0.05
ASA score 3–4	19 (63.3%)	43 (55.8%)	0.73 (0.31–1.75)	>0.05
≥1 premorbid condition	14 (46.7%)	44 (57.1%)	1.52 (0.65–3.56)	>0.05
WBC>10.0 g/dl	19 (63.3%)	39 (50.6%)	0.59 (0.25–1.41)	>0.05
Hb≥11.0 g/dl	24 (80.0%)	56 (72.7%)	0.67 (0.24–1.86)	>0.05
Urea>9.3	11 (36.7%)	19 (24.7%)	0.57 (0.23–1.40)	>0.05
Creatinine>110	6 (20.0%)	17 (22.1)	1.13 (0.40–3.22)	>0.05
Unhealthy cecum	13 (43.3%)	0 (0.0%)	NA	<0.001[a]
Creation of stoma	5 (16.7%)	51 (66.2%)	9.81 (3.36–28.60)	<0.001[a]
Duration of surgery>120 min	24 (80.0%)	55 (71.4%)	0.63 (0.23–1.74)	>0.05
Stage III or IV disease	19 (63.3%)	56/72 (77.8%)	2.03 (0.80–5.12)	>0.05
GOC III–V	13 (43.3%)	33 (42.9%)	0.98 (0.42–2.30)	>0.05

[a] Statistically significant after multivariate analysis

Table 6 Predictors of Worse Outcome (GOC 0–II Against GOC III–V)

Characteristics	GOC 0–II (n=78)	GOC III–IV (n=40)	GOC V (death) (n=16)	OR (95% CI)	p value
>60 years old	50 (64.1%)	35 (87.5%)	15 (93.8%)	4.67 (1.78–12.25)	0.001[a]
Male gender	41 (52.6%)	19 (47.5%)	14 (87.5%)	1.30 (0.65–2.59)	>0.05
ASA score 3–4	30 (38.5%)	31 (77.5%)	16 (100.0%)	8.36 (3.58–19.48)	<0.001[a]
≥1 premorbid condition	38 (48.7%)	24 (60.0%)	12 (75.0%)	1.90 (0.94–3.83)	>0.05
WBC>10.0 g/dl	45 (57.7%)	19 (47.5%)	6 (37.5%)	0.59 (0.30–1.18)	>0.05
Hb ≥11.0 g/dl	61 (78.2%)	26 (65.0%)	9 (56.3%)	0.46 (0.22–1.00)	>0.05
Urea>9.3	15 (19.2%)	16 (40.0%)	9 (56.3%)	3.39 (1.57–7.32)	0.002[a]
Creatinine>110	14 (17.9%)	9 (22.5%)	8 (50.0%)	1.99 (0.89–4.49)	>0.05
Left-sided malignancy	61 (78.2%)	35 (87.5%)	11 (68.8%)	1.28 (0.54–3.06)	>0.05
Competent ileocecal valve	35 (44.9%)	13 (32.5%)	7 (43.8%)	0.68 (0.34–1.38)	>0.05
Unhealthy cecum	9 (11.5%)	5 (12.5%)	4 (25.0%)	1.47 (0.54–3.97)	>0.05
Stage III or IV disease	56/74 (75.7%)	26/39 (66.7%)	14 (87.5%)	0.86 (0.39–1.90)	>0.05
Duration of surgery>120 min	52 (66.7%)	30 (75.0%)	10 (62.5%)	1.25 (0.59–2.64)	>0.05

[a] Statistically significant after multivariate analysis

Also seen in our series, surgery in these patients who are older and those with worse ASA score resulted in worse perioperative outcome.[21,22] Their limited physiological reserves are likely accountable for the abysmal results. Apart from these factors, patients who are in septic shock, had renal impairment, immunocompromised, and had higher APACHE II score and those who had blood

Table 7 List of Complications in Our Series

Complications	Number of patients (n, %)
Death	16 (11.9)
Pulmonary complications	
Ventilatory support post surgery	−16 (11.9)
Pleural effusion	−5 (3.7)
Pneumonia	−12 (9.0)
Atelectasis	−10 (7.5)
Cardiovascular complications	
Myocardial infarction	−3 (2.2)
Arrhythmia	−8 (6.0)
Gastrointestinal complications	
Anastomotic leak	−4 (3.0)
Ileus	−15 (11.1)
Upper gastrointestinal tract hemorrhage	−4 (3.0)
Wound complication	
Wound dehiscence	−3 (2.2)
Superficial wound infection	−11 (8.2)
Other complications	
Urinary tract infection (UTI)	−3 (2.2)
Cerebrovascular accident (CVA)	−3 (2.2)
Septicemia/septic shock	−10 (7.5)
Deep venous thrombosis/pulmonary embolism	−2 (1.5)

transfusion are also likely to do worse.[22,23] Thus, though it would be prudent to optimize the patients' conditions preoperatively as best as possible, this must be balanced against the risks of delaying surgery.

While some may question the role of CT scan in the presence of radiological evidence of complete obstruction, its advantages in these patients must not be neglected.[24,25] In patients who were diagnosed preoperatively with metastatic or unresectable disease, proper counseling to the patient and the family could be performed to handle their expectations. In these situations, the possibility and implications of stoma creation or bypass surgery or palliative stent should be discussed.

Even if the diagnoses of advanced or metastatic disease were only achieved intraoperatively without preoperative imaging, extensive surgery in these patients should be minimized as they are unlikely to improve the long-term outcome and often result in unnecessary perioperative morbidity and mortality. As seen in our series, 13 (9.7%) patients underwent a bypass procedure or defunctioning stoma without resection of the malignancy. In addition, like in two of our patients with advanced rectal malignancy, defunctioning colostomy was performed initially, and this allowed neoadjuvant chemotherapy and radiotherapy to be administered subsequently. These two patients eventually had potentially curative surgeries performed.

CT scan is also useful to confirm the diagnosis especially in patients who had previous abdominal surgery or in those with known history of pseudo-obstruction. Other techniques that could ascertain the presence of the malignant obstruction include contrast enema or gentle endoscopic evaluation.[26,27] Furthermore, CT scan can garner information regarding the possibility of insertion of an endoscopic stent.[10,11] The site and length of the primary lesion and the severity of

obstruction are important considerations. Though left-sided tumors are preferred, low rectal lesions might not be suitable due to possibility of stent-related perianal trauma and severe tenesmus.[10,11] Stenting is usually preferred in patients who are not ideal surgical candidates due to disseminated disease and extremely high operative risk or simply to act as a bridge to convert an emergency surgery to an elective one by relieving the obstruction. The rate of technical and clinical success has been reported in up to 100%,[10,11] but some of its complications would include that of colonic perforation, tumor overgrowth, stent migration, and the cost of the stent itself.[10,11] Our institution favors one-stage resection with decompression and primary anastomosis where feasible and Hartmann's procedure if not, but in the light of recent randomized studies, we have started deploying endoscopic stenting as a bridge to surgery more frequently in recent years. This series documents our experience prior to adoption of stenting, and hence the few patients who were stented and managed nonoperatively were not included. The authors recognized this as a significant shortcoming of our study.

Not unlike other series, the majority of our patients (79.9%) who presented with acute obstruction had left-sided malignancy, with the sigmoid colon being the most common site. Resection and ileocolic anastomosis for right-sided obstructed tumor has always been considered safe and sound.[25,28] On the other hand, it was not surprising to note that patients who had surgery for left-sided obstruction were more likely to have a stoma created. The underlying rationale can be attributed to the reported higher associated anastomotic dehiscence rates in colocolonic or colorectal anastomoses compared to ileocolonic or ileorectal anastomoses.[24,29] Hence, as seen in our series, a defunctioning stoma after primary resection and anastomosis or an end colostomy is an attractive alternative in these circumstances.

Interestingly, a sizeable proportion of our patients (30.0%) with left-sided pathology underwent concurrent resection of the cecum despite the fact that only less than half of these patients had unhealthy cecum. In patients with unhealthy cecum such as associated perforation or gangrene, the decision to perform an extensive surgery is obvious. However, in the absence of these conditions, some of the justifications for concurrent resection of the right colon in left-sided malignancies would include the following: the appeal of an ileocolonic or ileorectal anastomosis as discussed above; the removal of any possibility of synchronous lesions in the right colon, which has been quoted to be in the region of 3–10%[29–31]; and easier manipulation and subsequent anastomosis through an en bloc resection of the feces-filled right colon as this would reduce the risks and implications of fecal spillage and contamination.[32] As shown by our series, despite the more extensive resection, it was not associated with higher perioperative complication rates or longer surgery. Unfor-

tunately, one of the main longer-term complications following such extensive resection is usually severe diarrhea, but this often improves significantly with time and medications.[33,34]

The surgical procedures in tackling left-sided colonic obstruction have changed significantly in the past few decades. From an initial three-stage operation to the two-stage operation (Hartmann's procedure) to the increasing adopted one-stage primary resection and anastomosis without stoma.[9,35,36] This trend has been attributed to factors such as increased utilization of subtotal or total colectomy and encouraging data from centers that performed primary anastomosis after resection for obstructed left-sided malignancy, with or without on-table colonic lavage.[9,35,36] Some of the advantages of a one-stage resection and anastomosis would include avoidance of complications of a stoma, the risk of a second operation, and also offering a better quality of life especially for patients with incurable malignancies.[9,36]

In our institution, Hartmann's procedure is still frequently performed as it has been shown to be a safe surgical option in our patients, who are mostly of advanced age.[37] This procedure allows complete oncologic clearance and minimizes the risks associated with primary anastomosis and on-table lavage and shortens the operative time.[37,38] Unfortunately, reversal of Hartmann's procedure is often challenging and fraught with difficulties, resulting in numerous patients having a permanent stoma[37,38] as seen in over 90% of our patients with only three patients having their end colostomy reversed.

As with most studies, there were several limitations in the present study. This series of patients was enrolled from a single institution, and any retrospective study has inherent flaws. The relative small number of patients in our series may mask several other important factors that could be accountable for the outcomes measured. In addition, patients that were managed nonoperatively for obstructed colorectal malignancy were not included in our series as our focus was to uncover factors that could predict perioperative outcome and to highlight the various surgical options in these patients.

Although these limitations are significant, this study remains important in highlighting the various surgical issues surrounding acute malignant colorectal obstruction. The impact of the site of obstruction was also illustrated in our series. This study also highlighted the various factors that could account for significant morbidity and mortality after surgery in these patients.

Conclusion

In an Asian population, surgery in patients with acute colorectal malignant obstruction is associated with signif-

icant morbidity and mortality rates. Though left-sided malignant obstruction occurs more frequently (11.5% vs. 8.1%) and is associated with a higher incidence of stoma creation, primary resection and anastomosis is a safe option in selected patients.

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ORIGINAL ARTICLE

The Diagnosis of Diverticulitis in Outpatients: On What Evidence?

Erin S. O'Connor · Glen Leverson · Gregory Kennedy · Charles P. Heise

Abstract

Purpose Diverticular disease is common in the outpatient setting; yet, rigorous study of diagnosis and management strategies is currently limited to hospitalized patients. Here, we characterize the clinical assessment generating the diagnostic label of diverticulitis in outpatients.

Methods Encounters for diverticulitis were identified using ICD-9 diagnosis codes (562.11/562.13) from the electronic medical record system of a tertiary referral hospital and its regional clinics. The frequencies of various demographic and clinical variables were compared between patients presenting in the emergency room (ER) or outpatient Clinic.

Results Between 2003 and 2008, 820 inpatients and 2,576 outpatients met inclusion criteria (328 [13%] ER, 2,248 [87%] Clinic). Compared to ER patients, Clinic patients were less likely to undergo urgent abdominal/pelvic computed tomography (CT) scan (14% vs. 85%, $p<.0001$) or have an abnormal WBC count (35% vs. 69%, $p<.0001$). Twenty-four-hour events, including inpatient admission (30% ER vs. 3.5% Clinic, $p<.0001$) and colectomy (1.2% ER vs. 0.4% Clinic, $p=0.08$) were rare in both groups.

Conclusion Diverticulitis in the outpatient setting is often characterized by infrequent use of CT scans, lack of leukocytosis, and rare need for urgent surgery or early admission. As this diagnostic label appears to be commonly applied without objective evidence, further study is needed to evaluate its validity.

Introduction

Diverticular disease is well-recognized as a common entity in Western culture, affecting nearly two thirds of the population over age 80.[1–3] While many individuals with diverticulosis remain symptom-free, it is estimated that between 10% and 25% will develop diverticulitis in their lifetime.[2,4,5] Given the frequency of this diagnosis, its association with increased age, and the changing demographics of the US population, there is a clear need for evidence-based recommendations regarding diagnosis and optimal timing of surgical management.

Unfortunately, the natural history of diverticulitis makes the development of such recommendations quite challenging. This is a heterogeneous disease, with a spectrum of presentations ranging from mild abdominal pain and fever, to gross rupture with ensuing peritonitis and shock.[6] Afflicted patients may initiate and maintain their care in either the outpatient or inpatient arena and require treatment approaches involving both primary care and surgery. Because outpatient presentation is a common component of the natural history of diverticulitis, appropriate diagnostic strategies and management recommendations for new and recurring outpatients are

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important. However, outside of anecdotal reports, extremely little is known regarding the typical assessment used by providers in the outpatient population.[7] This results from the challenges inherent in studying this patient subgroup, unlike hospitalized patients on whom current practice guidelines are based.[8–11] Although some studies have included patients evaluated in the emergency room (ER), the ability to capture this group or those evaluated in the clinic setting is limited. In fact, some have suggested that establishing a national registry would lead to a more thorough understanding of the complete spectrum of diverticulitis.[12] Regardless of the approach, a clearer understanding of the outpatient aspect of this disease is essential for the development of comprehensive recommendations addressing diagnosis, management, and optimal timing of surgical intervention.

Crucial unanswered questions regarding diverticulitis in the outpatient setting remain at this time. Number of annual visits, location of outpatient presentation, and range of disease severity are currently unknown. In addition, the diagnostic approaches used by outpatient providers are poorly described, as adherence to recommendations presented in clinical practice guidelines has not been well-studied. In order to address these important knowledge gaps, we implemented an analysis of outpatient encounters detected through the electronic medical record (EMR) in a statewide medical system. The goal of this study was to identify and characterize patients presenting with diverticulitis in the outpatient setting and to critically evaluate the clinical assessment generating that diagnostic label.

Methods

Data Sources

After study protocol approval was obtained from the University of Wisconsin Institutional Review Board, data were extracted from the electronic medical record system of the University of Wisconsin Hospital and Clinics (UWHC) and the University of Wisconsin Medical Foundation (UWMF). Data extraction was performed by programming resources in the Department of Family Medicine (DFM), who have developed the Clinical Data Warehouse (CDW) as a platform for accessing medical record data for research and educational purposes. The DFM-CDW is maintained under separate IRB approval.

Patients

All patients receiving care at a UWHC or UWMF medical facility were considered for inclusion in our study. Inclusion criteria included those patients with an inpatient or outpatient encounter (defined as occurring in the ER or Clinic) with an associated diagnosis code for diverticulitis (International Classification of Diseases, ninth revision, Clinical Modification [ICD-9-CM] code 562.11 or 562.13) during the time period from January 1, 2003 through October 16, 2008. Exclusion criteria included those patients under 40 years old at the time of the initial encounter. The number of inpatients meeting the same inclusion and exclusion criteria was recorded in order to define the total population of diverticulitis patients in our medical system during the study period; comparison of demographic and clinical features was not conducted for the purposes of this analysis.

Encounters of Interest

In several cases, there were multiple encounters per patient meeting inclusion criteria during the study period. In order to assess independent events, we chose to examine only the first chronological encounter, thus allowing each episode to represent a unique patient. In addition, the aim was to focus on outpatient encounters which represented initial assessment of symptoms rather than follow-up from a prior outpatient visit or hospitalization. It was assumed that patients would be seen in follow-up within 6 weeks of their first visit or inpatient stay. Therefore, encounters occurring between 1/1/03 and 2/14/03 were excluded from the study, as the goal was to evaluate new episodes of diverticulitis.

Variables

Stratification Variables

Patients with an outpatient visit for diverticulitis were selected using ICD-9-CM diagnosis codes 562.11 and 562.13 identified from the administrative and billing databases. Point-of-service location was also determined, allowing these patients to be separated into two groups, those whose encounters occurred in an ER and those who were seen in an outpatient clinic (Clinic).

Descriptive Variables

For each patient, we obtained demographic data including age and gender from the clinical databases. Comorbid conditions (diabetes, COPD/asthma, renal failure, and rheumatoid conditions) were identified using predefined DFM-CDW diagnosis groups, which categorize related conditions and their associated ICD-9-CM codes.

Outcome Variables

Using ICD-9-CM and CPT codes (Table 1), we identified pertinent clinical elements occurring in the 48-h period surrounding the encounter of interest. These elements included imaging (abdominal X-ray, abdominal/pelvic computed tomography (CT) scan) and procedures (colectomy, colostomy, and abscess drainage). Laboratory data became part of the electronic medical record system for many outpatient locations beginning in 2006. Thus, for the subset of encounters occurring between February 15, 2006 and October 16, 2008, we determined the presence or absence of laboratory values including white blood cell count, hematocrit, and creatinine. Inpatient admission within 24 h, defined as hospitalization with a primary visit reason of diverticulitis subsequent to the outpatient encounter of interest, was identified.

Statistical Analysis

The demographic characteristics and outcomes of diverticulitis patients by location (ER vs. Clinic) were described, and statistical differences evaluated using χ^2 tests for categorical variables and one-way ANOVA tests for continuous variables. Analyses were performed using SAS 9.1 software (SAS Institute, Cary, NC). All tests of significance used two-sided p values at the 0.05 level.

Results

Overall, 3,396 unique encounters meeting inclusion and exclusion criteria during the 5-year study period were identified (Table 2), with 820 inpatient visits and 2,576 outpatient visits (76%). Of the encounters which occurred in the outpatient setting, 328 (13%) took place in the ER,

Table 1 Diagnostic and Procedure Codes

ICD-9-CM Codes[a]	
Diverticulitis	562.11, 562.13
CPT Codes[b]	
Imaging	
Abdominal/pelvic CT scan	74150, 74160, 74170, 72192, 72193, 72194
Abdominal X-ray	74000, 74010, 74020, 74022
Procedures	
Colectomy	44140, 44141, 44143, 44144, 44145, 44146, 44204, 44005, 44206, 44207, 44208
Colostomy	44320, 44188
Abscess drainage	49020, 49021, 49060, 49061, 49423, 49424

[a] International Classification of Disease, 9th Edition, Clinical Modification

[b] Current Procedural Terminology

Table 2 Descriptives of Diverticulitis Cases by Outpatient Encounter Location ($n=2,576$)

Characteristic	ER $n=328$ (13%)	Clinic $n=2248$ (87%)	P value
Mean age (years)	56.2	60.0	<.0001
Male (%)	53.1	44.8	0.02
Comorbid condition (%)			
Any comorbid condition	23.8	12.4	<.0001
Diabetes	3.7	3.4	0.77
COPD/asthma	3.0	2.5	0.59
Renal failure	18.6	5.4	<.0001
Rheumatoid conditions	1.8	2.4	0.49

Patients with an encounter for diverticulitis were identified in two outpatient locations: ER and Clinic. Mean age, proportion of males, and frequency of comorbid conditions are presented for each group. Comparisons were made with χ^2 and one-sided ANOVA tests, using two-sided p values at the 0.05 level for significance

ER emergency room

while the remaining 2,248 (87%) encounters were in the Clinic. The mean age of patients seen in the ER was 56 years, compared to 60 years in the Clinic ($p<.0001$). Patients presenting to the ER were more likely to be male (53% vs. 45%, $p=.02$) and to have at least one predefined comorbid condition (24% vs. 12%, $p<.0001$).

Figure 1 displays the proportion of patients seen in each outpatient setting who had imaging studies in the 48-h period surrounding the encounter date. ER patients were more likely than Clinic patients to undergo abdominal imaging, including abdominal X-ray (27% vs. 9%, $p<.0001$) and abdominal/pelvic CT scan (85% vs. 14%, $p<.0001$). The use of both imaging modalities was more frequent among ER patients than Clinic patients (23% vs. 3%, $p<.0001$).

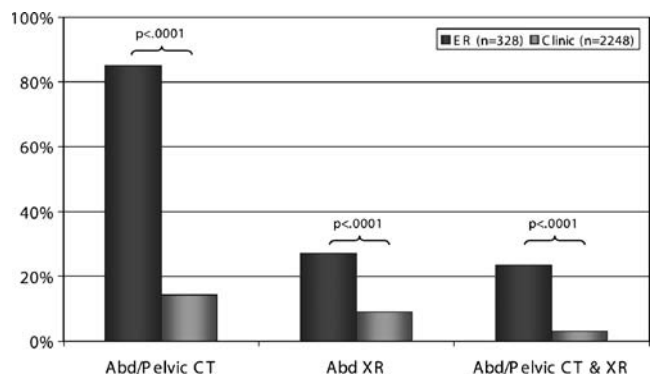**Figure 1** Imaging obtained by outpatient encounter location. Proportion of patients seen in the emergency room (*ER*) or Clinic undergoing abdominal imaging within the 48-h period surrounding the encounter of interest is presented. Comparisons were made with χ^2 tests, using two-sided p values at the 0.05 level for significance. *Abd/Pelvic CT* abdominal/pelvic computed tomography scan; *Abd XR* abdominal X-ray.

The proportion of patients in each outpatient setting who underwent a procedure in the 48-h period surrounding the encounter date was also evaluated. Urgent colectomy was performed in 1.2% of ER patients compared to 0.4% of Clinic patients ($p=.07$). Although infrequent among all patients, ER patients were more likely than Clinic patients to require colostomy (1.2% vs. 0.2%, $p<.01$) or abscess drainage (1.2% vs. 0.3%, $p=.01$).

Laboratory data became available through the electronic medical record for various UWHC and UWMF clinics beginning in 2006. Of the 36 ER patients and 315 Clinic patients who had laboratory values identified, the mean WBC count was significantly higher among patients evaluated in the ER compared to those seen in Clinic (12.5 vs. 9.9 K/μL, $p<.0001$). Mean hematocrit was lower among ER patients (39.9% vs. 41.5%, $p=.03$), but mean creatinine values did not differ by encounter location (1.08 vs. 0.96 mg/dL, $p=.19$). Figure 2 displays the proportion of patients in each outpatient setting who had abnormal lab values recorded. Emergency room patients more frequently had abnormal WBC values identified (69%) compared to Clinic patients (35%, $p<.001$).

Inpatient admission within 24 h was identified for those patients with an outpatient encounter included in our analysis. Of patients evaluated in the ER, 30% were hospitalized for diverticulitis within 24 h of their outpatient visit, compared to 3.5% of Clinic patients ($p<.0001$).

Discussion

This report identifies patients in a statewide medical system presenting with diverticulitis in the outpatient setting and describes the evaluation utilized by providers to support that diagnostic label. These results begin to address several
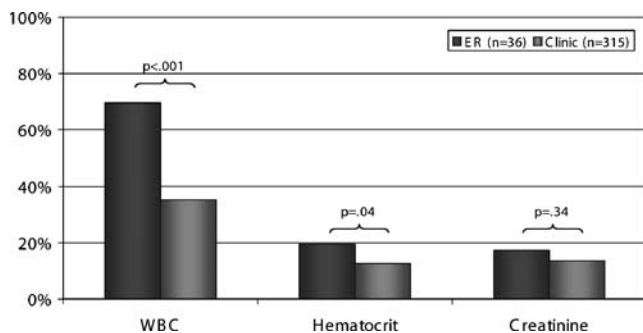
Figure 2 Abnormal laboratory test results by outpatient encounter location. Patients seen in the emergency room (*ER*) or Clinic with a diagnosis of diverticulitis between 2/15/2006 and 10/16/2008 were eligible for review of laboratory data in the electronic medical record. Proportion of patient seen in each location who had abnormal results defined by local laboratory reference ranges is presented. Comparisons were made with χ^2 tests, using two-sided p values at the 0.05 level for significance. *WBC* white blood cell.

knowledge deficits in understanding the outpatient portion of the diverticulitis spectrum, as prior study has been limited to hospitalized patients or anecdotal evidence from outpatient providers.[4,7,12–15] The current analysis identified more than 2,500 unique outpatient encounters for diverticulitis within a 5-year period, the great majority of which were in a clinic setting, rather than the emergency room. By comparison, we noted a total of 820 inpatient visits for diverticulitis in the same time period, far less than the number of outpatient encounters. With a lack of similar prior published reports, it is impossible to determine if this is comparable to the experience of other medical systems. However, given that only one diverticulitis episode per patient was included to ensure independence in this analysis, these counts are likely to underestimate the true number of visits initiated in the outpatient setting. As one considers the potential number of repeat outpatient visits and associated resources utilized (imaging, laboratory tests, medications, etc.), not to mention often-overlooked patient factors, such as time lost from work and decreased quality of life due to symptoms, the probable burden of diverticulitis on the healthcare system becomes quite large. Data from a US survey conducted in 1980 attributed $300 million in annual health care costs to diverticular disease; yet, this figure still primarily reflects those costs generated in the inpatient setting.[16,17] The impact of the outpatient aspect of this disease process has not been estimated until this analysis, and even this crude measure indicates that the resource drain on providers, patients, and the healthcare system itself is likely to be quite substantial.

Current practice guidelines state that the diagnosis of diverticulitis may be made on clinical grounds alone and that imaging should be used in select patients with severe or atypical symptoms as confirmatory tests.[9–11] Indeed, the only study which specifically reports attributes and outcomes of "office practice" patients with diverticulitis dates back to the 1950s and identified patients exclusively through clinical features including abdominal pain, fever, and leukocytosis.[7] Our analysis focused on the objective measures of abdominal imaging and leukocytosis, due to data limitations preventing assessment of more subjective elements such as pain. We found that despite being labeled with the ICD-9-CM code for diverticulitis, the overwhelming majority of patients seen in a clinic setting (86%) did not undergo abdominal imaging. In addition, for those with laboratory values recorded in the EMR, most Clinic patients (65%) did not have an abnormal WBC count. The patterns observed in this analysis imply that abdominal imaging and leukocytosis may not drive the diagnostic label of diverticulitis in the clinic setting. Instead, the diagnostic approach to patients presenting in Clinic with possible diverticulitis appears to be based on physical exam characteristics or other clinical evidence not captured in this analysis.

In contrast, patients seen in the emergency room frequently underwent abdominal imaging, especially CT scan. If outpatient practitioners are assumed to choose imaging in accordance with practice guidelines, this suggests that these patients were likely to have evidence of more severe disease, perhaps manifested by an elevated WBC count. However, leukocytosis was only observed in 69% of ER patients in this analysis. Thus, other factors, such as increased pressure to rule out confounding diagnoses and the ready availability of a variety of imaging modalities, likely contribute to the increased utilization of CT scans by ER practitioners. Although CT scan results were not reviewed during this analysis, the fact that all patients were labeled with diverticulitis suggests that the imaging findings were consistent with that diagnosis and may have proven more influential to the provider's decision than a lack of elevated WBC count in some cases. Thus, in contrast to the clinic setting, CT scans appear to be a key component of the diagnostic workup for patients presenting with presumed diverticulitis in the ER setting. Leukocytosis does not appear to be essential for diagnosis in either setting, as a significant proportion of patients were found to have normal WBC counts. This is in contrast to published practice guidelines, which indicate that leukocytosis is critical to the clinical diagnosis of diverticulitis, especially in the absence of abdominal imaging.[7–11]

While clinical assessment alone may be sufficient for successful medical management of diverticular disease, and potentially more economical in the short-run, the lack of objective evidence, particularly imaging, is likely to raise concerns and questions should this patient be referred for surgical evaluation and treatment. Most surgeons hesitate to offer an elective operation to a patient whose prior diverticulitis episodes have no confirmatory imaging. The current findings reveal that a substantial number of patients, especially those evaluated in an outpatient clinic, do not receive such imaging as part of their diagnostic workup. By extension, these patients may not be strongly considered for elective colectomy upon initial referral for surgical consultation. While the cost-effectiveness of abdominal imaging for outpatient presentations of diverticulitis is not addressed in this study, the large proportion of patients diagnosed without imaging suggests that such an analysis will be important for future studies investigating the optimal outpatient management of this disease.

Urgent surgical intervention was rarely needed in our study population, with fewer than 2% of all patients requiring colectomy or abscess drainage in the 48-h period surrounding diagnosis. This has been suggested previously, as large cohort studies have demonstrated successful nonoperative management of acute diverticulitis in approximately 80% of hospitalized patients.[18,19] Low rates of emergent operation are expected in the present study, given the less severe

disease presentation anticipated in this cohort of outpatients. Inpatient admission rates within 24 h following outpatient presentation were similarly low, with 30% of ER patients and 3.5% of Clinic patients requiring hospitalization after the encounter of interest. Early admissions were likely initiated for a trial of conservative medical therapy, including intravenous antibiotics, as rates of colectomy within 24 h of the initial encounter are low. These findings suggest that while diverticulitis in the outpatient setting may be typically considered "uncomplicated" due to the rare need for emergent operation, a subset of patients will require more aggressive medical therapy on the basis of their clinical presentation. Further, as this analysis is limited to examining one outpatient episode and one subsequent inpatient episode, the full extent of the financial and quality of life burdens incurred by outpatients due to recurrence is assuredly underestimated. Although unique medical record numbers were available for all patients, investigation of recurrence events requiring ER evaluation or hospitalization beyond 24 h was not reliably possible, due to potential losses-to-follow-up as patients might be anticipated to seek urgent or emergent care in facilities not affiliated with our medical system. A more in-depth evaluation as to the recurrence patterns of outpatient diverticulitis will, therefore, be crucial in making optimal management and treatment decisions for these patients and should be taken into consideration in future analyses.

There are some limitations to this study. Implementation and adoption of the EMR has been an ongoing process in our medical system throughout the study period. Use of the EMR may create particular challenges related to missing data, as the absence of a test or other variable of interest may either indicate that the test was never ordered or that the test results were simply not recorded in the EMR. This is especially true for laboratory data, as clinics have only adopted electronic reporting of results in recent years. Manual chart abstraction could be used to clarify the implications of missing data in future studies but was not implemented in this analysis. In addition, this EMR-based analysis is limited to variables with discrete coding, and excludes information found in free-text format (i.e., finding of abdominal tenderness during the physical exam) due to the difficulty of exact matches and, thus, may not fully capture all clinically relevant elements characterizing the patients seen in various outpatient settings. Finally, this retrospective EMR analysis relies on ICD-9-CM diagnosis codes to identify patients with diverticulitis. Although we presume that this diagnosis is correctly given, the accuracy is unknown, as validated studies on the identification of diverticulitis patients are lacking and will, therefore, be the impetus of future study.

In conclusion, this analysis describes the subpopulation of diverticulitis patients presenting in the outpatient setting and the extent to which objective data (abdominal imaging

and WBC count) contributes to making this diagnosis. In our medical system, patients labeled with diverticulitis are more commonly seen in an outpatient setting and rarely require urgent surgical intervention or admission. Consistent with practice guidelines, abdominal CT scans are infrequently used in Clinic patients; in contrast, leukocytosis (when identified) is often absent. Thus, many outpatients are labeled with diverticulitis despite a lack of objective evidence, suggesting that other clinical factors persuade provider decision-making in this setting. These results motivate further investigation into the diagnostic criteria for diverticulitis, as accurate definition of this condition will be essential to answer remaining questions regarding the frequency and timing of recurrence, the influence of elective or emergent surgical management, and the quantifiable impact of outpatient diverticulitis on healthcare costs and patient quality of life.

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Author/Coauthor Contribution

Erin S. O'Connor MD—study design, data extraction, manuscript preparation; Glen Leverson PhD—study design, statistical analysis; Gregory Kennedy MD PhD—manuscript preparation; Charles P. Heise MD—study design, manuscript preparation

ORIGINAL ARTICLE

Predictive Factors for Negative Outcomes in Initial Non-operative Management of Suspected Appendicitis

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Ryo Kobayashi · Takeyuki Hiramatsu**

Abstract

Background Acute appendicitis has been reported to be managed with non-operative therapy at relatively high successful rate. However, risk factors for negative outcome have not been established.

Method Three hundred eighty consecutive patients who underwent initial therapy for suspected appendicitis were reviewed. They were divided into three groups: operation group, the group successfully managed with non-operative therapy (success group), and the group required surgical conversion (failure group). Preoperative clinical data were compared among the groups and risk factors for negative outcomes were investigated.

Result Thirteen patients were excluded due to contraindication for non-operative therapy. Of the remaining 367 patients, 143 patients (39.0%) were primarily treated with surgery, and 224 patients (61.0%) were initially managed with antibiotics. Among the 224 patients, 91 patients (40.6%) were refractory to antibiotics and converted to surgery after more than 24 h usage of antibiotics. Multivariate analysis revealed that elevated C-reactive protein (CRP) level (>4 mg/dL) and presence of appendicolith were significant risk factors for conversion. Morbidity rate showed no significant difference between the operative and failure groups.

Conclusion Elevated CRP concentration and appendicolith may predict the negative outcome in non-operative management. However, immediate appendectomy can possibly be avoided at least 24 h without increasing morbidity under the usage of antibiotics.

Introduction

Early diagnosis and surgical resection have been a mainstream in the treatment of acute appendicitis for over

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120 years since Fitz[1] published a classic paper on 237 patients with perforated appendicitis. However, recent increasing body of evidence has suggested that early surgical intervention may not always be necessary, and acute appendicitis can be managed in conservative way at relatively high successful rate with adequate doses of antibiotics.[2–4]

Initial non-operative management and/or delayed appendectomy seem preferable especially in perforated case because this strategy possibly decreases postoperative complications compared with a conventional approach by emergent operation.[2,5–6] However, non-operative management with antibiotics is not always effective especially in patients with advanced inflammation. There have been only a few evidences on the risk factors for negative outcomes in non-operative management of appendicitis.[4,7–8] Hence, optimal selection of initial treatment for acute appendicitis are still debatable.

To clarify the factors that may contribute to the negative outcomes in initial non-operative management, we have retrospectively reviewed consecutive 380 patients who underwent initial treatment for suspected appendicitis in a single institute and analyzed the factors that might be associated with failure in initial non-operative management.

Material and Methods

From January 2004 through December 2007, 380 patients were clinically diagnosed with acute appendicitis and treated at Yaizu City General Hospital. Data collected included patient demographics, histories, physical findings, laboratory findings, and CT scan findings prior to initial treatment. All data analyses were performed in accordance with the ethical guidelines for clinical studies at Yaizu City General Hospital.

Clinical Diagnosis of Appendicitis

Our diagnostic algorithm for acute appendicitis is summarized in Fig. 1. In short, both right lower quadrant pain and elevated inflammatory markers [white blood cell (WBC) count or C-reactive protein (CRP) concentration] are
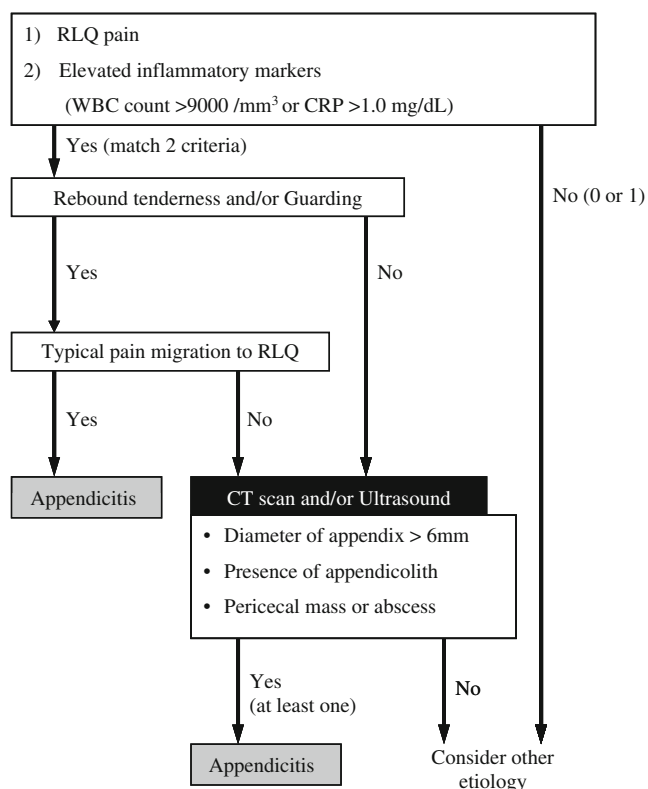
Figure 1 Diagnostic algorithm for suspected acute appendicitis at Yaizu City General Hospital (Yaizu algorithm).

prerequisites for suspecting appendicitis. If patients present several signs suggesting local peritonitis (rebound tenderness or guarding) accompanied with typical history of pain migration to right lower quadrant (RLQ), appendicitis is strongly suspected. Otherwise, image modalities such as CT scan or ultrasound, are required for confirmation. Enlarged appendix (>6 mm), presence of an appendicolith, and pericecal mass or abscess are typical radiographic signs suggesting the presence of appendicitis. At least one of these findings is required for radiographic confirmation in our algorithm. If all of these findings are absent both in CT scan and ultrasonography, appendicitis is less likely, and other etiology should be concerned. Subsequently, the present study population consisted of patients who were strongly suspected of having appendicitis.

Selection of Initial Treatment

Selection of the initial treatment for clinically suspected appendicitis was based on our routine treatment policy (Yaizu criteria; Fig. 2). First, if the patients did not conform to the exclusion criteria for non-operative management, they were initially managed with treatment options "they voluntarily selected" after they were well informed of the diagnosis, estimated pathologic severity, predicted outcomes, and risks of each treatment option. Based on hundreds of experiences, we have empirically known that most patients can be managed with IV antibiotics at relatively high successful rate even in gangrenous or perforated appendicitis. However, only a few solid evidences[2] are available regarding the effectiveness of conservative treatment for non-perforated appendicitis. Therefore, if the patients refused to undergo operation and selected initial non-operative management, their treatments were started under strict observation for at least 24 h stood by emergent operation in every case.

In this study, there are three groups to be analyzed: (1) operation group (OP group), (2) non-operative group successfully managed with antibiotics (success group), and (3) group required conversion to surgery during non-operative management (failure group). In the operation group, appendectomy or drainage was performed within 24 h after diagnosis. In the non-operative groups, patients received oral or IV antibiotics and were re-evaluated at 24 h after the first administration of the antibiotics. If the symptoms and/or inflammatory markers got worse at the time of re-evaluation, appendectomy or drainage was considered. Attempted non-operative therapy was defined by at least 24 h of antibiotic treatment prior to surgery. All patients who received non-operative management were followed up at least 1 year, and conversion rate for surgery and recurrence rate after successful non-operative management were recorded.
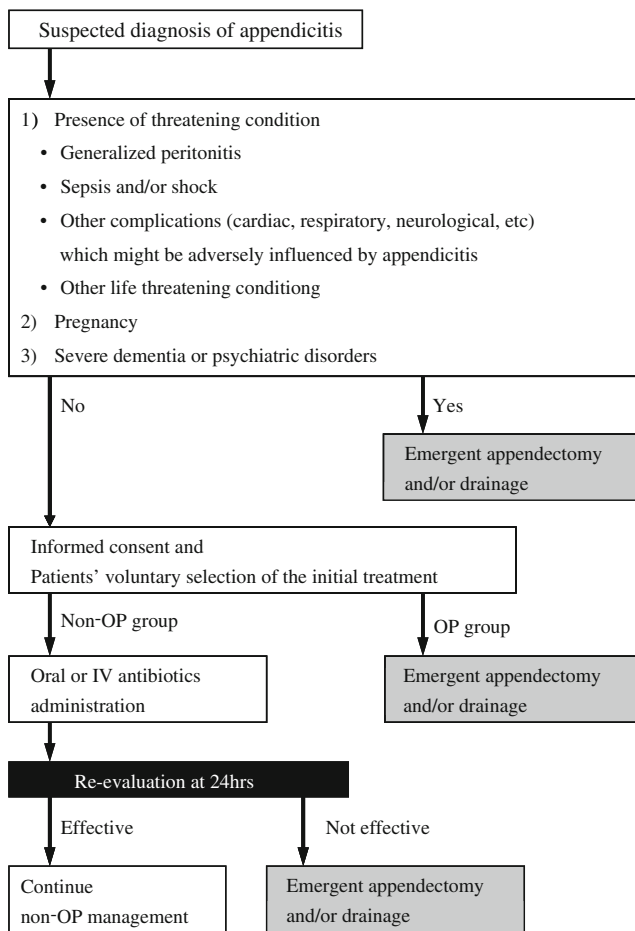
Figure 2 Treatment strategy for suspected acute appendicitis at Yaizu City General Hospital (Yaizu criteria).

Clinical data were recorded using an Excel (Microsoft) spreadsheet and analyzed using statistical software JMP 8 (SAS Institute Japan Ltd, Tokyo, Japan). For statistical analysis, Mann–Whitney U test was used for continuous data, and chi-squared test or Fischer's exact test were adopted for proportional data where appropriate. A value of $p<0.05$ was considered as statistically significant. Factors that were significant on the univariate analysis were included in multivariate analysis using logistic regression model.

Results

Among 380 patients, 13 patients [generalized peritonitis ($n=7$), incarcerated right obturator hernia ($n=1$), pregnancy ($n=2$), severe dementia ($n=2$), and autism ($n=1$)] were excluded from this study because they conformed to the exclusion criteria for non-operative management. Of the remaining 367 patients, 143 (39.0%) were initially treated with surgery and 224 (61.0%) were managed with antibiotic therapy primarily.

Among the non-operative cases, 91 patients (40.6%) were refractory to the conservative therapy and finally converted to the surgery.

All 380 patients were clinically diagnosed with appendicitis following our diagnostic algorithm. Because CT scan images taken before April 2004 were not available, frequency of radiographic findings were calculated using the data after May 2004. CT scan was required for diagnosis in 69.8% (97/139) of patients in the OP group and 65.3% (139/213) of patients in non-OP groups, respectively ($p=0.377$; chi-squared test). In non-OP groups, percentage of patients who required CT scan in the success and failure groups were 64.6% (82/127) and 66.3% (57/86), respectively ($p=0.797$). Negative appendectomy rate was 3.5% (5/143); one patient presented terminal ileitis without appendiceal inflammation, another patient showed mild swelling of the appendix due to diverticulitis, and appendiceal neoplasm was histopathologically proven in the remaining three patients. All of these patients were included in the present analysis.

Demographics of OP and non-OP groups are shown in Table 1. There was no significant difference in age, sex, and history of appendicitis, respectively. In operation cases, frequency of typical pain migration (umbilical region to RLQ) and WBC count were slightly higher than those in the non-operation case. However, the other physical findings or serum CRP concentration, which may be the most sensitive parameter to reflect the severity of appendicitis (our unpublished data), showed no significant difference between the groups. Presence of calcified appendicolith was more frequent in operation cases. Median durations of symptom prior to surgery were 27.4 h [interquartile range (IQR), 13.6–42.6] in the initial operation cases and 53.2 h (IQR, 32.6–98.5) in conversion cases, respectively ($p<0.0001$).

Table 2 shows clinical parameters of success and failure groups in primary non-operative management ($n=224$). Regarding the demographics, no significant difference was observed in age, sex, and history of appendicitis between these two groups. In the failure group, abdominal guarding was more frequent at presentation, and serum CRP level was significantly higher than that in success group (5.5 vs. 1.0 mg/dL). On CT findings, maximum diameter of the appendix, incidence of fluid collection, and presence of calcified appendicolith showed significant difference between the two groups. In multivariate analysis, elevated CRP level [>4 mg/dL; odds ratio (OR), 5.55; 95% confidence interval (CI), 1.94–17.29] and presence of appendicolith (OR, 4.65; 95% CI, 1.15–24.46) were significant factors, which might be associated with failure in non-operative therapy (Table 3).

Median length of hospital stay was slightly longer in failure group than that in success group (7 vs. 6 days; $p=$

Table 1 Demographics and Clinical Findings at Presentation

	OP cases (n=143)	Non-OP cases (n=224)	p value
Age	29 (15–50)[a]	30 (17–48.5)	0.50
Sex (male/female)	86/57	122/102	0.28
History of appendicitis	9.8% (14/143)	8.9% (20/224)	0.78
Physical findings	(n=140)	(n=155)	
Tender to RLQ	100% (140/140)	100% (155/155)	1.00
Rebound tenderness	65.7% (92/140)	59.3% (92/155)	0.22
Guarding	21.4% (30/140)	25.8% (40/155)	0.38
Migration of pain	80.0% (112/140)	67.7% (105/155)	0.049
Nausea/vomiting	41.4% (58/140)	31.6% (49/155)	0.08
Inflammatory markers	(n=143)	(n=224)	
WBC count (/mm^3)	13,370 (10,440–15,810)	12,030 (9,230–14,920)	0.005
CRP (mg/dL)	2.0 (0.2–6.9)	2.9 (0.4–9.6)	0.10
CT scan findings	(n=97)	(n=139)	
Diameter (mm)	10.5 (8.3–12.5)	10.0 (8.0–12.0)	0.23
Wall thickness (mm)	1.8 (1.5–2.3)	2.0 (1.6–2.6)	0.08
Fat stranding	30.9% (30/104)	39.6% (55/139)	0.17
Abscess	9.3% (9/104)	10.1% (14/139)	0.98
Fluid collection	21.6% (21/104)	20.9% (29/139)	0.88
Appendicolith	44.3% (43/104)	28.3% (41/138)	0.019

[a] Figures represent median (interquartile range)

0.019), whereas it was 5 days in primary surgery case (Table 4). No significant difference was observed in postoperative morbidity rate between primary operation and conversion cases (Table 4). Recurrence of appendicitis was observed in 17 patients (4.7%/years) during median follow-up period of 1,075 days (range, 75–1,778 days).

Discussion

The effective strategy to manage the appendicitis through conservative treatment and/or delayed appendectomy was firstly proposed in 2004 in pediatric perforated cases.[6] Since then, several articles regarding the feasibility and effective-

Table 2 Clinical Variables of Success and Failure Groups in Primary Non-operative Management

	Success group (n=133)	Failure group (n=91)	p value
Age	27 (16.5–46.5)[a]	34 (17–55)	0.10
Sex (Male/Female)	71/62	51/40	0.70
History of appendicitis	8.3% (11/133)	9.9% (9/91)	0.81
Physical findings	(n=64)	(n=91)	
Tender to RLQ	100% (64/64)	100% (91/91)	1.00
Rebound tenderness	54.7% (35/64)	61.5% (56/91)	0.39
Guarding	17.2% (11/64)	31.9% (29/91)	0.04
Migration of pain	60.9% (391/64)	72.5% (66/91)	0.13
Nausea/Vomiting	34.4% (22/64)	30.0% (27/91)	0.54
Inflammatory markers	(n=133)	(n=91)	
WBC count (/mm^3)	12,360 (10,090–15,210)	11,430 (7,900–14,350)	0.051
CRP (mg/dL)	1.0 (0.2–4.1)	5.5 (2.6–14.2)	<0.001
CT scan findings	(n=82)	(n=57)	
Diameter (mm)	8.8 (8.0–10.9)	11.2 (9.0–13.2)	0.001
Wall thickness (mm)	2.1 (1.7–2.6)	1.8 (1.4–2.8)	0.32
Fat stranding	32.9% (27/82)	49.1% (28/54)	0.05
Abscess	8.5% (7/82)	12.3% (7/57)	0.84
Fluid collection	14.6% (12/82)	29.8% (17/57)	0.03
Appendicolith	22.0% (18/82)	40.4% (23/57)	0.019

[a] Figures represent median (interquartile range)

Table 3 Multivariate Analysis for Negative Outcomes in Non-operative Management

	OR	95% CI	p value
Guarding	1.02	[0.26, 3.59]	0.97
Elevated CRP concentration (>4 mg/dL)	5.55	[1.94, 17.29]	0.001
Diameter of the appendix (>9.5 mm)	1.34	[0.23, 4.12]	0.61
Pericecal fluid collection	4.56	[0.92, 35.09]	0.06
Presence of appendicolith	4.65	[1.15, 24.46]	0.03

OR odds ratio, 95% CI 95% confidence interval

ness of primary non-operative management have been published both in pediatric and adult cases.[2,6–9] Successful rate of non-operative management has been reported to be relatively high, ranging from 80% to 95%.[2–4,9]

In the present series, however, non-operative management was not always effective, and the conversion rate to surgery was higher (40.3%) than those in reported cases (5-20%).[2–4,9] This might be partially because the present group possibly consisted of more advanced pathology compared with those in reported cases due to patients' voluntary selection of their initial treatment regardless of the severity of inflammation. Although there was no statistical difference in postoperative complication between primary operation and conversion cases, length of hospital stay was significantly longer, and accordingly, medical costs might be increased in conversion case (data not collected). Therefore, prediction of negative outcome is important in the selection of the initial non-operative management.

Regarding risk factors, absence of abscess,[4,8] presence of appendicolith,[4] and elevated percent bands[7] have been reported to be correlated with the failure in non-operative management. The multivariate analysis in our series showed similar results; elevated serum CRP concentration (>4 mg/dL) and the presence of appendicolith were significantly correlated with the negative outcomes in initial non-operative management.

Serum CRP concentration is an objective and quite sensitive parameter reflecting the severity of appendiceal inflammation (our unpublished data). It is easy to evaluate and is reliable compared with physical or radiographic findings in terms of its objectiveness. Although actual pathologic severity cannot be assessed in non-operative cases, elevated CRP concentration might imply advanced pathology. Therefore, severity of inflammation might be a risk factor for failure in conservative therapy. As for the calcified appendicolith, it has been reported as a potent risk factor for complicated appendicitis[10–11] or recurrence after non-operative management.[12–13] Furthermore, the appendicolith might have accelerating potential in progression of this disease (our unpublished data). Based on these facts, elevated serum CRP concentration and presence of appendicolith can be relative indications for surgery.

Regarding the timing of surgery, the optimal timing of surgery for appendicitis is still controversial, though it has been actively discussed so far.[14–16] In this study, however, no remarkable difference was observed in postoperative morbidity rate regardless of the timing of surgery, even though the time to surgery was apparently longer in the conversion cases compared with the primary operation cases (53.2 vs. 27.4 h; $p < 0.0001$). This result suggests that "immediate" operation may possibly be avoided at least 24 h under the usage of adequate doses of antibiotics.

Because the present study was not randomized and the initial treatment was, in principle, based on the patient's voluntary selection, it might be affected with selection bias to some extent. However, our clinical diagnosis of appendicitis was based on the strict algorithm (Fig. 1) to seek patients who are truly suffered from appendicitis. In addition, CRP concentration was similar between OP cases and non-OP cases (Table 1), suggesting that the two groups consisted of patients with similar backgrounds in terms of severity of the disease. Therefore, the influence of selection bias might be minimal in the present analysis.

Our results provided promising predictors for negative outcomes in initial non-operative management and also suggested the possibility that immediate appendectomy might not always be necessary under the usage of adequate doses of antibiotics. To develop solid criteria for the selection of initial management for acute appendicitis and optimal timing of surgery, further investigation including randomized controlled trial may be required.

Table 4 Post-treatment Outcomes

	LOH[a]		Postoperative morbidity	
Non-OP cases (n=224)				
Success group (n=133)	6 (5–8)[b]		–	
Failure group (n=91)	7 (5–12)	$p < 0.0001$	13.2% (12/91)	$p = 0.24$
OP cases (n=143)	5 (4–7)		8.4% (12/143)	

[a] Length of hospital stay
[b] Figures represent median (interquartile range)

Conclusion

Elevated CRP concentration and presence of appendicolith may predict the negative outcome in non-operative management. However, immediate appendectomy can possibly be avoided at least 24 h without increasing morbidity rate under the usage of adequate doses of antibiotics.

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ORIGINAL ARTICLE

C. difficile Colitis—Predictors of Fatal Outcome

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David A. Etzioni · Andreas M. Kaiser

Abstract

Purpose Clostridium difficile colitis (CDC) has a clinical spectrum ranging from mild diarrhea to fulminant, potentially fatal colitis. The pathophysiology for this variation remains poorly understood. A total abdominal colectomy may be lifesaving if performed before the point of no return. Identification of negative prognostic factors is desperately needed for optimization of the clinical and operative management.

Methods In-patients with CDC between 1999 and 2006 were identified through the discharge database (ICD-9: 008.45). Of these, patients with positive ELISA toxin or biopsy were included. Excluded were ELISA-negative patients. Data collected included general demographics, underlying medical conditions, APACHE II score, clinical and laboratory data, and duration of the medical treatment. Mortality and cure were the two endpoints. Regression analysis was used to identify parameters associated with mortality.

Results Three hundred ninety-eight patients (mean age 59, range 19–94) with CDC were analyzed. Fourteen patients (3.52%) underwent surgery. Mortality in the cohort was 10.3% (41/398 patients). Patients with fatal outcome had a longer pre-CDC hospital stay (11 vs. 6 days). Mortality was significantly ($p<0.05$) associated with a higher APACHE II score, a higher ASA class, a lower diastolic blood pressure, preexisting pulmonary and renal disease, use of steroids, evidence of toxic megacolon, higher WBCs, and clinical signs of sepsis and organ dysfunction (renal and pulmonary). Parameters without significant difference ($p>0.05$) included patient age, albumin, clinical presentation/examination parameters, and transplant status, other than the mentioned comorbidities. Of the 41 fatal outcomes, five patients (12.2%) underwent surgery, and 36 did not (87.8%). Mortality rate of the surgical group was 35.7% (four out of 14 patients). Comparison of the fatalities not undergoing surgery with the survivors revealed decreased clinical signs, suggesting a masking of the disease severity.

Conclusions Our study identified several clinical factors, which were associated with mortality from CDC. Future clinical studies will have to focus on the disease progression and the fatalities occurring either without an attempt for or despite surgical intervention, as an earlier intervention might have proven lifesaving.

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Abbreviations

ASA	American Society of Anesthesiologists
CDC	*C. difficile* colitis
LOS	Length of hospital stay
WBC	White blood cells

Introduction

Clostridium difficile infection has been associated with a wide spectrum of clinical presentations ranging from mild diarrhea to fulminant and potentially fatal toxic colitis.[1,2]

Alteration of the colonic flora as a result of antibiotic medications or other host factors allows for selection and overgrowth of toxin-producing *Clostridium* strains.[3] The severity of symptoms presumably is a function of the balance between bacterial virulence and host defense mechanisms.[4] The treatment of *C. difficile* colitis (CDC) in the majority of patients is conservative. Common measures include discontinuation of the causative antibiotics (if possible), administration of toxin binders (e.g., cholestyramine), and administration of antibiotics against the *C. difficile*. These antibiotics are given via oral–gastric (e.g., vancomycin), for some drugs (metronidazole) via oral or intravenous route.[5] A relatively small group of individuals with aggressive fulminant disease, however, will only have a chance to survive if they undergo an urgent radical surgery (total abdominal colectomy).[6,7]

The factors leading to these two stark contrasting forms of the same disease continue to be poorly understood. Several recent reports from the USA, Canada, and Europe have documented a growing numbers of both community- and hospital-acquired regional outbreaks of *C. difficile* colitis, which led to the identification of different virulence subtypes.[4,6,8–10] The emergence of hypervirulent strains has been associated with a higher incidence of the severe form of the disease with increased 30-day mortality due to reduced responsiveness to antibiotics and a higher incidence of toxic megacolon, intestinal perforation, and of the rate of needed colectomy.[6,9,11]

Insights into the pathophysiology of fulminant CDC remain sparse, and the current knowledge of predictive parameters for a fatal outcome is limited.[12] A total abdominal colectomy with end ileostomy may be lifesaving if performed before the point of no return has been crossed[7]; however, the decision for recommending such a big operation with an ostomy before a patient is visibly crashing remains in many instances an intellectual and emotional challenge. The literature with reports of high morbidity and mortality associated with the surgical treatment for fulminant colitis is counterproductive in that situation,[12] as these unreflected statements with wrongly superficial conclusions risk pushing physicians into a harmful direction. However, a total abdominal colectomy or even more extensive resections are very safe procedures under different circumstances.[13] The adverse outcome in the setting of fulminant colitis therefore seems to rather reflect the impact of inadequate timing with a delay of surgery rather than a risk of the surgery as such.[13–16] Hence, identification of negative prognostic factors is desperately needed for optimization of the decision-making process for the clinical and operative management.

In the current communication, we attempted to address some of these issues by reviewing all cases of *C. difficile* colitis at our 293-bed institution within an 8-year period. In contrast to other authors, we did not limit our study population to just the fulminant cases or the surgical patients but included all patients with a confirmed CDC. The objectives of our analysis were to develop a better understanding of the dynamics of unfavorable outcomes of CDC with and without surgery and to define predictive clinical parameters and constellations associated with failure of surgical or non-surgical management and with mortality.

Material and Methods

Patients who were treated for acute *C. difficile* colitis within the 8-year period between January 1999 and December 2006 at the USC University Hospital were identified from the inpatient discharge database and retrospectively analyzed. Included were all inpatients with an ICD-9 code of "*C. difficile* colitis" (008.45), whose *C. difficile* diagnosis was confirmed by means of a positive toxin ELISA, or a biopsy consistent with pseudomembranous colitis. Excluded were patients whose *C. difficile* was only diagnosed on a clinical basis but who remained test negative. After identification of the patients, the full medical records were reviewed by a group of three physicians (HD, ES, and CGR) using our institutional electronic medical record (Electronic Patient Folder, version 4.50.4, HBO & Company). Data were entered into a datasheet that had been generated with Microsoft Access XP and Excel XP. Among 124 parameters recorded were patient demographics, symptoms and duration of symptoms, underlying disease and comorbidities, clinical signs, white blood cells, ASA class, APACHE II scores, medical and surgical treatments, complications, outcome during the hospital stay, as well as other clinical, imaging, and laboratory data. For serial datapoints, the maximum value within the period related to the CDC treatment was used for data analysis.

The study protocol and data collection were approved by the Institutional Review Board of the University of Southern California and were compliant with HIPAA regulations.

Statistical Analysis

Results were reported in descriptive statistics and expressed as mean±standard deviation for continuous values, as median for nominal values (e.g., ASA). Statistical analysis was performed with SigmaStat software (Version 3.11, Systat Software Inc, Richmond, CA, USA) to compare groups of patients. The X^2 test or Fisher's exact test was used for nominal variables, the unpaired Student's t test or Mann–Whitney rank sum test for comparison of two groups, and one-way analysis of variance with Mann–Whitney rank sum test and Dunn's test as a post hoc test for comparison of more than two groups. Multivariate logistic

regression analysis was used to determine the predictive impact of multiple factors on mortality. Observed differences were considered statistically significant if $p < 0.05$.

Results

Patient Characteristics

Data from 398 patients (190 men, 208 women) with a mean age of 59.4 ± 16.3 years (range 19–94 years) were included in the study analysis. Based on the inclusion criteria, 97% of patients were toxin positive, and the remaining 3% were diagnosed by means of the pathology. Patient characteristics of the whole patient collective as well as of the four subgroups (survivors vs. fatal, medical vs. surgical) are shown in Table 1. A severe or fulminant/toxic course, defined as a presentation requiring surgery or resulting in death, occurred in 50 out of 398 patients (12.6%). The subgroups were equal and represented similar patients except for their size and the ASA classification.

Surgical Approach

Of the 14 surgical patients, 11 patients (78.6%) underwent a subtotal colectomy with diversion, one a colectomy without diversion, one underwent colostomy alone, and one underwent an exploration with colotomy and washout. Mortality in the subtotal colectomy with ileostomy group was 36.4% (four patients), while mortality with the other surgeries were 33.3% (one patient). It is of note that the patient treated only with a colonic washout did very well after surgery.

Mortality and Predictive Parameters

The whole cohort had a mortality of 41 out of 398 patients (10.3%). In five of them (12.2%), curative surgery was attempted but failed, resulting in a surgical mortality of 35.7% (five out of 14 patients who underwent surgery for toxic/fulminant CDC).

Direct comparison of survivors with the patients who died revealed a number of significant differences (as shown in Table 2) in both preexisting factors and parameters related to the acute presentation. In the survivors, most notably, the length of hospital stay prior to the diagnosis of CDC was significantly shorter, and the frequency of steroid use and renal or respiratory insufficiency were lower. The non-survivor group had statistically significantly higher APACHE II scores, a higher ASA class, lower diastolic blood pressures, a higher mean WBCs, and a higher percentage of them revealed clinical signs of sepsis and organ dysfunction (renal, pulmonary). Regression analysis confirmed the disease scores as well as preexisting renal and pulmonary dysfunction and steroid use as independent risk factors for mortality.

Correlation of the mortality with four ranges of WBCs is shown in Fig. 1, while the differences were not significant, and in the same range between WBCs <10, 10–15, and 16–20, there was a sharp increase of the mortality for WBCs >20. Figure 2a, b shows the impact of the ASA class and the APACHE II score on the mortality and the needed surgery.

A number of other examined factors (including the patients' age), however, did not reveal any significant difference (see Table 2). While steroid use remained associated with poor outcome on multivariate regression analysis, post-transplant status, other forms of immunosuppression or a history of cancer or chemotherapy did not show an impact on outcome.

Thirty-six of the 41 patients (87.8%) who died were not offered surgical treatment for various reasons, not all of which were apparent on the retrospective data review. This subgroup of severely ill patients represents the major target of the attempt to define predictors of negative outcome, as a

Table 1 Patient Characteristics

	Survivors medical	Survivors operative	Fatal medical	Fatal operative	Total	p<0.05
Number of patients (%)	348 (87.4)	9 (2.3)	36 (9.0)	5 (1.3)	398	-
Male/Female	165/183	5/4	17/19	3/2	190/208	-
Age	59±16	57±21	61±15	66±17	59±16	no
ASA [median]	3	3	4	3	3	yes[1]
% of patients with CDC after previous surgery	50.9	44.4	50.0	60.0	50.8	no
LOS before diagnosis of CDC	10.7	5.2	15.6	15.0	11.1	no

[a] Fatal med vs. SV surg; Fatal med vs. SV cons (Mann–Whitney rank sum test).

Table 2 Comparison of Survivors and Non-survivors

Factors	Parameters	Survivors ($n=357$)	Non-survivors ($n=41$)	p value
Preexisting	Pre-CDC LOS [days]	11.2	16.4	0.013
	Renal Insufficiency [%]	17.9	36.6	0.009
	Steroid use [%]	21.0	41.5	0.006
	Cancer [%]	29.4	19.5	0.251[a]
	COPD [%]	12.3	19.5	0.294[a]
	Diabetes [%]	31.1	43.9	0.138[a]
	Hypertension [%]	46.8	51.2	0.708[a]
	CAD [%]	21.8	24.4	0.863[a]
	Immunosuppression [%]	33.1	39.0	0.559[a]
	Transplant [%]	19.0	26.8	0.329[a]
	Chemotherapy [%]	11.2	4.9	0.327[a]
	Patient age [years]	59.0±16.4	62.4±15.2	0.097
Scores	ASA [median]	3	4	<0.001[b]
	APACHE II	6.1	8.1	0.006
Clinical signs	Diastolic BP	68.9	61.9	0.009
	Respiratory rate	20.1	22.9	0.034
	Sepsis [%]	2.0	17.1	<0.001
	Organ failure [%]	22.4	68.2	<0.001
	Renal failure [%]	16.0	43.9	<0.001
	Respiratory Failure [%]	5.0	39.0	<0.001

[a] Low power due to low number of patients detected in study

[b] Mann–Whitney rank sum test

timely decision for surgery might potentially have resulted in a different outcome. We therefore compared this group with the group of surgical survivors (see Table 3). Even if not all differences achieved statistical significance due to the relative small sample size, the medical non-survivors overall appeared to represent a sicker subcohort from the beginning with a longer pre-CDC length of stay and more comorbidities; in addition, however, they also displayed decreased clinical signs, hence suggesting a masking of the disease severity, or the patients were in a condition where the clinical exam was less reliable (e.g., on the respirator).
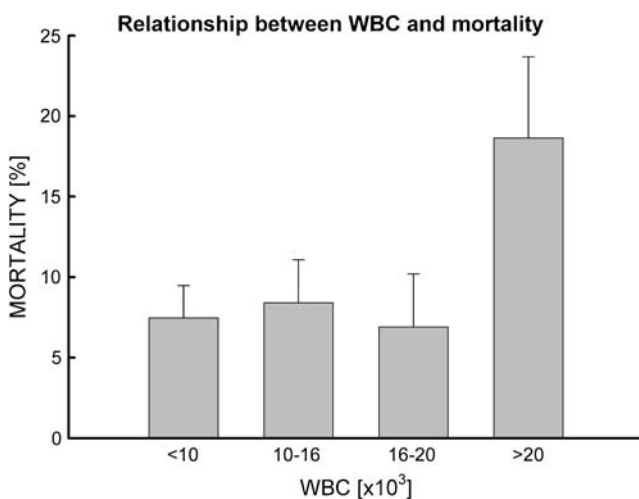

Figure 1 Correlation between WBC and mortality.

Comparison of CDC in Surgical/Fatal Group vs. Medical Survivors Group

A similar analysis was carried out by comparing patients with recognized severe disease, i.e., patients with *surgical/fatal* CDC on one hand with patients who survived with medical management on the other hand. As shown in Table 4, there were statistically significant differences between the two groups, which involved both preexisting parameters, scores, and elements of the clinical presentation. Yet, one also has to acknowledge that the medical survivor group contained 68 patients with an ASA of 4, and even two patients with an initial ASA of 5, which they paradoxically survived.

Medical vs. Surgical Management Group

Last but not least, we analyzed the impact of the various parameters on the probability to undergo surgery. The combination of metronidazole + vancomycin was used more frequently in the surgical group ($p<0.05$). Furthermore, pre-illness pulmonary disease and respiratory failure at time of CDC were significantly associated with a need for surgery. In addition, the following factors showed statistical significance (as shown in Table 5a): temperature, heart rate, WBC, abdominal pain, tenderness, and distention, and the APACHE II score., whereas differences among other factors did not achieve statistical significance (Table 5).
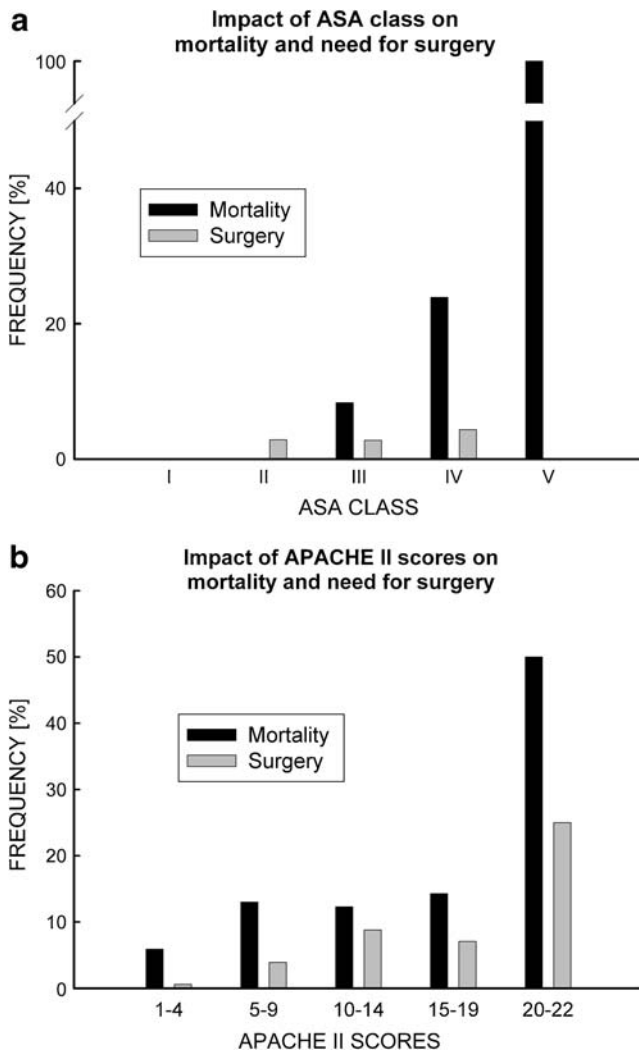
Figure 2 **a** Impact of ASA class on mortality and the needed surgery. **b** Impact of APACHE II class on mortality and the needed surgery.

Discussion

Fulminant colitis has been reported to develop in 3–8% of patients with *C. difficile* infection,[14] but in our cohort, it occurred in roughly 12.6% of all patients. Predictors of fatal outcome continue to be unsatisfactorily delineated. Recent surgical publications have focused on subsets of patients who underwent colectomy for fulminant pseudomembranous colitis.[7,12,15–18] Invariably, these surgical series with a median of 37 reported patients (range 14–130) demonstrated a high mortality rate of 34–47%. However, given that even more extensive colorectal resections such as a proctocolectomy are safely performed with minimal overall mortality of 2.3% (0.7–5.4%) in the elective and emergency context of ulcerative colitis,[13] one has to speculate that not the procedure per se is responsible for the poor outcome but that the surgical intervention for the reported

CDC patients simply came too late. This view is shared by other authors who, based on their series, suggested that operative intervention for fulminant *C. difficile* colitis earlier in the course and prior to multi organ failure was associated with decreased mortality.[7,16]

Key to implementing such a strategy to the clinical management is to identify parameters that predict unfavorable outcomes before the point of no return has been crossed. Some authors reported factors such as mental status changes, length of medical treatment, and hemodynamic instability with vasopressor requirement to correlate with poor outcome.[12,17,19] A recent critical care review on CDC equally concluded that emergent colectomy prior to vasopressor therapy was beneficial in preventing patient death.[14] Other authors, analyzing CDC in the critical care setting in 165 patients, suggested that operative intervention provided little benefit to patients with WBC less than 20,000 and normal lactate levels.[15] However, a closer look at those data with 38 surgical and 127 non-surgical patients provided inadequate power (only two patients) to substantiate the stated conclusion and revealed an even higher mortality rate in non-operated patients (41–95%),[15] hence rather suggesting an invariably unfavorable outcome if any critical care treatment is needed.

Nonetheless, the clinical paradigm that "sicker patients do worse" has not been uniformly confirmed either. Immunosuppression after kidney or pancreas–kidney transplantation in 702 patients, for example, was neither associated with a higher incidence of CDC overall (5.5%) nor of fulminant colitis with a need for a colectomy in particular (5.7%, 2/35 patients).[20] Similarly, Gellad et al. showed no significant difference between the development of complicated CDC between notably more morbid solid organ transplanted patients as compared to a non-transplanted reference groups.[21]

Our own study was undertaken to further investigate the issue. Even though it is not the largest series with regards to the reported colectomy patients, it is unique in the sense that we eliminated the selection bias of surgery- or fulminant-only populations by including and analyzing all inpatients of a well-defined single tertiary institution in order to look at clinical parameters and outcomes. It was our goal to analyze a large number of parameters in an attempt to define host constellations, which would lead to the development of the more aggressive form of this disease and hence justify an early or earlier surgical intervention. Our data were able to identify a number of host factors, which were significantly associated with a poorer outcome when we compared our survival group versus the non-survivors. An increased mortality on one hand was associated with the patients' preexisting conditions (e.g., renal and pulmonary insufficiency, a higher ASA class, and use of steroids). On the other hand, specific clinical

Table 3 Comparison of Medical Non-survivors to Surgical Survivors

Factors	Parameter	Surgical survivors ($n=9$)	Medical non-survivors ($n=36$)	p value
Preexisting	Pre-CDC LOS [days]	5.2	15.6	0.055
	Renal insufficiency [%]	0	36	0.098
	Diabetes [%]	0	47	0.031[a]
	Hypertension [%]	11	47	0.255
	Steroid use [%]	11	42	0.163
Scores	ASA [median]	3	4	0.018[a]
	APACHE II	8.1	7.1	0.187
Presentation	Abdominal pain [%]	78	28	0.022[a]
	Tenderness [%]	89	19	0.001[a]
	Abdominal distention [%]	67	22	0.075
	Acute abdomen [%]	56	0	0.010[a]
	Normal abdominal exam [%]	22	58	0.099
	Mental status change [%]	0	11	0.617
	Sepsis [%]	22	19	0.909
	Organ dysfunction [%]	33	69	0.099
	Renal failure [%]	22	44	0.312
	Respiratory failure [%]	33	42	0.711
	WBC	19.0	15.5	0.055

[a] Statistically significant difference

findings, e.g., a lower diastolic blood pressure, a higher APACHE II score, evidence of toxic megacolon, higher WBCs, and clinical signs of sepsis and organ dysfunction (renal, pulmonary, and cerebral), were negative predictors. As shown by others,[15] we found that the WBCs above 20,000 were associated with a higher mortality rate, even if there was not strictly a linear correlation.

The pre-CDC length of hospital stay was repeatedly found to have an impact on survival. The reasons for this observation are not clearly apparent. However, these patients were often more seriously ill from other causes, and

furthermore, one might also speculate that the CDC might have smoldered for a longer period under the radar screen. This interpretation is supported by the data that show a masking of clinical parameters in the medical non-survivors. This important finding emphasizes the need to be on high alert in patients with the mentioned preexisting conditions and who are inadequately assessable, e.g., because they show neurological impairment, sedation, or are otherwise intensive care dependent as demonstrated by higher ASA and APACHE II scores. It is of note that the mortality among our mid-classification of APACHE II (5–19) scores was

Table 4 Comparison of Medical Survivors to Surgical and/or Fatal CDC

Factors	Parameter	Medical survivors ($n=348$)	Surgical or fatal CDC ($n=50$)	p value
Preexisting	Pre-CDC LOS [days]	10.7	13.6	0.021
	Renal insufficiency [%]	18.4	30.0	<0.001
	Steroid use [%]	21.3	36.0	<0.001
Scores	ASA [median]	3	3	<0.001
	APACHE II	6.0	8.1	0.005
Presentation	Acute abdomen [%]	1.1	10.0	<0.001
	Normal abdominal exam [%]	60.3	46.0	<0.001
	Mental status change [%]	3.7	8.0	<0.001
	Sepsis [%]	1.7	18.0	<0.001
	Organ dysfunction [%]	22.1	62.0	<0.001
	renal failure [%]	15.8	40.0	<0.001
	respiratory failure [%]	4.3	38.0	<0.001
	WBC	12.3	16.7	0.008

Table 5 Comparison of Surgical and Non-surgical Patients

Factors	Parameter	Non-surgical (n=384)	Surgical (n=14)	p value
Preexisting	Pre-CDC LOS [days]	11.8	8.1	0.810
	Age	59.5	60.2	0.878
	Cancer [%]	28.6	21.4	0.779
	Renal insufficiency [%]	20.1	14.3	0.849
	Diabetes [%]	33.3	7.14	0.077
	HTN [%]	47.6	35.7	0.514
	CAD [%]	21.9	28.6	0.791
	Steroid [%]	23.2	21.4	0.865
	Chemotherapy [%]	10.9	0	0.387
Presentation	Abdominal pain [%]	38.3	78.6	0.006
	Abdominal tenderness [%]	27.6	85.7	<0.001
	Abdominal distention [%]	26.8	71.4	<0.001
	WBC	12.7	19.8	0.014
	HR	91.6	111.1	0.003
	Temperature	99.1	100.3	0.010
	Respiratory rate	20.2	23.1	0.004
	Respiratory failure [%]	7.8	28.6	0.025
	Hematocrit	32.2	33.7	0.591
	Albumin	2.82	2.58	0.176
	Systolic BP [mmHg]	124.7	119.9	0.712
	Diastolic BP [mmHg]	68.2	67	0.871
	Nausea [%]	18.7	42.8	0.059
	Vomiting [%]	8.67	14.3	0.796
	Organ failure [%]	26.6	42.9	0.298
	Mental status change	3.7	0	0.895
	CNS failure [%]	3.7	0	0.895
	Hepatic failure [%]	3.6	0	0.599
Scores	APACHE II	6.1	10.6	<0.001
	ASA [median]	3	3	0.954

similar, and the mortality did not seem to correlate with increasing scores in this segment. In addition, we found the mortality rate in that APACHE range to be 14.3%, which is markedly less than the 25% originally reported when the APACHE scoring system was introduced.[22]

The mortality rates for our surgical subgroup were in the same range as reported by other authors.[7,12,15–18] This known fact and the new finding of a much larger group of non-survivors who were not even operated suggests that a distinct set of disadvantages might have prevented these individuals from getting timely access to surgery.

In summary, we identified a number of parameters that are associated with unfavorable outcome. Yet we continue to have a limited understanding when it comes to a subgroup of medically managed patients who survived despite seemingly poor prognostic indicators. While our data are encouraging, they should for now be interpreted with clinical caution when it comes to the actual recommendation to treat an individual patient more aggressively. We suggest to use these risk factors to sensitize clinicians to the need of carefully assessing these complex patients with the constant question in mind whether a more aggressive treatment, e.g., a life-saving operation, should be considered.

Conclusion

With independent impact from both host factors and the bacterial virulence, the pathophysiology leading to an unfavorable course and outcome of *C. difficile* colitis remains a challenge. Our study not only identified several clinical factors, which were associated with increased mortality from CDC, but more importantly pointed out a subset of sicker patients, who due to blunting of clinical signs and symptoms carries a higher risk of poor outcome. Future investigations should be designed in a prospective fashion using our current criteria to monitor the continuous disease progression and narrow down the actual "point of no return" in order to minimize potentially preventable fatalities.

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ORIGINAL ARTICLE

Comparative Study between Clipless Laparoscopic Cholecystectomy by Harmonic Scalpel Versus Conventional Method: A Prospective Randomized Study

Tharwat Kandil · Ayman El Nakeeb · Emad El Hefnawy

Abstract

Background This study was planned to compare the traditional method of laparoscopic cholecystectomy (LC) versus LC using harmonic as regard the safety and efficacy.

Material and methods This study included group A (70 patients) in whom LC was conducted using the traditional method (TM) by clipping both cystic duct and artery and dissection of gallbladder from liver bed by diathermy, and group B (70 patients) LC was conducted using harmonic scalpel (HS) closure and division of both cystic duct and artery and dissection of gallbladder from liver bed by HS. The intraoperative and postoperative parameters were collected including duration of operation, postoperative pain, and complications.

Results HS provides a shorter operative duration than TM (33.21+9.6 vs. 51.7+13.79, respectively, $p=0.001$), with a significant less incidence of gallbladder peroration (7.1% vs. 18.6, $p=0.04$) and less rate of conversion to open cholecystectomy but not reach a statistical significance. The amount of postoperative drainage is significantly less in HS (29+30 vs. 47.7+31, $p=0.001$). No postoperative bile leak was encountered in HS, but it occurred in 2.9% of patients in TM. VAS in HS at 12 h postoperative was 3.25+1.84 vs 5.01+1.2 ($p=0.001$) and at 24 h postoperative was 3.12+1.64 vs. 4.48+1.89 ($p=0.001$).

Conclusion HS provides a complete hemobiliary stasis and is a safe alternative to stander clip of cystic duct and artery. It provides a shorter operative duration, less incidence of gallbladder perforation, less postoperative pain, and less rate of conversion to open cholecystectomy.

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Introduction

The advantages of laparoscopic cholecystectomy (LC) have been published extensively, and LC has become the gold standard in treating benign gallbladder diseases.[1–3] LC has largely replaced conventional open cholecystectomy.[4–6]

The traditional LC is commonly performed by means of dissector, the electrosurgical hook, spatula, and/or scissors, and this method has been used in most centers. Simple metal clips are frequently used to achieve cystic duct and artery closure.[7–8] Alternative technique using sutures for cystic duct closure is infrequently used.[9]

Various energy sources are routinely used as cutting and coagulating aids in laparoscopic surgery. Risks involved with the use of monopolar electrosurgery are significantly

greater.[10] Nonetheless, monopolar electrosurgery is the preferred method in more than 85% of surgeons.[11] Bipolar electrosurgery, being as effective as monopolar electrosurgery, has not been widely used in the LC procedure.[12]

The majority of electrosurgical injuries manifests late or goes unrecognized. The incidence of accidental burns caused by unintentional energy transmission during a LC ranges between 0.06% and 0.3%. However, only one or two patients in 1,000 are recognized.[13]

Several studies have described the use of ultrasound dissection technology in the LC, which concluded that ultrasonic dissection was safe and easy to use.[14–16] Few studies reported the harmonic scalpel, though superior, is not immune from causing undesirable biological effects on the body.[14–17] However, current available studies on LC using harmonic ultrasonic dissector are too small to determine any statistically significant difference in outcomes between traditional LC and LC with harmonic.

This study planned to compare traditional method of LC versus LC using harmonic as regard the safety and efficacy.

Material and Methods

This study was carried out from January 2008 to December 2008. Patients with gallbladder stone were treated by LC at the Gastroenterology Surgical Center and Mansoura University General Hospital and were included in this prospective randomized trial. The exclusion criteria included patients above 80 years old, patients with history of upper laparotomy, patients with common bile duct stones, and pregnant women.

All patients were subjected to thorough history and clinical examination focused on manifestation of gallstone disease and chronic liver disease. The following investigations were performed [whole blood picture, liver function tests (serum albumin, ALT, AST, and prothrombine time "INR"), HCV and HBV markers, and abdominal ultrasound] to show the state of the liver, portal vein, gallbladder, and CBD.

Informed consent was obtained from all patients to be included in the study, after explaining the nature of the disease and operative steps and possible complications. This study was approved by the local ethical committee.

The patients were randomized into two groups using enclosed envelope. The envelopes were drawn and opened by a nurse not otherwise engaged in the study before operation. Group A LC was done using traditional method, which included 70 patients, and group (B) LC was done using harmonic scalpel, which included 70 patients.

Under general anesthesia and the same antibiotics (third generation cephalosporin), surgery was performed using conventional four ports umbilical port, port below xiphoid,

and two ports below right costal margin. Pneumoperitoneum at pressure 12 mmHg was used.

In group A, LC was done using traditional method by dissection of Calot's triangle and clipping of both cystic duct and artery by metal clips. After that, dissecting the gallbladder from its bed by hook using electrocautery technique was performed. Finally, we insert abdominal drain in Morrison pouch.

In group B, LC was done using harmonic ACE (Ethicon Endo-Surgery) by dissection of Calot's and then occlusion of both cystic duct and artery using harmonic ACE. For closure and division of cystic pedicle, we set the instrument at power 2, i.e., more coagulation, and do it at two levels and separate the duct at the proximal level toward the gallbladder. When dissecting the gallbladder from its bed, we set it to level 5, i.e., more cutting power, and control of any bleeding from the bed using the active blade of harmonic ACE. Finally, we insert abdominal drain in Morrison pouch.

The intraoperative parameter observed included duration of the operation, amount of CO_2 used in the operation, bile escape, saline irrigation during operation, and volume of blood loss were all recorded.

The patients started oral feeding 8 h postoperatively; abdominal ultrasound was done for all patients in both groups on day of discharge to show any collection or free fluid in the abdomen. The patients were usually discharged after removal of drain and when the patient is surgically free.

Postoperative pain (PP) was evaluated at 12 h, 24 h, 48 h, and 1 week after operation using a visual analog scale (VAS)[18] (with which each patients noted the severity of pain at each evaluated time using a linear between 0 (no pain) and 10 (severe pain). Postoperative analgesia in the form of nonsteroidal anti-inflammatory drug was administered intramuscularly when required. If the patients still complained of pain, strong analgesic (1 mg/kg pethidine intramuscularly) was administered. The total dose of these medications was recorded.

Postoperative maximum body temperatures were recorded at (24 and 48 h) for all patients.

Postoperative nausea and vomiting "PONV" were assessed after 24 and 48 h. Metoclopramide was given if the patients required reduction of nausea, and the total doses of this medication were recorded. The frequency of vomiting was recorded.

At the end of the first postoperative week, first month, and sixth postoperative month, patients underwent a clinical examination and an abdominal ultrasonography. In addition, blood sample was taken to show follow up of liver function.

The statistical analysis of the data in this study was preferred using the SPSS version 10. Analysis of data was by intension to treat. For continuous variables, descriptive statistics were calculated and reported as mean+SD. Cate-

Table 1 Demographic Data of Patients

Variables	Group A	Group B	p value
Age	41.38+11.91	40.97+11.56	0.835
	18–66	18–66	
Male/female	30/40	29/41	0.674
Body mass index	28.64+4.46	28.14+3.87	0.48
Co-morbid disease			
DM	12 (17.1%)	13 (18.6%)	0.826
Hypertension	7 (10%)	7 (10%)	0.892
Liver cirrhosis	15 (21.4%)	14 (20%)	0.796
Child A	13	12	
Child B	3	2	
Smoking	15 (21.4%)	13 (18.6%)	0.674

gorical variables were described using frequency distributions. The Student's t test for paired samples was used to detect differences in the means of continuous variables, and chi-square test was used in cases with low expected frequencies ($p<0.05$ was considered to be significant).

Results

This study was carried out from January 2008 to December 2008. One hundred fifty patients with gallbladder stone were treated by LC at the Gastroenterology Surgical Center and Mansoura University Hospital, and ten patients were excluded due to different reasons: three patients had common bile duct stones, three patients had previous history of laparotomy, two patients were above 80 years, and two patients refused to join in this study.

One hundred forty patients were included in this prospective randomized trial. They were randomly divided into two groups: group A, LC with conventional method that included 70 patients with a mean age of 41.38+11.91 and group B, LC using harmonic that included 70 patients with a mean age of 40.97+11.56. Demographic data of the patients on both groups are shown in Table 1.

Intraoperative and postoperative findings of both groups are shown in Table 2. The incidence of gallbladder perforation was significantly higher in the traditional group

than in the harmonic group (18.6% vs. 7.1%, respectively; $p=0.04$). The mean operative time was significantly shorter in the harmonic group than in the traditional group (33.21+9.62 min vs. 51.7+13.79 vs. respectively; $p=0.0001$). Intraoperative blood loss was significantly more in the traditional group than in the HS group (83.31+46.23 vs. 43.28+31.27; $p=0.0001$). In the traditional group, two cases (2.9%) were converted to open surgery (one due to unclear anatomy and one due to bleeding), but in the HS group, all cases were completed laparoscopically. The mean amount of postoperative drainage was significantly more in the traditional group than in the HS group (47.78+31.54 vs. 29+30.79 ml, $p=0.001$. The hospital stay was shorter in harmonic group (23.44+2.29 vs. 26.95+8.94 h, $p=0.002$).

The overall morbidity rate was 15.71% (11/70) in the traditional group versus 4.2% (3/70) in the HS group, with the difference being statistically insignificant. The rate of pulmonary and port site infection was higher in traditional group than HS group, but it did not reach statistical significance. There was bile leak encountered in two patients (2.9%) in the traditional group (one from accessory duct and the other from cystic duct), but no postoperative bile leak occurred in HS group. No bile duct injuries were encountered in the present study (Table 3).

The time course of changes in maximum body temperature from preoperative (baseline) values is shown in Table 3. There was no observed significant change in temperatures in both groups.

Although the total incidence of nausea and vomiting were higher in the traditional group, the number of patients who expressed suffering from nausea or vomiting did not differ significantly at different time points (Table 3).

The incidence of pain is significantly more in the traditional group at 12 h postoperatively (68.6% vs. 51.4%, $p=0.03$), but the incidence of PP at different postoperative time points (24 h, 48 h, and 1 week) differs but not did not reach a significance between both groups (Table 3). VAS in HS group was lower than in the traditional group; the difference is significant at 12 h postoperative (3.25+1.84 vs. 5.01+1.2, $p=0.0001$) and at 24 h postoperative (3.12+1.64 vs. 4.48+1.89, $p=0.0001$), but the difference was insignificant at 48 h and 1 week postoperative (Table 4).

Table 2 Intraoperative and Postoperative Parameter in Both Groups

Variables	Group A	Group B	p value
Intraoperative blood loss (ml)	83.31+46.23	43.28+31.27	0.0001
Bile spillage (patients)	13(18.6%)	5 (7.1%)	0.04
Duration of operation (min)	51.7+13.79	33.21+9.62	0.0001
Conversion rate	2 (2.9%)	0	0.156
Amount of drainage	47.78+31.54	29+30.79	0.001
Hospital stay (hours)	26.95+8.94	23.44+2.29	0.002

Table 3 Postoperative Complications

Variables	Group A	Group B	p value
Postoperative pulmonary complication	3 (4.3%)	1 (1.4%)	0.312
Port site infection	4 (5.7%)	1 (1.4%)	0.173
Postoperative collection	2 (2.9%)	1 (1.4%)	0.561
Postoperative bile leakage	2 (2.9%)	0	0.156
Body temperature			
Before the operation	36.6+0.5	36.74+0.4	0.310
24 h	37.6+0.6	37.29+0.4	0.01
48 h	37.6+0.6	37.36+0.4	0.901
Presence of postoperative nausea			
24 h	24 (34.3%)	16 (22.9%)	0.462
48 h	5 (7.1%)	3 (4.3%)	0.136
Presence of postoperative vomiting			
24 h	4 (5.7%)	2 (2.9%)	0.4
48 h	2 (2.9%)	1 (1.4%)	0.56

Discussion

LC is the gold standard treatment of gall stones. The ultrasonically activated (harmonic) scalpel has been proven to be an effective and safe instrument for dissection and hemostasis in both open and laparoscopic surgical procedures. To date, the primary use of the harmonic scalpel in LC has been for the division of cystic artery and liver bed dissection. Advancements in the harmonic scalpel blade tip now provide for the reliable ultrasonic division and closure of cystic duct.[19]

Ultrasonic scalpel causing three effects that act synergically: cavitation, coaptation/coagulation, and cutting. The lateral energy spread is minimal, and the risk of distant tissue damage is lower than that of electrosurgery.[20,21]

In our study, the mean operative time was significantly shorter in the harmonic group than in the traditional group (33.21+9.62 min vs. 51.7+13.79, respectively, $p=0.0001$). Samer et al. reported that statistically significant shorter mean operative time in the HS group can be attributed to several factors; the harmonic ACE is a multifunctional instrument. It replaces four instruments routinely used in the LC, namely, the dissector, clip applier, scissors, and electrosurgical hook or spatula. Finally, the activation of the harmonic ACE does not form smoke, therefore allowing the surgeon to work in a clear operative field throughout the operation.

In our study, intraoperative blood loss was significantly more in the traditional group than isn the HS group (83.31+46.23 vs. 43.28+31.27; $p=0.0001$). Westervalt[19] and Huscher et al.[22] reported that harmonic scalpel has been proven to be an effective and safe instrument for dissection and hemostasis.

The main finding of the present study is the absence of either minor or major bile leaks from the cystic-duct stump in the HS group, denoting that the harmonic shears are as safe and efficient as simple metal clips in achieving the closure of the cystic-duct stump in the LC. Samer et al. reported the same result about the absence of either minor or major bile leaks from the cystic-duct stump. Westervalt[19] found that no bile leaks from the cystic-duct stump in his 100 patients in whom the closure and division of the cystic duct was achieved solely by the harmonic shears. Huscher et al.[22] found that bile leaks were encountered in seven of the 331 patients (2.1%), in whom the closure and division of the cystic duct was achieved by the harmonic shears alone. This 2.1% cystic-duct leakage rate is comparable to the 2% rate reported in the literature when using other cystic-duct closure techniques.[22–24]

Table 4 Postoperative Pain

Variables	Group A	Group B	P value
Presence of pain			
12 h	48 (68.6%)	36 (51.4%)	0.03
24 h	40 (57.1%)	31 (44.3%)	0.13
48 h	25 (35.7%)	18 (25.7%)	0.2
1 w	5 (7.1%)	4 (5.7%)	0.73
Pain location (incisonal/shoulder)			
12 h	43/5	31/5	0.06
24 h	29/11	30/1	0.02
48 h	20/5	16/2	0.14
1 w	3/2	3/1	0.73
VAS			
12 h	5.01+1.2	3.25+1.84	0.000
24 h	4.48+1.89	3.12+1.64	0.000
48 h	1.77+0.83	1.65+1.08	0.487
1 week	1.07+0.25	1.05+0.23	0.733

Huscher et al.[22] stated that the blades were first applied more proximally for a few seconds to achieve a simple sealing of the lumen, then they were applied a few millimeters distal to the previous application site, holding the grasp until the division of the duct was accomplished.

Various examples of cystic-duct leakage are due to inadequate closure of the duct caused by mismatch of the clip arms, necrosis of the duct at the site of clipping, or slippage of the clips off the end of the duct and migration into the biliary tract.[25–30] The above-mentioned hazards inherent in the use of metallic clips were not encountered when closure and division of the cystic duct was achieved with the harmonic shears.

The use of ultracision was associated with a statistically significant lower incidence of gallbladder perforation compared to electrocautery (7.1% vs. 18.6%, respectively; $p=0.04$).[14] Samer et al. reported that the use of the harmonic ACE was associated with a statistically significant lower incidence of gallbladder perforation, compared to electrocautery (10% vs. 30%, respectively; $p=0.002$).

LC has become the standard treatment for gallbladder disease. However, despite its low degree of invasiveness, many patients complain of PP and PONV due to residual pneumoperitoneum.[31,32]

Many factors attributed to PP may be due to residual pneumoperitoneum, diaphragmatic stretch during laparoscopy, or duration of the operation with using large volume of gases.[32–34] The use of lower insufflations pressure (7.5 mmHg) has considerably decreased PP.[35] In our study, the incidence of pain is significantly more in the traditional group at 12 h postoperatively (68.6% vs. 51.4%, $p=0.03$), and VAS in HS group was lower than in traditional group; the difference is significant at 12 h postoperative (3.25+ 1.84 vs. 5.01+1.2, $p=0.0001$) and at 24 h postoperative (3.12+1.64 vs. 4.48+1.89, $p=0.0001$). This statistical difference may be attributed to several factors such as shorter duration of operation, so we use less amount of gasses, and less incidence of perforation of gallbladder in harmonic group so less escape of bile in the peritoneum.

The mean amount of postoperative drainage was significantly more in the traditional group than in the HS group (47.78+31.54 vs. 29+30.79 ml, $p=0.001$. The hospital stay was shorter in the harmonic group (23.44+2.29 vs. 26.95+ 8.94 h, $p=0.002$) as reported by Huscher et al.[22]

Conclusion

The harmonic scalpel provides complete hemobiliary stasis for all patients and is a safe alternative to stander clip of cystic duct and artery. It provides a shorter operative duration, less incidence of gallbladder perforation, less PP, and less rate of conversion to open cholecystectomy.

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ORIGINAL ARTICLE

Effectiveness of Prophylactic Antibiotics in a Population-Based Cohort of Patients Undergoing Planned Cholecystectomy

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Abstract

Background In the absence of randomized controlled trials with sufficient power to assess the effectiveness of prophylactic antibiotics (PA), the best evidence is provided by large population-based register studies.

Methods The Swedish Register of Gallstone Surgery and ERCP (GallRiks) started in May 2005 and reached 75% national coverage in 2007. During 2006 and 2007, a total of 16,400 operations were registered in GallRiks. In the present study, all elective procedures performed in 2006–2007 in units performing at least 25 operations annually were included in an analysis of the risk for postoperative infectious complications

Results Altogether 10,927 procedures were performed 2006–2007. Univariate logistic regression analysis revealed a paradoxical increase in postoperative infectious complications requiring antibiotic treatment and postoperative abscess if PA were given ($p<0.05$). This increase disappeared in multivariate analysis with adjustment for age, gender, presence of cholecystitis, accidental gallbladder perforation, and presence of bile duct stones.

Conclusion No benefit from PA was seen in this study on elective cholecystectomy. Although a randomized controlled trial could possibly show a reduction in the risk for postoperative infectious complications not detected in this study, such a reduction must be weighed against the risk of promoting drug resistance by the widespread use of PA.

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Introduction

Despite increasing concern about the risk of promoting the development of resistant bacteria,[1] prophylactic antibiotic treatment is widely used in routine surgery for gallstone disease.[2] The absence of evidence has led to uncertainty regarding the benefit of prophylactic antibiotics. A number of randomized controlled trials of prophylactic antibiotic treatment in laparoscopic cholecystectomy have been reported,[3–8] none of which have shown any reduction in the rate of infectious complications by the administration of prophylactic antibiotics. The inability of these trials to show significant outcome may, however, have been due to insufficient statistical power. In order to achieve a larger patient sample, two meta-analyses of low-risk patients undergoing laparoscopic cholecystectomy have recently been performed.[9,10] These meta-analyses were also not able to show any benefit from prophylactic antibiotics.

The effectiveness of prophylactic antibiotic treatment in cholecystectomy cannot be fully assessed without

stratification for low-risk and high-risk procedures. Whereas all randomized controlled trials so far published have focused on low-risk procedures, no study on high-risk procedures has been published. As high-risk cholecystectomies are more infrequent, it is difficult to assemble study samples of sufficient size for this group. On the other hand, the low incidence of infectious complications following low-risk procedures makes it impossible to reach sufficient statistical power in a trial on this group without a very large patient sample.[2] An alternative method covering large numbers of patients is through the use of a register study.

The question is not only whether or not prophylactic antibiotics prevent postoperative infectious complications but also whether it is worthwhile risking adverse reactions and the development of resistant bacteria in order to reduce the risk for rather infrequent and often harmless infectious complications after gallstone surgery. The purpose of this study was to explore the effectiveness of prophylactic antibiotics in preventing infectious complications in a population-based setting.

Materials and Methods

The Swedish Register for Cholecystectomy and ERCP (GallRiks) was started in May 2005 with the aim of registering indications, complications, results, and quality-of-life outcome of gallstone surgery on a national standardized basis. By the end of 2007, 56 hospitals were included in GallRiks. All surgical procedures for gallstone disease are registered online in GallRiks by the surgeon performing the procedure. Registered data include personal registration number, gender, medical history, American Society of Anesthesiologists (ASA) classification, data on indication for surgery, operation method, and perioperative complications. Administration of antibiotics is defined as prophylactic if it does not exceed 24 h. No standardized follow-up visit is performed, but 30 days after surgery, the local coordinator at each unit performs a review of the patient notes in order to detect and record any postoperative adverse event. The register is validated each year by blinded reassessment of a randomly selected sample of patient notes. The prevalence of errors has so far been lower than 2%. This study is based on data assembled in 2006–2007. All units where at least 25 procedures were performed annually were included.

Statistics

Two outcome measurements, any postoperative infection requiring antibiotic treatments and a postoperative abscess, were assessed. Postoperative abscess was defined as any localized infection that required percutaneous or surgical

drainage. They were used as dependent variable when univariate logistic analyses were performed, and gender, age, indication for surgery (gallstone disease with secondary complications versus uncomplicated gallstone disease), method of approach, and operative time were used as predictors. In order to assess the effectiveness of prophylactic antibiotics, we also performed multivariate logistic analyses with the same outcome measures and prophylactic antibiotics as covariate and adding potential confounding variables as covariates one by one.

We also did subgroup analyses of patients with accidental preoperative gallbladder perforation, patients undergoing open cholecystectomy, procedures lasting more than 90 min, and patients older than 60 years to see whether there was any group that had more benefit from prophylactic antibiotics than the rest of the group.

Results

By the end of 2007, 54 units were included in GallRiks. Between 2006 and 2007, altogether 15,652 cholecystectomies were registered comprising 4,725 emergency procedures and 10,927 planned procedures. The latter constitute the study group in this report—7,729 women and 3,198 men. Mean age was 49,9 years and standard deviation was 15,48 years. In 8,555 patients, surgery was performed because of pain attacks without complication secondary to the gallstone disease, and 2,372 underwent surgery because of secondary complications such as cholecystitis, pancreatitis, or cholangitis. Laparoscopic techniques were performed in 9,755 procedures, whereas 1,172 were conducted with an open approach. Prophylactic antibiotics were given to 2,715 patients. The distributions of the covariates included in the multivariate analyses are shown in Table 1. Altogether 377 patients were treated for postoperative infectious complications requiring antibiotics and 93 for postoperative abscess.

Univariate logistic regression analysis of postoperative infection requiring antibiotic treatment revealed a paradoxical increase in the risk for postoperative infection if prophylactic antibiotics were given (Fig. 1). This increase, however, disappeared if potential confounders were added in multivariate analysis. Nevertheless, the odds ratio never declined to a level significantly lower than 1, i.e., a significant reduction in the risk for postoperative infection if prophylactic antibiotics were given was not seen no matter how many covariates were added (Fig. 2). No additional variable had any significant impact on the confidence interval for the odds ratio for prophylactic antibiotics. The same outcome was seen with postoperative abscess as dependent variable (Figs. 3 and 4). No statistically significant reduction in the risk of postoperative

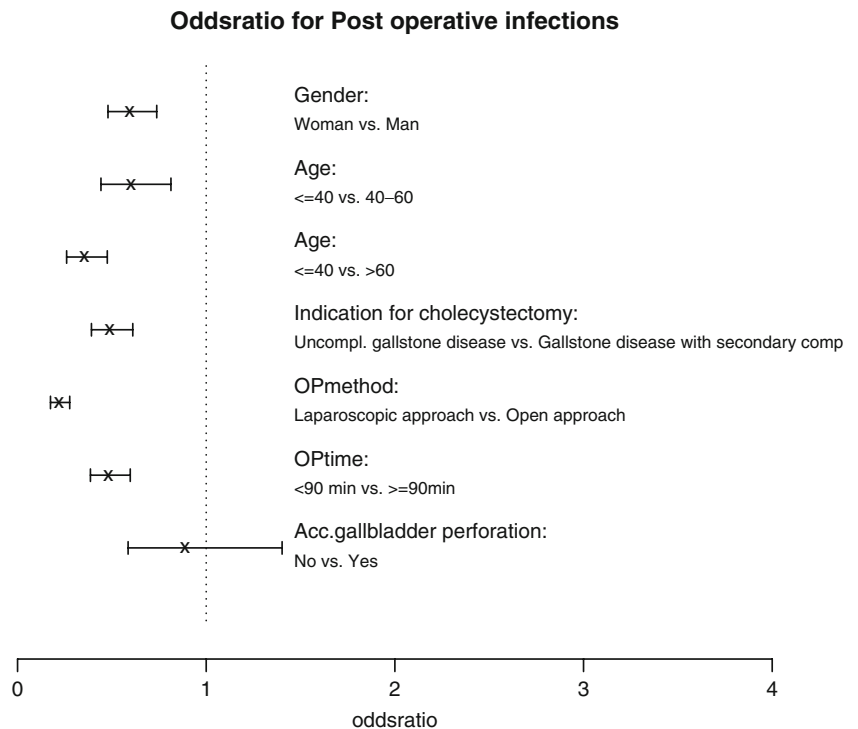**Table 1** Distributions of the Covariates Included in the Multivariate Analyses

	Prophylactic antibiotics	No prophylactic antibiotics	Total
Gender			
Men	1,004 (31%)	2,194 (69%)	3,198
Women	1,711 (22%)	6,018 (78%)	7,729
Age			
≤40 years	571 (17%)	2,762 (83%)	3,333
40–60 years	1,031 (22%)	3,562 (78%)	4,593
>60 years	1,113 (37%)	1,882 (63%)	2,995
Indication for cholecystectomies			
Uncomplicated gallstone disease	1,663 (19%)	6,892 (81%)	8,555
Gallstone disease with secondary complication	1,052 (44%)	1,320 (56%)	2,372
Operative approach			
Laparoscopic approach	2,091 (21%)	7,664 (79%)	9,755
Open approach	624 (53%)	548 (47%)	1,172
Operative time			
<90 min	961 (16%)	5,023 (84%)	5,984
≥90 min	1,754 (36%)	3,186 (64%)	4,940
Accidental gallbladder perforation			
No	2,461 (24%)	7,811 (76%)	10,272
Yes	241 (41%)	345 (59%)	586

infections from prophylactic antibiotics was seen in any of the subgroups (accidental preoperative gallbladder perforation, patients undergoing open cholecystectomy, procedures lasting more than 90 min, and patients older than 60 years).

Discussion

No benefit from prophylactic antibiotics was seen in the present study. Univariate analysis revealed a paradoxical

Figure 1 Odds ratios for the risk for developing postoperative infectious complications requiring antibiotic treatment determined from univariate logistic regression analyses.

Oddsratio for Post operative infections

Gender:
Woman vs. Man

Age:
<=40 vs. 40–60

Age:
<=40 vs. >60

Indication for cholecystectomy:
Uncompl. gallstone disease vs. Gallstone disease with secondary comp

OPmethod:
Laparoscopic approach vs. Open approach

OPtime:
<90 min vs. >=90min

Acc.gallbladder perforation:
No vs. Yes

oddsratio

increase in infectious complication rate. This increase is probably an effect of confounding factors that also increase the risk for postoperative infectious complications influencing the decision to give antibiotics. This increase did not remain significant if adjustment was made for the most important confounding factors. Indeed, we did not see any significant reduction in the incidence of infectious complications, no matter how many variables were considered.

Although theoretically there may be minor effects of prophylactic antibiotic treatment that remain obscured by confounding factors, any potential positive effect must be considered in the context of the risks of widespread use of

Outcome: post op. infections

Figure 2 Odds ratios for the risk for developing postoperative complications requiring antibiotic treatment if prophylactic antibiotics are given peroperatively. *Model 1* is derived from univariate analysis. In the subsequent models, the odds ratios for postoperative complication if prophylactic antibiotics are given peroperatively are determined in multivariate analysis with adjustment for gender, age, and indication for surgery (*model 2*), gender, age, and operative time (*model 3*), gender, age, operative time, and surgical approach (*model 4*), as model 4 + mode of admission (*model 5*), as model 4 + ASA (*model 6*), as model 4 + presence of common bile duct stones (*model 7*), as model 4 + presence of cholecystitis (*model 8*), as model 4 + presence of pancreatitis (*model 9*), as model 4 + elevated bilirubin (*model 10*), as model 4 + previous history of cholecystitis (*model 11*), as model 4 + previous history of pancreatitis (*model 12*), as model 4 + previous history of elevated bilirubin (*model 13*), as model 4 + pathological preoperative cholangiography (*model 14*), as model 4 + preoperative drainage applied (*model 15*), as model 4 + diagnosis at histopathological examination (*model 16*), as model 4 + accidental gallbladder perforation (*model 17*), as model 4 + accidental bowel perforation (*model 18*), as model 4 + preoperative necessitating intervention (*model 19*), and as model 4 + bile duct injury detected intraoperatively (*model 20*).

antibiotics, in particular the development of antibiotic resistance. The only definite way of confirming a decrease in infectious complications would be a randomized controlled trial with sufficient statistical power. Assuming a reduction from 4% to 3%, for example, a randomized controlled trial would require a sample of more than 10,000 patients in order to achieve an 80% chance of detecting a significant reduction at the $p<0.05$ level. Considering the fact that 4.8% of patients receiving prophylaxis and 3.3% of those who did not receive prophylaxis developed postoperative infectious complications that warranted antibiotic treatment, this seems a reasonable assumption. Although such a study may provide a better evidence base than a cohort study, it would have to be performed not only with the aim of revealing the potential effectiveness of prophylactic antibiotics but also taking the beneficence of the hypothesis of the study into consideration. Infectious complications that are avoided by prophylactic antibiotics are very few and seldom severe, whereas the use of prophylactic antibiotics on a wide scale carries the risk of increasing antibiotic resistance.

Another possible source of bias is patients with ongoing infection not related to the surgical procedure, such as pneumonia or urinary tract infection. Although this group is very small, the presence of infections of other locations may have affected the decision to give antibiotics as well as the postoperative course.
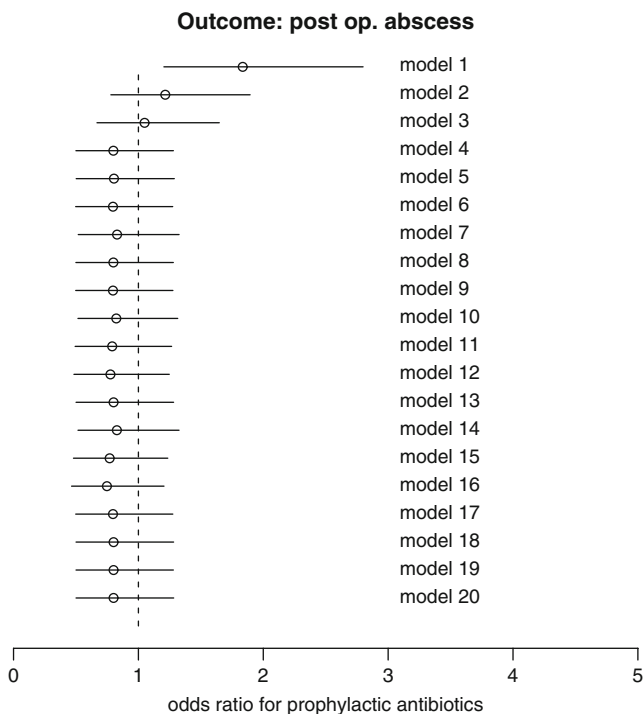
No matter how many potential confounding factors the outcome is adjusted for, the results of a nonrandomized

Oddsratio for abscess

Figure 3 Odds ratios for the risk for developing postoperative abscess determined from univariate logistic regression analysis.

Figure 4 Odds ratios for the risk for developing postoperative abscess. *Model 1* is derived from univariate analysis. In the subsequent models, the odds ratios for postoperative abscess if prophylactic antibiotics are given peroperatively are determined in multivariate analysis with adjustment for gender, age, and indication for surgery (*model 2*), gender, age, and operative time (*model 3*), gender, age, operative time, and surgical approach (*model 4*), as model 4 + mode of admission (*model 5*), as model 4 + ASA (*model 6*), as model 4 + presence of common bile duct stones (*model 7*), as model 4 + presence of cholecystitis (*model 8*), as model 4 + presence of pancreatitis (*model 9*), as model 4 + elevated bilirubin (*model 10*), as model 4 + previous history of cholecystitis (*model 11*), as model 4 + previous history of pancreatitis (*model 12*), as model 4 + previous history of elevated bilirubin (*model 13*), as model 4 + pathological preoperative cholangiography (*model 14*), as model 4 + preoperative drainage applied (*model 15*), as model 4 + diagnosis at histopathological examination (*model 16*), as model 4 + accidental gallbladder perforation (*model 17*), as model 4 + accidental bowel perforation (*model 18*), as model 4 + preoperative necessitating intervention (*model 19*), and as model 4 + bile duct injury detected intraoperatively (*model 20*).

study has to be interpreted with great caution when considering the effect of a treatment or intervention. Although multivariate analysis in this study included many of the most important confounding factors, there are some factors that are not covered by the register. Macroscopic contamination in the wound may have a strong impact on the decision to give prophylactic antibiotics as well as the risk for developing infectious complications. Prophylactic antibiotics may also have been given on indications related to concurrent conditions such as cardiac valve disease, immunosuppression, or presence of prosthetic devices. These confounding factors

may to some extent explain the paradoxical increase in infection rate with prophylactic antibiotics, although hardly to the extent that they would obscure a strong relationship between antibiotic prophylaxis and infectious complications.

The outcome of our study is in line with previous randomized controlled trials[3–8] and meta-analyses[9,10] that also failed to show that prophylactic antibiotics reduce the risk for postoperative infectious complications. Although neither the present nor previous studies can rule out the possibility that there may be some benefit from antibiotics in the case of massive bacterial contamination, immunosuppression, or high comorbidity, there is no room for routine administration of antibiotics in uncomplicated cholecystectomy. In our multivariate analysis, we adjusted for several of the factors that are often suggested as risk factors for infectious complications, i.e., open approach,[11] gallbladder perforation,[12] and high age.[13] Not even with these adjustments, however, could a favorable impact of antibiotics be seen.

In conclusion, no benefit from prophylactic antibiotics was seen in elective cholecystectomy. Even though a minor benefit in terms of reduced risk for postoperative infection may theoretically have been obscured by confounding factors in the absence of randomization, the low incidence of postoperative infections raises the issue not only of the effectiveness of prophylactic antibiotics but also whether a potential reduction in incidence is worthwhile when the negative effects of the widespread use of antibiotics are taken into consideration.

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ORIGINAL ARTICLE

Surgical Management of Infrahilar/Suprapancreatic Cholangiocarcinoma: an Analysis of the Surgical Procedures, Surgical Margins, and Survivals of 77 Patients

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Abstract

Background Optical surgical management of infrahilar/suprapancreatic cholangiocarcinoma remains controversial.
Methods Between 1988 and 2006, 77 patients with infrahilar/suprapancreatic cholangiocarcinoma underwent curative surgical resections following our intention-to-treat strategy. The clinicopathological factors affecting survival were evaluated using univariate and multivariate analyses with regard to the surgical procedures and surgical margins.
Results The surgical procedure included extrahepatic bile duct resection alone (EHBD; $n=17$), major hepatectomy combined with extrahepatic bile duct resection (MHx; $n=26$), pancreaticoduodenectomy (PD; $n=28$), and MHx and concomitant PD (HPD; $n=6$). Performance of MHx and/or PD in addition to EHBD increased surgical morbidity ($p=0.001$). Among patients undergoing the four surgical procedures (EHBD, MHx, PD, and HPD), no significant difference was found in the incidence of positive overall surgical margins (53%, 65%, 46%, and 67%, $p=0.51$) or long-term survivals (median survival time, 51, 27, 41, and 22 months, $p=0.60$). A multivariate analysis revealed that perineural invasion (95% confidence interval, 1.1–12.3, $p=0.009$), nodal metastasis (1.6–6.8, $p=0.001$), and blood transfusion (1.1–3.9, $p=0.02$) were independent predictors of a poor outcome. Perineural invasion was associated with positive radial margins ($p=0.045$) and submucosal ductal infiltration ($p=0.03$).
Conclusion Perineural invasion, rather than the type of surgical procedure, had a significant impact on surgical curability and survival of patients with infrahilar/suprapancreatic cholangiocarcinoma treated according to our intention-to-treat strategy.

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Introduction

Recent advances in imaging modalities and surgical strategies have improved the outcome of the surgical treatment for cholangiocarcinoma. Surgical resection for perihilar cholangiocarcinoma often involves major hepatectomy combined with extrahepatic bile duct resection (MHx) following preoperative biliary drainage and portal vein embolization.[1–7] In highly select institutions, the mortality of MHx for perihilar cholangiocarcinoma has been reduced to less than 1%,[3–5,7] and the 5-year survival rate has been increased up to 40%.[4,7] Pancreaticoduodenectomy (PD) has long been a standard procedure for middle or distal cholangiocarcinoma. The reported 5-year survival rate is 24–39%, and the surgical mortality is reported to be 2–7%.[8–15]

However, surgical treatment for infrahilar/suprapancreatic cholangiocarcinoma has never been fully discussed. Infrahilar/suprapancreatic bile duct can be classified into the superior or middle bile ducts according to the Japanese classification.[16]

Superior cholangiocarcinoma corresponds to Bismuth types I and II cholangiocarcinoma,[17] and middle cholangiocarcinoma corresponds to classical middle third cholangiocarcinoma.[18,19] These types of cholangiocarcinoma are originated from the bile duct in the hepatoduodenal ligament and not only do they often involve the adjacent hepatic artery and portal vein but they also often extend along the biliary tract in a mucosal and/or submucosal fashion.[20] Therefore, extrahepatic bile duct resection (EHBD) is sometimes insufficient to secure negative surgical margins and MHx and/or PD in addition to EHBD is required to obtain a favorable prognosis.[21,22] MHx with concomitant PD (HPD) enables the extensive resection of biliary trees, but it is associated with significantly higher morbidity and mortality rates. Consequently, surgical procedure for the removal of infrahilar/suprapancreatic cholangiocarcinoma should be determined on the balance of the surgical curability and safety. In this study, we reviewed the medical records of 77 patients undergoing curative surgical resection with pathologically proven infrahilar/suprapancreatic cholangiocarcinoma and determined the prognostic factors for survival with regard to the impact of surgical procedures and surgical margins.

Patients and Methods

Between 1988 and 2006, 212 patients with extrahepatic cholangiocarcinoma underwent resectional surgery in the Hepatobiliary and Pancreatic Surgery Division, National Cancer Center Hospital, Tokyo. They were classified into hilar ($n=92$), superior or middle ($n=77$), and distal ($n=43$) cholangiocarcinoma, as determined using the final pathological diagnosis according to the Japanese classification as follows[16]: hilar, arising from the hilar bile duct; superior or middle, arising from the infrahilar/suprapancreatic bile duct; and distal, arising from the intrapancreatic bile duct. The infrahilar/suprapancreatic bile duct was equally divided into two parts; the upper part was termed as the "superior" bile duct, and the lower part was termed the "middle" bile duct.[16] In the present study, clinicopathological data on patients with superior or middle cholangiocarcinoma was reviewed.

Indication of Surgical Procedures

Preoperatively, the predominant location of the tumor and the extent of the tumor along the biliary tract were evaluated using imaging studies, including an enhanced computed tomography scan, ultrasonography, magnetic resonance imaging, cholangiography, and angiography. The surgical procedure was decided by each attending surgeon after considering the balance between the tumor extent and the safety of each surgical procedure, following our intention-to-treat strategy. All patients with obstructive jaundice underwent preoperative

biliary drainage with a percutaneous approach via the future remnant hemiliver, in principle.[3,4,7] MHx and PD were performed after the serum total bilirubin concentrations had decreased to less than 2 and 5 mg/dL, respectively.

After laparotomy and the exclusion of distant metastasis, all of the following four surgical procedures included a regional lymphadenectomy at the hepatoduodenal ligament, the upper part of the retropancreatic area, and the common hepatic artery. In patients with localized cholangiocarcinoma in the hepatoduodenal ligament, EHBD with lymphadenectomy was adopted, especially in patients with a poor general condition or high-risk factors.

When the tumor was predominantly located in the superior bile duct or tumor involvement in the right hepatic artery was observed on the preoperative images, an extended right hemihepatectomy combined with EHBD was scheduled, following preoperative portal embolization of the right hemiliver. The caudate lobe was completely removed during extended right hemihepatectomy. When the tumor was predominantly located in the left hemiliver, an extended left hemihepatectomy with EHBD was performed without preoperative portal vein embolization. The Spiegel lobe and part of the paracaval portion of the caudate lobe were removed, but part of the caudate process was sometimes preserved during extended left hemihepatectomy. The indications for preoperative portal vein embolization and the fundamental strategy for major hepatectomy are described elsewhere.[1,3,4,7]

When the tumor was predominantly located in the middle and distal bile duct, PD was performed.[15] Since 2000, pylorus-preserving PD (PPPD) has become the standard procedure, rather than the standard Whipple procedure.

HPD was indicated in patients with widespread cholangiocarcinoma or in patients with infrahilar/suprapancreatic cholangiocarcinoma involving the right hepatic artery, when the general condition of the patient was favorable. Preoperative portal embolization was performed in all the patients undergoing HPD.

When the tumor involved the major portal vein, aggressive combined resection of the portal vein and reconstruction was performed. When the tumor involved the future remnant main hepatic artery, hepatic arterial resection and reconstruction was performed under a surgical microscope by plastic surgeons.[23]

Diagnosis and Definition of Surgical Margins

Intraoperative evaluation of the hepatic-side and/or duodenal-side ductal margins was performed using frozen sections in all patients. When the duodenal-side ductal margin was positive, additional resection of the intrapancreatic bile duct was performed, as far as possible and in principle. When the hepatic-side ductal margin was positive, additional resection of the hepatic duct was performed, if possible. Positive surgical margins were classified into two categories: "mucosal

infiltration" and "submucosal infiltration".[20] When the ductal margins were positive for both mucosal and submucosal infiltration, they were defined as positive for submucosal infiltration in the following discussion: Radial margins were defined as surgical margins other than the ductal margins of the resected specimen.

Definition of Surgical Complications

Postoperative pancreatic fistula was defined according to the definition proposed by an international study group on pancreatic fistula[24]: an amylase concentration in the drain fluid (obtained on or after postoperative 3) greater than three times the standard serum amylase concentration. Pancreatic fistulas were classified into grades A, B, or C according to severity: briefly, grade A, a "transient fistula" that was not associated with a delay in hospital discharge; grade B, a fistula that led to a delay in discharge, with persistent drainage for more than 3 weeks; and grade C, a fistula that was usually associated with major complications. Grades B and C fistulas were considered significant complications. Delayed gastric emptying (DGE) was classified into grades A, B, and C according to the definitions used in the recently report[25]: grade A, unable to tolerate solid oral intake by postoperative day 7 but vomiting is uncommon; grade B, unable to tolerate solid oral intake by postoperative day 14 and vomiting is common; and grade C, unable to tolerate solid oral intake by postoperative day 21 and vomiting is common. Grades B and C DGE were considered significant complications. Postoperative hepatic insufficiency was defined as an increase in the postoperative total bilirubin value of more than 10 mg/dL.

Comparison of Clinicopathological Variables Among the Four Procedures

The clinicopathological variables were compared among the four groups of patients undergoing each procedure: EHBD, MHx, PD, and HPD. All of the sequential parameters were dichotomized at the median value of each variable.

Univariate and Multivariate Analyses of the Predictors of Survival

A univariate analysis of the two groups was performed using the following categorized variables: age (≥ 65, < 65 years), gender, period of surgical resection (1988–2000 vs. 2001–2006), surgical procedures (EHBD, MHx, PD, HPD), operative time (≥ 10, < 10 h), blood loss ($\geq 1,200$, $< 1,200$ mL), blood transfusion, morbidity, dominant location of the tumor (superior vs. middle bile duct), tumor differentiation (papillary to well-differentiated adenocarcinoma vs. moderately to poorly differentiated adenocarcinoma), depth of tumor infiltration (T1 vs. T2 or T3 in TNM classification[26]), presence or absence of pathological lymphatic invasion, venous invasion, perineural invasion, nodal metastasis (N factor in TNM classification[26]), distant metastasis (M factor in TNM classification[26] including hepatic metastasis and para-aortic nodal metastasis), status of hepatic-side ductal margin, duodenal-side ductal margin, overall ductal margins, radial margin, and overall surgical margins. Each threshold value was determined according to the median value of each category. A multivariate analysis was performed using factors that proved to be significant in the univariate analysis.

Statistical Analysis

The results are reported as the median and range unless otherwise specified. A parametric statistical analysis was performed using the chi-square analysis or Fisher's exact test. The cumulative survival rates were generated using the Kaplan–Meier method, and the difference between the rates of the groups was assessed using the log-rank test. Statistical significance was defined as a p value of less than 0.05. The statistical analyses were performed using a statistical analysis software package (SPSSII 11.0, SPSS Inc., Chicago, IL, USA).

Results

Summary of Surgical Procedures

The surgical procedures for infrahilar/suprapancreatic cholangiocarcinoma included EHBD alone ($n=17$), MHx combined with EHBD ($n=26$), PD ($n=28$), and MHx and concomitant PD ($n=6$). The hepatectomy procedures consisted of extended right hemihepatectomy ($n=19$), right trisegmentectomy ($n=1$), extended left hemihepatectomy ($n=5$), left trisegmentectomy ($n=1$), and right HPD ($n=6$). The PD procedures consisted of PPPD ($n=18$) and the standard Whipple procedure ($n=10$). Combined portal vein resection was performed in 12 patients (16%), and hepatic artery resection and reconstruction was performed in two patients (3%).

Overall surgical morbidity was 71%, and two patients (2.6%) died as a result of surgery; one patient who underwent an extended left hemihepatectomy plus EHBD died of intra-abdominal bleeding and hepatic insufficiency on day 6, and another patient who underwent EHBD died of intra-abdominal bleeding on day 8. No deaths occurred after 1999. The overall 5-year survival arte and median survival time of the 77 patients were 32% and 38 months, respectively.

Comparison of the Four Surgical Procedures

Table 1 showed the comparative results among the four surgical procedures. Selection of the surgical procedure was

Table 1 Comparison of Clinicopathological Variables Among the Four Groups of Patients Undergoing Four Types of Surgical Procedures in the Management of Superior or Middle Cholangiocarcinoma

		EHBD ($n=17$)	MHx ($n=26$)	PD ($n=28$)	HPD ($n=6$)	p value
Surgical period	1988–2000	11	10	8	1	0.06
	2001–2006	6	16	20	5	
Age (year)	<65	5	11	12	3	0.76
	≥65	12	15	16	3	
Predominant location	Bs	6	18	3	3	<0.001*
	Bm	11	8	25	3	
Operative time (min)	<600	14	11	16	0	0.003*
	≥600	3	14	12	6	
Blood loss (mL)	<1,200	15	8	16	0	<0.001*
	≥1,200	2	18	12	6	
Additional ductal resection	Performed (%)	9 (53%)	13 (50%)	12 (43%)	4 (67%)	0.73
Blood transfusion	Performed	2 (12%)	7 (27%)	9 (32%)	3 (50%)	0.27
Morbidity	Overall	6 (35%)	19 (73%)	24 (86%)	6 (100%)	0.001*
	POPF	2 (12%)	4 (15%)	22 (79%)	5 (83%)	<0.001*
	Bile leakage	1 (6%)	12 (46%)	3 (11%)	2 (33%)	0.004*
	DGE	6 (35%)	4 (15%)	13 (46%)	4 (67%)	0.035*
	Cholangitis	2 (12%)	5 (19%)	3 (11%)	1 (17%)	0.84
	Hepatic failure	0	0	0	1 (17%)	0.007*
Hospital stay (days)	<35	11	12	10	2	0.26
	≥35	6	14	18	4	
Mortality		1 (6%)	1 (4%)	0	0	0.62
T factor	T1	4	1	1	1	0.09
	T2–4	13	25	27	5	
Nodal status	Positive	7 (41%)	18 (69%)	14 (50%)	5 (83%)	0.13
M factor	M1	0	4 (15%)	1 (4%)	0	0.15
Perineural invasion	Positive	12 (71%)	22 (85%)	25 (89%)	6 (100%)	0.25
Clinical stage	I, II	17	21	26	6	0.13
	III, IV	0	5	2	0	
Overall surgical margin	Positive	9 (53%)	17 (65%)	13 (46%)	4 (67%)	0.51
Hepatic-side ductal margin	Positive	8 (47%)	11 (42%)	10 (36%)	2 (33%)	0.87
	Mucosal	4	5	6	1	
	Submucosal	4	6	4	1	
Duodenal-side ductal margin	Positive	5 (29%)	14 (54%)	0	0	<0.001*
	Mucosal	3	8	0	0	
	Submucosal	2	6	0	0	
Radial margin	Positive	6 (35%)	3 (8%)	6 (21%)	2 (33%)	0.28

Three M1 patients in hepatectomy group were found to have hepatic metastasis, and the remaining two M1 patients had para-aortic nodal metastasis

EHBD extrahepatic bile duct resection, *MHx* major hepatectomy, *PD* pancreaticoduodenectomy, *HPD* major hepatectomy plus pancreaticoduodenectomy, *Bs* superior bile duct, *Bm* middle bile duct, *POPF* postoperative pancreatic fistula (grade B or C), *DGE* delayed gastric emptying (grade B or C)

related to the predominant location of the tumor. Performance of MHx and/or PD in addition to EHBD was associated with increased operative time, blood loss, and surgical morbidities, but not with the hospital stay and mortality. PD guaranteed negative duodenal-side ductal margin; however, the incidence of positive overall surgical margin was comparable among the four groups of patients undergoing four types of surgical procedures.

Univariate and Multivariate Analyses of Prognostic Factors

In the univariate analysis of prognostic variables, blood transfusion, depth of tumor invasion (T2, T3), nodal metastasis, distant metastasis, perineural invasion, and a positive radial margin were significantly predictors of a poor outcome (Table 2). A multivariate analysis of these six variables revealed that blood transfusion, nodal metastasis,

Table 2 Univariate Analysis of Prognostic Factors of 77 Patients Undergoing Surgical Resection for Superior or Middle Cholangiocarcinoma

		Number	Overall 5-year survival rate (%)	MST (months)	p value
Patient characteristics					
Age	≤65	35	41	51	0.21
	>65	42	24	34	
Gender	Male	57	36	38	0.25
	Female	20	23	29	
Operative period	1988–2000	30	30	38	0.85
	2001–2006	47	37	33	
Surgical parameters					
Surgical procedure	EHBD	17	29	51	0.60
	MHx	26	32	27	
	PD	28	35	41	
	HPD	6	44	22	
Operative time	<10 h	41	32	51	0.18
	≥10 h	35	38	33	
Blood loss	<1,200 mL	39	30	51	0.29
	≥1,200 mL	38	38	29	
Blood transfusion	Not performed	56	36	51	0.02*
	Performed	21	21	24	
Morbidity	Absent	22	29	51	0.37
	Present	55	33	34	
Pathological factors					
Dominant location	Bs	30	30	28	0.36
	Bm	47	35	51	
Differentiation[a]	pap, well	29	32	51	0.24
	mod, por	47	34	33	
T factor in TNM	T1	7	100	ND	0.01*
	T2, T3	70	27	33	
Lymphatic invasion	Absent	11	60	83	0.054
	Present	66	28	33	
Venous invasion	Absent	20	53	75	0.15
	Present	57	24	33	
Perineural invasion	Absent	12	132	69	0.005*
	Present	65	23	33	
N factor in TNM	N0	33	52	75	0.0004*
	N1	44	16	26	
M factor in TNM	M0	72	33	36	0.048*
	M1	5	ND	15	
Hepatic-side ductal margin	Negative	46	34	33	0.98
	Positive	31	24	39	
Duodenal-side ductal margin	Negative	58	31	33	0.58
	Positive	19	34	51	
Overall ductal margins	Negative	41	30	33	0.52
	Positive	36	35	51	
Radial margin	Negative	60	38	51	0.03*
	Positive	17	10	23	
Overall surgical margin	Negative	34	35	38	0.47
	Positive	43	29	39	

T and N factors are determined by TNM classification of malignant tumors, 6th edition

EHBD extrahepatic bile duct resection, *MHx* major hepatectomy, *PD* pancreaticoduodenectomy, *HPD* major hepatectomy plus pancreaticoduodenectomy, *Bs* superior bile duct, *Bm* middle bile duct, *pap* papillary adenocarcinoma, *well* well-differentiated adenocarcinoma, *mod* moderately differentiated adenocarcinoma, *por* poorly differentiated adenocarcinoma

*p<0.05

[a] Excluding one patient with mucinous carcinoma

and perineural invasion were independent predictors of a poor outcome (Table 3). No significant survival difference was found among the four groups of patients undergoing the four procedures (Fig. 1).

Perineural invasion was positive in all the patients with a positive radial margin (*n*=17) or a positive intestinal ductal margin (*n*=19). There was a significant relationship between perineural invasion and a positive radial margin (*p*=0.045) and between perineural invasion and a positive submucosal ductal margin (*p*=0.03; Table 4).

Ductal Margin Status and Local Recurrence

The frozen section was positive in 32 patients, but it turned to be negative on the permanent section in one patient. The positive predictive value was 0.97. The frozen section was negative in 45 patients, but it turned to be positive on the frozen section in five patients. Thus, the negative predictive value was 0.89. Among the 36 patients with a positive ductal margin, 17 patients had only mucosal infiltration, while 19 patients had submucosal infiltration. One of the 17 patients with positive mucosal infiltration (6%) developed a ductal recurrence, while eight of the 19 patients with positive submucosal infiltration developed a ductal recurrence (42%); the incidence of ductal recurrence was significantly higher in patients with submucosal infiltration than in patients with mucosal infiltration (*p*=0.01). Among the 31 patients with positive hepatic-side ductal margins, the survival of patients with positive submucosal infiltration (*n*=16) was worse than that of patients with only positive mucosal infiltration (*n*=15, *p*=0.004; Fig. 2).

Discussion

Although many authors have discussed the surgical treatment of perihilar and distal cholangiocarcinoma,[1–15] optical surgical management of infrahilar/suprapancreatic cholangiocarcinoma has not been discussed in a lump. As infrahilar/suprapancreatic cholangiocarcinoma is located midway of the biliary tree, four types of surgical procedures, i.e., EHBD, MHx, PD, and HPD, can be indicated for removal of the

Table 3 Multivariate Analysis of Prognostic Factors of 77 Patients Undergoing Surgical Resection for Superior or Middle Cholangiocarcinoma

Variables	β	Risk ratio	95% CI	*p* value
Perineural invasion	1.433	4.191	1.428–12.295	0.009*
Nodal metastasis	1.210	3.354	1.644–6.843	0.001*
Blood transfusion	0.722	20.59	1.102–3.847	0.024*

*p<0.05
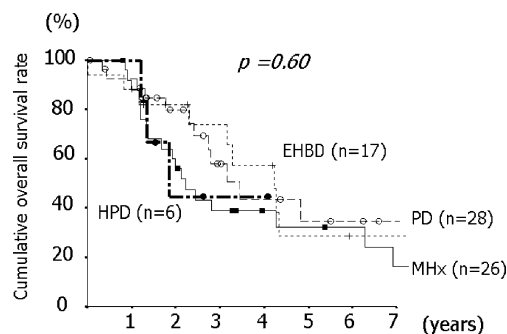
Figure 1 Cumulative overall survival of 77 patients with infrahilar/suprapancreatic cholangiocarcinoma treated with four types of surgical procedures. No significant difference was found in the survival of patients undergoing extrahepatic bile duct resection (*EHBD*; *dotted line with cross* (*n*=17)), major hepatectomy (*MHx*; *solid line with black square* (*n*=26)), pancreaticoduodenectomy (*PD*; *dotted line with white circle* (*n*=28)), or major hepatectomy plus pancreaticoduodenectomy (*HPD*; *bold line with black circle* (*n*=6); *p*=0.60).

tumor. The present study clearly showed that the perineural invasion was significantly associated with incidence of positive radial and submucosal ductal margins and was also an independent predictive factor for survival together with blood transfusion and nodal metastasis. Performance of MHx and/or PD in addition to EHBD increased surgical morbidities, but the type of procedure did not have significant impact on the incidence of positive overall surgical margins or on the long-term survivals of patients, if applied according to our intention-to-treat strategy.

Regarding the surgical treatment of perihilar cholangiocarcinoma, it has been repeatedly advocated that the surgical margin is an important prognostic factor.[1–4,6,9,11,13,14] In these reports, the ductal and radial margins were discussed together as the "surgical margin". We previously reported that when treating middle or distal cholangiocarcinoma, it is important to secure a negative radial margin, although it may be less beneficial to obtain a negative hepatic-side ductal margin.[15] In this study, the radial margin, which was associated with perineural invasion, was a predictor of survival according to a univariate analysis, but the ductal margin was not. Considering these results, when the radial margin is apparently positive, additional ductal resection for a positive ductal margin may not improve the survival of patients.

On the other hand, it is noteworthy that a positive submucosal ductal margin resulted in a higher incidence of local recurrence and a worse survival outcome, compared with the results for a positive mucosal ductal margin. The hepatic-side submucosal margin was a possible prognostic factor as shown in Fig. 2. As shown in Table 4, positive submucosal infiltration and positive ductal margins are strongly associated with perineural invasion, which proved to be an independent and significant prognostic factor. That is, the prognosis of patients might be largely influenced not by the ductal status but by the presence or absence of perineural

Table 4 Relationship Between the Perineural Invasion and the Radial and Ductal Margins

|		Perineural invasion		p value
		Negative	Positive	
Radial margin	Negative	12	48	0.045*
	Positive	0	17	
Ductal margin	Negative or mucosal positive	12	46	0.03*
	Submucosal positive	0	19	

*p<0.05

invasion. This strong relationship between perineural invasion and the extent of cancer invasion has been reported by other authors.[8,20,27] Bhuiya et al.[27] reported that perineural invasion was an independent prognostic factor that was not influenced by the site, size of the tumor, or lymph node metastasis. These results may suggest that the presence of perineural invasion represents a malignant behavior of the tumor and that this type of tumor infiltrates the adjacent structures in the hepatoduodenal ligament via neural pathways and also exhibits replacement growth along the ductal wall, resulting in positive radial or submucosal ductal margins. This importance of perineural invasion on a negative prognosis has been reported not only in patients with cholangiocarcinoma[2,4,8,20,27] but also in patient with gallbladder cancer.[28,29]

Ikeyama et al. reported that an extended right hemihepatectomy is essential for nodular and infiltrating (as classified according to the gross type on a cholangiogram) Bismuth type I or II hilar cholangiocarcinomas but that EHBD with or without limited hepatectomy is adequate in patients with papillary tumors.[21] In our series, 28 patients with macroscopically nodular and infiltrating Bismuth type I or II tumors underwent surgical resection (detailed data not shown). The median survival of the 14 patients who underwent extended right hemihepatectomy or right trisectionectomy was 2.0 years, while that of the 14 patients who underwent other procedures (EHBD, PD, or other types of hepatectomies) was 3.3 years ($p=0.38$). Therefore, we

Figure 2 Cumulative overall survival of 31 patients with positive hepatic-side ductal margins. The survival of patients with positive mucosal infiltration (*dotted line*, n=16) was significantly better than that of patients with positive submucosal infiltration (*solid line*, n=15; p=0.004).

cannot conclude that extended right hemihepatectomy is necessary for Bismuth type I or II hilar cholangiocarcinoma. The 5-year survival rate of the patients undergoing extended right hemihepatectomy in Ikeyama's series was favorable (63%) probably because negative surgical margins were obtained in 18 patients who underwent extended right hemihepatectomy.

MHx combined with EHBR enables the resection of the hilar bile duct including the biliary trees in the caudate process and should be one of the ideal surgical procedures for hilar cholangiocarcinoma.[1–7] The introduction of preoperative adequate biliary drainage and portal vein embolization of the future resectable liver[30] has dramatically increased the safety of the extensive hepatectomy. We previously reported a zero mortality rate for major hepatectomy for hilar cholangiocarcinoma between 2000 and 2004.[7] When the duodenal-side ductal margin is positive, additional resection of the intrapancreatic bile duct can be performed as far as the confluence with the main pancreatic duct. We have examined the ductal margins using frozen sections, and the positive and negative predictive values of frozen section were 0.97 and 0.86. We believe that these predictive values are acceptable and frozen sections are useful for intraoperative decision making. Nevertheless, the hepatic and duodenal sides of the ductal margins are positive in 41% and 44% of cases, respectively. These percentages illustrate the difficulty in obtaining a negative ductal margin against the ductal spread of cholangiocarcinoma.

Historically, we have been increasingly performed PD rather than other resections since 2001 mainly because of the improved safety of this procedure. The most troublesome drawback of PD for biliary cancer is the high incidence of pancreatic fistula from normal and soft pancreas tissue. HPD enables a broad resection of the biliary system, but is associated with a considerable risk of major pancreatic leakage and subsequent hepatic insufficiency. A two-staged PD may be an alternative approach that could enable life-threatening complications associated with pancreatic fistula to be avoided,[31] but we have only performed a one-stage pancreatic reconstruction using mucosa-to-mucosa anastomosis of the pancreatic duct. Although the incidence of pancreatic fistula was very high in our series (79%), no deaths occurred among the 34
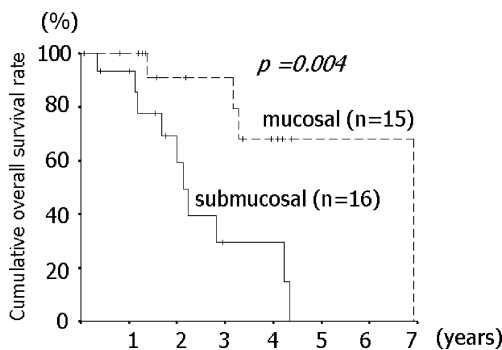
patients undergoing PD. This outcome may be partly due to the adequate postoperative drainage of pancreatic juice and the prevention of bleeding by wrapping the stump of the gastroduodenal artery with the falciform ligament.[32] PD guaranteed a negative duodenal-side ductal margin, but the overall incidence of a positive surgical margin was comparable between the PD group and the non-PD group (50% vs. 60%, $p=0.36$). These results indicate the difficulty in achieving a complete resection of cholangiocarcinoma arising in the hepatoduodenal ligament and also suggest that HPD should be indicated in highly select patients with widespread cholangiocarcinoma who have an acceptable general condition for surgery. All of the six patients undergoing HPD for widespread cholangiocarcinoma had perineural invasion, and five of them had nodal metastases, but the 5-year survival rate was 44%, which was relatively favorable. HPD could be indicated in patients with positive submucosal ductal margins and negative radial margins following MHx or PD because the submucosal infiltration on the hepatic-side ductal margin was a possible prognostic factor, associated with the presence of perineural invasion (Fig. 2; Table 4). In this sense, aggressive and safe application of HPD in selected patients might improve the curability and the survival.

Blood transfusion is reportedly associated with a poor outcome in several other malignancies, such as colorectal,[33] breast,[34] gastric,[35] periampullary,[36] and hepatocellular carcinoma.[37] The immunosuppressive effect of transfusion has been proposed as a possible reason.[38] Blood transfusion might also reflect the extensiveness of the operation, and extensive surgery, rather than blood transfusion itself, may be related to a poor outcome. Surgeons should make all possible efforts to prevent blood loss during the surgery and to avoid blood transfusion.

In summary, in the surgical management of infrahilar/suprapancreatic cholangiocarcinoma, no significant difference was found in the incidence of positive overall surgical margins or long-term survivals among the four groups of patients undergoing four types of surgical procedures if applied within our intention-to-treat strategy. Perineural invasion, rather than the type of surgical procedure, had a significant impact on surgical curability and survival.

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ORIGINAL ARTICLE

Surgical Management of Segmental and Sectoral Bile Duct Injury After Laparoscopic Cholecystectomy: a Challenging Situation

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Massimo Malago · Christoph Erich Broelsch

Abstract

Background Injury to a segmental or sectoral bile duct is a rare event in laparoscopic cholecystectomy; its diagnosis and management may be difficult.

Patients and Methods Between April 1998 and December 2006, 73 patients referred to the author's tertiary center for management of postcholecystectomy biliary complications were studied. The patients with segmental/sectoral bile duct injury were divided into two groups: injury to a duct which drains at least one Couinaud segment (type 1) or injury to a minor biliary radical in the gallbladder fossa (type 2). Beside the management of concomitant vascular or other biliary injury, type 1 segmental/sectoral duct injury was repaired by biliary–enteric anastomosis and type 2 by oversewing.

Results Ten out of 73 referred patients had segmental/sectoral duct injuries (eight type 1, two type 2). Despite multiple radiological imaging and endoscopic procedures, in seven patients, the lesion was identified only by precise surgical dissection. The median length of hospital treatment was 26 (range 9–47) days. One patient died due to sepsis before any definitive treatment. During the mean follow-up of 43 (range 27–111) months, seven patients remained asymptomatic while two patients developed biliary anastomotic strictures requiring intervention.

Conclusion Segmental/sectoral duct injury is difficult to be assessed by conventional radiological diagnostics and should be taken into consideration in every case of bile leakage. Surgical treatment, adapted to the type of lesion, generally results in a favorable outcome.

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Introduction

Bile duct injury (BDI) is a matter of ongoing concern in patients after laparoscopic cholecystectomy (LC).[1–3] Injury to a segmental or sectoral bile duct, mainly from the right biliary system, is a rare, but troublesome, event.[4–23] A low insertion of a right segmental/sectoral duct into the common bile duct or a short cystic duct, which joins the right biliary system presenting in 3.2% to 36.1% of the population studied, are important variants that place the right duct at risk of being injured.[9,14,24–28] An unrecognized segmental/sectoral duct injury (SDI) leads to an intricate postoperative course after LC, most often complicated by bile peritonitis, sepsis, or even secondary vascular complications.

Along with the development of laparoscopic surgery, an increasing experience in the management of bile duct injury after laparoscopic cholecystectomy has been achieved.[29–36] Nevertheless, duly diagnosis of the injury to the right

segmental/sectoral bile duct is still a dilemma, and the ideal treatment algorithm remains ill-defined.[7,13,36] The aim of this study is to present our experience in the management of SDI with special reference to the diagnostic pitfalls and surgical treatment.

Patients and Methods

Of 73 consecutive patients with LC-related biliary injuries referred to the author's tertiary center between April 1998 and December 2006, ten (13.7%) had SDI. Medical records from the referring institutions were reviewed for demographics, clinical data, and findings during and after LC. The diagnosis and the management in the author's institution, together with the data available from the referring hospitals, were recorded in a prospective database. The outcome was analyzed.

Management at the Primary Institute

In nine patients, LC was documented as uneventful. Postoperatively, however, further investigations were mandatory due to persistent abdominal pain (on days 7, 9, 9, 10, and 18, respectively), jaundice (on day 7), or evident bile leak (on days 2, 3, and 3, respectively). Endoscopic retrograde cholangiopancreaticography (ERCP; $n=6/9$), diagnostic laparoscopy ($n=2/9$), and open surgery ($n=5/9$) only revealed a cystic duct leak and an occlusion of the common bile duct (CBD) in one and two patients, respectively. In none of the nine patients, SDI was recognized. In one patient, SDI was noticed by the surgeons after conversion from laparoscopic to an open procedure. Despite oversewing, bile leakage persisted and made a referral necessary. The patients were referred on median day 12 after LC (range from 8 to 85 days) with persistent bile leak ($n=8$), unexpected abdominal pain ($n=1$), or jaundice ($n=1$).

Management at the Author's Institute

Helical computerized tomography (CT) or magnetic resonance cholangiography (MRC) was carried out for con-firmation and further characterization of the BDI. Multidetector computed tomographic cholangiography (MDCT-CA) with a biliary contrast agent, meglumine iotroxate (Biliscopin, Schering, Berlin, Germany),[37] was performed to delineate the intra- and extrahepatic biliary tree. Bile duct injuries others than SDI were classified according to Strasberg et al.[29] and Bismuth.[38] Vascular integrity was confirmed by Doppler ultrasonography or by angio-CT or magnetic resonance imaging in case of inconclusive findings of ultrasonography.

Intraoperative cholangiography (IOC) was performed to delineate the biliary tree whenever technically feasible. According to the size and drainage territory of the injured duct, two types of SDI were defined: (type 1) injury to the right anterior/posterior sectoral duct or any other segmental bile duct, which drains at least one Couinaud segment and (type2) injury to a minor biliary radical in the gallbladder fossa, i.e., injury to the subvesical bile duct or the duct of Luschka, which is usually smaller than 1 mm in diameter (Fig. 1). In patients with type 1 SDI, the respective liver segments were delineated by IOC through the injured duct. In patients with type 2 SDI, ICG was technically not feasible due to the small caliber of the duct.

Roux-en-Y biliary–enteric anastomosis to the segmental/sectoral duct was carried out in patients with type 1 SDI. A transanastomotic stent (silastic tube, Marquar, Boissy-Saint-Léger, France) was placed into the segmental/sectoral duct through the transjejunum route. If there was a concomitant injury at the main biliary tree, a biliary–enteric anastomosis to the common hepatic duct including the orifice of the segmental/sectoral duct was performed. Type 2 SDI was oversewn with 5–0 polypropylene sutures. In the presence of a concomitant injury of the right hepatic artery (RHA), arterial reconstruction or right hepatectomy was carried out as previously described in detail by the authors.[31,39]

Liver biochemistry was monitored, and ultrasonography was performed regularly during the postoperative hospital stay and every 6 to 12 months thereafter. Further follow-up information was collected by telephone, written questionnaire, and contact with the general practitioner. In patients

Figure 1 According to the size and drainage territory of the injured duct, two types of segmental/sectoral duct injury were defined: (type 1) injury to the right anterior/posterior sectoral duct or any other segmental bile duct, which drains at least one Couinaud segment and (type 2) injury to a minor biliary radical in the gallbladder fossa, i.e., injury to the subvesical bile duct or the duct of Luschka, which is usually smaller than 1 mm in diameter.
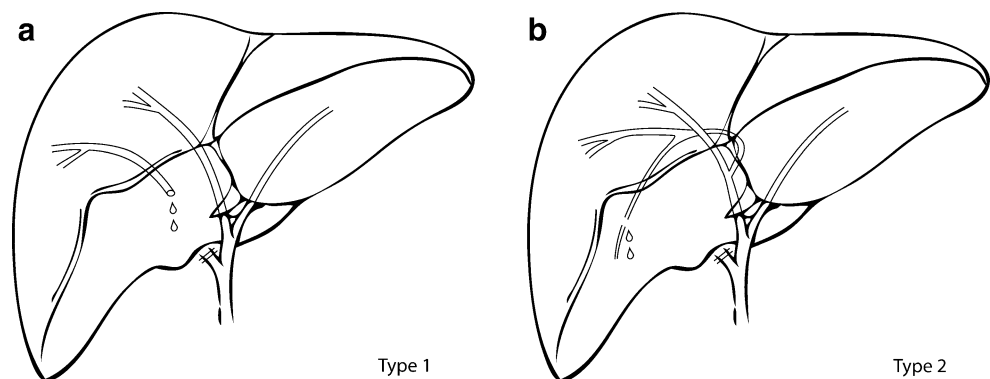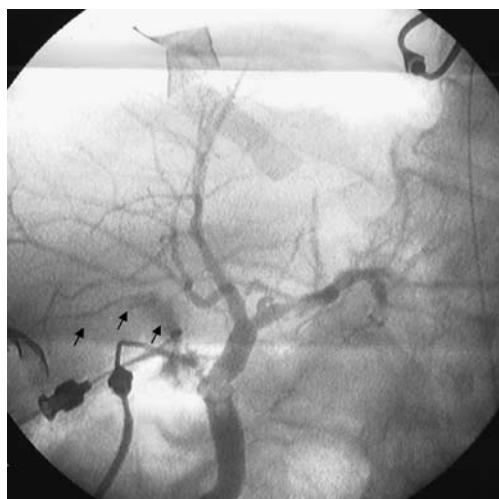
Figure 2 Type 1 segmental duct injury. The intraoperative cholangiography confirmed the drainage of the segmental liver parenchyma (*arrows*), which was not communicating with the main biliary tree after the injury.

with recurrent episodes of cholangitis, MRC was undertaken. Percutaneous transhepatic cholangiography (PTC) and balloon dilatation were performed in patients with anastomotic strictures. Reoperation with redo biliary enteric

anastomosis was attempted when conservative treatment failed.

Results

Of the eight patients with type 1 SDI, two patients had an injury to the right posterior sectoral bile duct. Six patients had an injured right segmental bile duct, which drains liver segment 6 and part of segment 5. Two patients presented with type 2 SDI. Concomitant biliary or vascular injury was found in eight patients: a cystic duct leak in two, a combined injury to RHA and common hepatic duct in one, isolated injuries to the common hepatic duct underneath or at the bifurcation in four, and an isolated injury to the RHA in one patient.

Identification of SDI

Using MDCT-CA (*n*=1) was the only successful preoperative diagnostics to reveal an SDI, while ERC (*n*=6), CT without biliary contrast agent (*n*=6), and MRC (*n*=2) failed. SDI was suspected by IOC in one patient only, showing an open orifice above the cystic duct. In the other
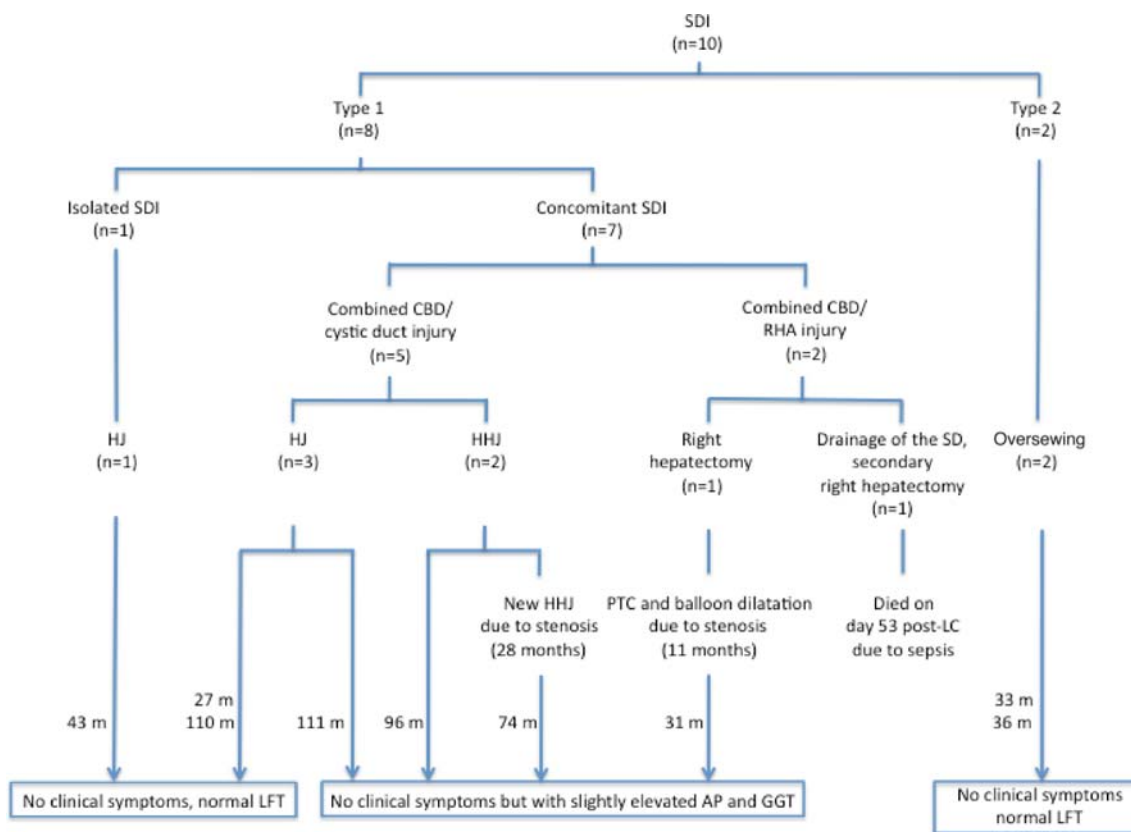
Figure 3 Flow chart of definitive treatment and follow-up in ten patients with segmental/sectoral duct injury in the author's institution. The follow-up time is presented as months (*m*). *AP* alkaline phosphatase, *CBD* common bile duct, *GGT* gamma glutamyl trans-

peptidase, *HJ* hepaticojejunostomy, *HHJ* hepatohepaticojejunostomy, *LC* laparoscopic cholecystectomy, *LFT* liver function test, *PTC* percutaneous transhepatic cholangiography, *RHA* right hepatic artery, *SD* segmental/sectoral duct, *SDI* segmental/sectoral duct injury.

Table 1 Published Series of Patients with Segmental/Sectoral Bile Duct Injury After Cholecystectomy

Series	Subtype	Recognition Methods	Timing (postcholecystectomy)	Treatment Surgical or nonsurgical	Methods	Results
Christensen et al.[6]	Type 1 (n=5)	All by PTC ERCP were negative in 4/4	2 weeks, 8, 68, 106, 126 months	Surgical in all	First percutaneous drain then open surgery HJ (n=4) Liver resection (n=1)	Uneventful postoperative course No follow-up data
Peters et al.[7]	Type 2 (n=8)	By HIDA scan + ERCP (n=7) Surgical exploration in 1	Median 3.5 (range 2–24) days	Nonsurgical (n=7) Surgical (n=1)	ERCP, sphincterotomy ± stent (n=7, two with additional CT-guided drain) Laparotomy with drainage (n=1)	Uneventful postinterventional course No follow-up data
Schipper et al.[8]	Type 1 (n=4)	By percutaneous fistulography (n=3) By surgery (n=1) ERCP was negative in 3/3	1, 1, 1, 5 weeks	Surgical in all	By postponed elective laparotomy with HJ. Three patients had preoperative drainage	Uneventful postoperative course No follow-up data
Meyers et al.[9]	Type 1 (n=13)	By fistulography (n=7)	Data were not provided	Nonsurgical (n=2)	Percutaneous drain (n=4, but two of them required HJ due to persistent bile leak)	In mean follow-up time of 26 months: surgical group with normal LFT, nonsurgical group with elevated LFT
Suhocki and Meyers[12]		By CT-cholangiogram (n=3)		Surgical (n=11)	HJ after preoperative drainage (n=9, one of them required liver resection as the definitive therapy)	
Ohtsuka et al.[11]	Type 1 (n=1)	By percutaneous fistulography	5 days	Surgical	Right hepatectomy after unsuccessful management by percutaneous drainage and sealing the duct with glue	Uneventful after right hepatectomy No follow-up data
Mergener et al.[13]	Type 1 (n=2)	By ERCP (n=11)	Median 4 (range 0–29) days	Nonsurgical (n=13)	ERCP, sphincterotomy/stent (n=12)	Two patients with repeated ERCP and stent change after 5 weeks, uneventful postinterventional course in others
	Type 2 (n=13)	By PTC (n=2) By percutaneous fistulography (n=2)		Surgical (n=2)	Percutaneous drain (n=1) HJ (n=2)	No follow-up data
Lillemoe et al.[14]	Type 1 (n=9)	All by PTC and two also by fistulography	Median 1.4 (range 0.8–3.5) months	Surgical in all	HJ after preoperative drainage by PTC with additional percutaneous drain of the abdominal fluid collection	Uneventful postinterventional course Median follow-up time of 36 months: 6/9 are well, 3/9 had recurrent stricture at the HJ anastomosis, which needed PTC and balloon dilation
Wills et al.[15]	Type 2 (n=10)	All by surgical	Median 5.5 (range 3–14) days	Surgical in all	Relaparoscopy in all with laparoscopic suture in 8 and drainage in 2, one needs laparotomy afterward	All patients are well in 2.5 years follow-up
Sandha et al.[16]	Type 2 (n=26)	All by ERCP	Median 9 (range 1–50) days	Nonsurgical in all	ERCP: sphincterotomy alone in low-grade bile leak, and with stent in high-grade bile leak	Uneventful postinterventional course No follow-up data
Kaffes et al.[17]	Type 2 (n=15)	All by ERCP	Median 7 (range 1–51) days	Nonsurgical in all	ERCP: sphincterotomy ± stent, some may need surgery (data can not be further stratified)	Data can not be stratified
Perini et al.[18]	Type 1 (n=12)	By PTC in 6/6,	Median 32 (range 8–731) days	Nonsurgical (n=7)	ERCP with stent (n=1)	One patient undergoing HJ developed stricture in 24 m. Two patients undergoing metallic stent had stricture at 12 and 72 m.

Study	SDI type	Diagnosis	Time	Treatment	Definitive treatment	Outcome
		By ERCP in 2/6 (4/6 with suspicion)		Surgical (n=5)	PTC with metallic biliary stent (n=5, including 2 patients with unsuccessful surgery) HJ (n=4, all had preoperative drainage) Liver resection (n=2, all had preoperative drainage)	All three patients received PTC and balloon dilation. Other patients are well in 44 (rang 2–90) months follow-up
Williams et al.[21]	Type 1 (n=1)	By PTC not by ERCP/HIDA/CT	20 days	Surgical	After preoperative percutaneous drainage and PTC, HJ was performed to repair the injury	Uneventful postinterventional course No follow-up data
Hwang et al.[22]	Type 1 (n=1)	By ERCP and MRCP	35 days	Nonsurgical	By percutaneous drainage and PTC to reduce the biliary collection. Hepatic atrophy was then induced by selective portal vein embolization	The patient is well in follow-up time of 30 months
Tantia et al.[23]	Type 1 (n=1) Type 2 (n=16)	By ERCP in 6/7 By MRCP in 1 By relaparoscopy in 4	Data can not be further stratified	Nonsurgical in all	ERCP with stent (n=11) US guided repeated aspiration (n=6)	The patients are all well in 4 to 12 months follow-up

CT computerized tomography, ERCP endoscopic retrograde cholangiopancreaticography, HIDA scan Tc-99m iminodiacetic acid cholescintigraphy, HJ hepaticojejunostomy, LFT liver function test, MRCP magnetic resonance cholangiopancreaticography, PTC percutaneous transhepatic cholangiography, US ultrasonography

patients, IOC was performed after surgical identification of the SDI, to illustrate the territories drained by the segmental/sectoral duct (Fig. 2). Precise surgical exploration identified the SDI in all patients.

Definitive Treatment

The definitive repair was carried out according to the type of SDI and concomitant injury (Fig. 3). In both patients with type 2 SDI, bile leak was successfully managed by oversewing. In six patients with type 1 SDI, who did not have any vascular injury, biliary–enteric anastomosis including enteric drain of the injured segmental/sectoral duct was performed. The two remaining patients with type 1 SDI had a concomitant RHA injury. In one patient, a right hepatectomy was carried out because of hepatic ischemia revealed 35 days after LC. In the other patient, a right hepatectomy would have been indicated by evidence of necrotic changes at the segmental duct 7 days after LC. However, the volume of the left liver lobe was not large enough to sustain a safe right hepatectomy. Thus, liver resection was planned in a second step after demarcation of the ischemic zone and potential lobe atrophy. The segmental duct was drained by a percutaneous transhepatic cholangiodrainage. This strategy failed, however, since the patient died due to sepsis 6 weeks after the first procedure.

Postoperative complications consisted of wound infection in two patients undergoing biliary enteric anastomosis. The median length of hospital treatment was 26 days (range, 9 to 47 days).

Long-Term Follow-up

At the median follow-up time of 43 months (range, 27 to 111 months), seven patients, five with type 1 SDI and two with type 2 SDI, were clinically asymptomatic. Among them, two patients with type 1 SDI had slightly elevated serum alkaline phosphatase and gamma glutamyl transpeptidase.

Two other patients with type 1 SDI developed biliary anastomotic strictures at follow-up of 11 and 28 months, respectively. The first patient had a concomitant RHA injury, which had initially led to right hepatectomy and a left hepaticojejunostomy. The stricture was successfully managed by PTC and balloon dilatation. The other patient had had a hepatohepaticojejunostomy due to concomitant CBD transection. He underwent hepatohepaticojejunostomy again after two ineffective procedures of PTC with balloon dilatation. Both patients are clinically asymptomatic, nevertheless with slightly elevated serum alkaline phosphatase and gamma glutamyl transpeptidase serum levels at follow-up of 31 and 75 months, respectively,

Discussion

Among a significant body of literature on BDI during LC, SDI has been mainly addressed in case reports only.[4–23] Apart of the rare occurrence, a more general recognition of the condition is hampered by the various terms for SDI, as they are injury to the "accessory bile duct", "aberrant right hepatic duct", "right posterior bile duct", "subvesical bile duct", "subserosal ducts", "subcholecystic ducts", "hepatocholecystic ducts", or "duct of Luschka".[4–23] Because this troublesome event usually has a significant delay in diagnosis and appropriate treatment, it is of paramount importance to summarize the clinical manifestation of SDI and define the optimal diagnostic approach as well as the appropriate therapy.

While occlusion of a segmental/sectoral duct leads to cholestasis and recurrent cholangitis,[6,22] transection of the segmental/sectoral duct mainly presents with right upper abdominal pain or with evident bile leaking from the drain.[7–23] In the current study, it is found that ERC, being the first diagnostic and therapeutic procedure in most cases of post-LC bile duct injury, had limitations in identifying type 1 SDI. Similar results were reported by most other authors, with correct diagnosis of type 1 SDI by ERC in less than 20% of patients[6–8,11–14,18] except injury to the Luschka duct[16,17,23] (Table 1). The reason could be misinterpreting the distal end of the transected segmental/sectoral duct as an insufficiently closed cystic duct or misinterpretation due to a lack of intercommunication between a torn segmental/sectoral bile duct and the CBD, especially in patients with transected CBD.[6,14] However, SDI could still be suspected as a relative absence of complete filling of the right hepatic ductal system after careful review in some of "normal" cholangiograms.[8,14] In absence of dilatation of the bile duct, SDI is difficult to be detected either by CT or MRC in a normal setting. Two patients in the current study had ERC as well as MRC. Both of them failed to illustrate the SDI. These limited data do not show the advantage of MRCP in identifying SDI over ERCP. By using Biliscopin as contrast medium, MDCT-CA did successfully identify type 1 SDI in one patient in the current study. However, MDCT-CA might not be useful in the diagnosis of type 2 SDI because the injured duct drains only a very limited portion of the liver parenchyma that could cause false negative result.[27] Fistulography through a surgically or radiologically placed percutaneous drain in an extrahepatic bile collection has been reported to be effective in the diagnosis by retrograde flow of contrast material into the torn ductal system.[8,9,12–14] The bilioma collection should be mature enough, which takes usually more than 2 weeks, to ensure the contrast material flows toward the source of the bile leakage and not toward other parts of the abdomen. Successful identifying

the SDI in selected patients by PTC has been reported.[12–14] Data showed that dilation of the segmental/sectoral duct is not a prerequisite for successful PTC.[14,18]

The author's data found that precise surgical exploration is more efficient in identifying SDI than conventional radiological diagnostics. Without fistulography or PTC, SDI was found preoperatively only in one patient In this cohort. Identification of SDI by open surgery was the most common pattern for diagnosis. When an SDI is identified macroscopically, IOC is helpful for the choice of treatment by illustrating the topography of the segmental biliary tree. During the surgical exploration, the following points should be taken into consideration: (1) in patients with suspicion of cystic duct leak, the culprit might be the distal end of a segmental/sectoral duct and (2) when a principal injury has been found in the main biliary tree, a concomitant SDI may be easily overlooked.

Successful management of SDI by nonsurgical and surgical treatment has been reported (Table 1).[4–23] Nonsurgical treatment options include percutaneous drainage, placement of an endoscopic stent, or intrabiliary catheterization with metallic stent, which usually is a multisessional procedure.[8] The reported length of nonsurgical treatment in type 1 SDI is rather long, ranging from 3 to 12 months[13,18,22] while it is more efficient in treatment of type 2 SDI.[7,16,17,23] Accompanying the long duration of treatment, the nonsurgical approach is not without danger. The presence of drainage may not totally protect from the occurrence of bile peritonitis. In a recent Italian national survey, 27.3% of the patients with BDI undergoing nonoperative drainage developed bile peritonitis.[2]

In the author's institute, early surgical repair of BDI is advocated in general, avoiding complications such as peritonitis and sepsis. Surgical management of SDI depends on the subtype, timing of recognition of the injury as well as on any concomitant injury. Injury to the subvesical bile duct or "duct of Luschka" can be managed by oversewing. Due to large amounts of bile production, a transected major sectoral duct or segmental bile duct definitely needs a biliary–enteric anastomosis in a Roux-en-Y hepaticojejunostomy or hepatojejunostomy fashion. Illustration of the entire biliary tree by IOC allows differentiating type 1 SDI from type 2 SDI. In patients with delayed onset of SDI, which usually presents as a clipped duct leading to hepatic atrophy, liver resection would be the treatment of choice.[10,11,22] Concomitant transection of the CBD has been found in half of the patients with SDI in the current study. Biliodigestive reconstruction, including the injured segmental duct and the common hepatic duct, through a single jejunal loop is achieved in most cases. Placing a transanastomotic stent into the segmental/sectoral duct is found to be helpful.[6,8,9,14] In patients with vascular injury, the therapy options depend on the extension of ischemia.[39]

Biliary reconstruction without restoration of the arterial supply results in either bile leak or anastomosis stricture. Sufficient drainage with timely hepatectomy would be the only promising choice in this setting.

The outcome after surgical treatment of SDI is generally satisfying. Compared to the length of nonsurgical treatment that ranges from 3 to12 months,[13,18,22] the median duration of treatment in the cohort presented in our report is much shorter, being 26 days (range, 9 to 47 days). The long-term results of surgical repair of SDI in the current study are favorable. Only two patients developed biliary strictures at the site of hepaticojejunostomy during follow-up of 27 to 111 months.

In summary, SDI is a rare event among BDI caused by LC. The lesion is difficult to be assessed properly by ERCP or conventional CT or MRI. Fistulography, PTC, and precise surgical exploration have been reported as efficient diagnostics. Whenever a bile leak occurs after LC, SDI should be taken into consideration. Patients with bile leak should be strongly suspected of having SDI when ERCP is interpreted as "normal" or when bile leakage persists after an endoscopic stent is placed for a "cystic duct leak". A type-guided surgical treatment of SDI results in a favorable outcome in most patients. Management of SDI with concomitant arterial injury is still a challenging scenario.

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ORIGINAL ARTICLE

Eleven Cases of Postoperative Hepatic Infarction Following Pancreato-Biliary Surgery

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Abstract

Background Postoperative hepatic infarction is rare; therefore, clinical characteristics and outcomes of postoperative hepatic infarction after pancreatobiliary surgery have not been obvious.

Methods Eleven patients encountered hepatic infarction after pancreato-biliary surgery. Management, clinical course, and outcome of these 11 patients were retrospectively analyzed.

Results Possible causes of the hepatic infarction were inadvertent injury of the hepatic artery during lymph node dissection in five patients, right hepatic artery ligation in two patients, long-term clamp of the hepatic artery during hepatic arterial reconstruction in two patients, suturing for bleeding from the right hepatic artery in one patient, and celiac axis compression syndrome in one patient. Five of the 17 infarcts extended for one whole section of the liver, and distribution of the other 12 was less than one section. Ten patients discharged from hospital; however, one patient died of sepsis of unknown origin.

Conclusions Attention should be paid to inadvertent injury of hepatic artery to prevent hepatic infarction. Hepatic infarctions after pancreato-biliary surgery seldom extend to the entire liver and most of them are able to be treated without intervention.

Abbreviations

TACE	Transcatheter arterial chemoembolization
RFA	Radiofrequency ablation
TIPS	Transjugular intrahepatic portosystemic shunt
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
PE	Plasma exchange
CHDF	Continuous hemodiafiltration
ARDS	Acute respiratory distress syndrome
RCT	Randomized controlled trial

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Introduction

Liver infarction is relatively rare, as it is commonly believed that the liver's dual blood supply and extensive collateral pathways serve to protect this organ from ischemic insult. Once hepatic infarction occurs, however, it is sure to be life-threatening, so appropriate management is paramount. With recent advances in nonsurgical intervention for hepatic tumors including TACE, RFA, and TIPS, reports describing hepatic infarction have been gradually increasing.[1–4] However, to our knowledge, there are hardly any detailed reports on post-surgery hepatic infarction. In this article, we retrospectively analyzed our experience with 11 patients in order to clarify clinical characteristics and outcomes of patients encountered hepatic infarction following pancreato-biliary surgery.

Material and Methods

Between August 1981 and June 2008, 812 patients underwent major pancreato-biliary surgery including total panceatectomy, pancreatoduodenectomy, and extrahepatic bile duct resection with lymph node dissection in the hepatoduodenal ligament at the Department of Surgery, Teikyo University Hospital. Fourteen of these 812 patients developed postoperative hepatic infarction. Three patients in whom hepatic infarction developed after coil embolization to the common hepatic artery for postoperative hemorrhage from the stump of the gastroduodenal artery was excluded from this study. Therefore, 11 patients were identified in the study. The medical records of these 11 patients were retrospectively reviewed.

Diagnosis of hepatic infarction was based on the co-existence of contrast enhanced CT findings (wedge-shaped and clearly demarcated regions of non-perfusion within the liver parenchyma that extend to the liver capsule) (Fig. 1) seen in association with an acute increase in serum amino transaminase more than 500 IU/L. All arterial reconstructions were done under microvascular techniques by an experienced plastic surgeon. One patient undergoing combined resection of common hepatic artery originating from the superior mesenteric artery and the superior mesenteric artery, the superior mesenteric artery, and the proper hepatic artery were anastomosed to the splenic artery in an end-to-end and side-to-end fashion, respectively. The superior mesenteric artery in one patient was anastomosed to the splenic artery in end-to-end fashion. The right hepatic artery in one patient was reconstructed using right gastro-epiploic artery graft interposition. Systematic administration of heparin was not performed routinely.
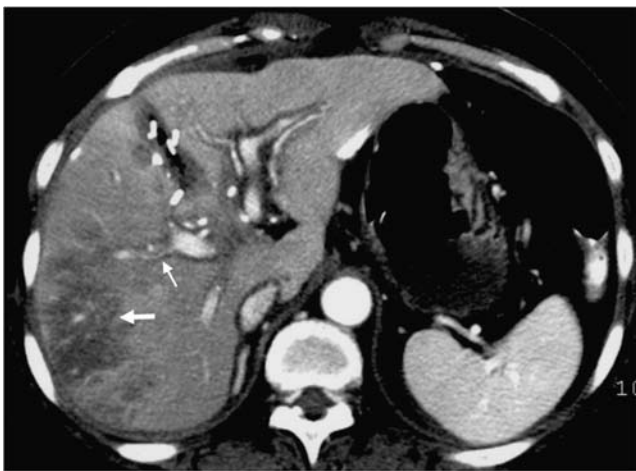
Figure 1 CT revealed wedge-shaped low-attenuation area without mass effect in S6 and S7 (*thick arrow*). The right hepatic artery (*thin arrow*) was demonstrated at the hepatic hilum on CT performed on postoperative day 1.

The terms of the hepatic anatomy were based on Couinaud's numbering system[5] and the Brisbane 2000 system.[6] Postoperative hepatic failure was defined as appearance of hepatic encephalopathy, hyperbilirubinemia to more than 10 mg/dl total bilirubin without hemolytic or obstructive mechanism, and a decreasing activity of blood coagulation assessed by a prothrombin time. Leakage of pancreatojejunostomy was defined as amylase-rich exudate (amylase more than three times normal serum concentration) from the drainage tube placed at the pancreatojejunostomy. Leakage of hepaticojejunostomy was diagnosed when drainage of >50 mL of bilious fluid after postoperative day 4 was recorded or a leak was shown by contrast radiology. Hospital mortality was defined as death from any cause during the hospital stay.

Results

Characteristics of 11 patients who encountered hepatic infarction after pancreato-biliary surgery are listed in Table 1. They consisted of four men and seven women with a mean age of 63.6±8.5 years (range 49 to 82 years). None of the patients had a history of chronic liver disease.

For preoperative workup, conventional angiography and three-dimensional CT angiography were performed in two and nine patients, respectively. Preoperative biliary drainage was performed in five of the patients for obstructive jaundice: endoscopic tube stent in two, endoscopic naso-biliary drainage in two, and expandable metallic stent in one. In two patients, the minimum serum total bilirubin level before surgery was above 3.0 mg/dl. Extrahepatic bile duct resection was performed in all patients. Incisions of the proximal hepatic duct were performed at the right and left hepatic ducts in three patients with gallbladder carcinoma. In the other eight patients, bile duct was cut in the common hepatic duct, that is to say, Glissonian sheath around the hepatic duct confluence was preserved. Hepatic resection was performed in three patients: wedge resection of the liver bed in two, S4b+S5 in one.

Clinical Presentation

The results are shown in Table 2. Serum AST and ALT levels were maximally elevated to mean 4,162.5± 3606.0 IU/L (range 422 to 11,810 IU/L) and mean 3,026.4±1991.0 IU/L (range 504 to 6,620 IU/L), respectively, 1 or 2 days after causal surgery. In all patients, the levels of serum aminotransferase decreased to within normal limits after several days.

Eight of the 11 patients had concomitant complications in relation to initial surgery. Acute respiratory distress syndrome in two, leakage of pancreatojejunostomy in two,

Table 1 Profiles of Eight Patients Encountering Hepatic Infarction After Surgical and Interventional Treatments

Pt. No.	Age (year)/gender	Diagnosis	Initial operation	Combined vascular resection
1	55/F	Gallbladder carcinoma	Hepatectomy (S4b+S5)+PPPD	SM-PV
2	69/F	IPMN	TP	SM-PV
3	64/F	Pancreatic adenocarcinoma	TP	SM-PV, SMA, CHA
4	62/F	Gallbladder carcinoma	Hepatectomy (Liver bed)+PPPD	SM-PV
5	66/F	Pancreatic adenocarcinoma	TP	SM-PV, SMA
6	59/F	Gallbladder carcinoma	Hepatectomy (Liver bed)+BDR	RHA
7	49/F	Pancreatic adenocarcinoma	PD	SM-PV
8	82/M	Pancreatic adenocarcinoma	TP	SM-PV
9	72/M	Chronic pancreatitis	PPPD	None
10	57/M	Chronic pancreatitis	PPPD	SM-PV
11	65/M	Bile duct carcinoma	PPPD	None

IPMN intraductal papillary mucinous neoplasm of the pancreas, *PPPD* pyloruspreserving pancreatoduodenectomy, *TP* total pancreatectomy, *SMA* superior mesenteric artery, *BDR* extrahepatic bile duct resection, *SM-PV* superior mesenteric-portal vein, *CHA* common hepatic artery, *RHA* right hepatic artery

leakage of hepaticojejunostomy in one, pleural effusion in one, and stricture of hepaticojejunostomy in one were noted. Three patients encountered no complications other than hepatic infarction.

CT Evaluation

Initial CT was performed after the rapid increase of serum aminotransferase was identified. Seventeen infarcts were found in the 11 patients. One patient had three infarcts, four patients had two infarcts, and six patients had single infarcts. The maximum axial diameters of infarct on CT were mean 8.6 ± 3.0 cm (5–15 cm). In five of the 17 infarcts, distribution of the low-attenuation area extended for one section of the liver: left lateral section in three and right anterior section in two. Distribution of the other 12 infarcts was less than one section. These 12 infarcts occurred within S8 ($n=3$) and S7 ($n=3$), between S6 and S7 ($n=2$), S5 ($n=2$), S2 ($n=1$), and S8 ($n=1$). No infarcts contained gas during the entire clinical course.

Possible Cause of Hepatic Infarction

Obvious blockage of hepatic arterial inflow was documented in two of the 11 patients. In two patients, the right hepatic artery was ligated during the initial operation. In a patient, the replaced right hepatic artery from superior mesenteric artery was ligated for curability. Reconstruction was not performed since it was thought the interlobar hepatic artery running into the Glissonian sheath around the hepatic duct confluence could be preserved. In another patient, an inadvertent ligation of the right hepatic artery was performed and not identified during the initial surgery. In two patients with hepatic artery resection, long-term

clamp of the proper hepatic artery (64 and 55 min) could have caused the hepatic infarction. In these two patients, patency of the hepatic artery was confirmed on CT. In one patient, right hepatic artery was injured, and bleeding was stopped by suturing during the operation. In the other six patients, no episode of obvious ligation or embolization of the hepatic artery was noted. In one of these six patients, who underwent pancreatoduodenectomy with combined resection of portal vein, celiac axis compression syndrome, which was present on the CT performed preoperatively, could not be released because of dense inflammatory adhesions. Inadvertent injury of the hepatic artery might have occurred during lymph node dissection in other five patients. In four of these five patients, the hepatic artery was demonstrated at least at the hepatic hilum on the initial and follow-up CT (Fig. 1). In one patient, the hepatic artery was not demonstrated on CT, and the proper hepatic artery was obstructed on celiac angiography performed 7 months after the initial surgery. Portal vein thrombus was not recognized in any of the patients on CT.

Management and Outcome

For the treatment of hepatic infarction, intravenous administration of prostaglandin E1 (PGE1) was performed in three patients, in addition to basic treatment including intravenous fluid resuscitation, antimicrobial agent, and transfusion of fresh frozen plasma. In one patient, plasma exchange (PE) and continuous hemodiafiltration (CHDF) were performed for the treatment of sepsis and acute respiratory distress syndrome (ARDS) and the prevention of hepatic failure. Hepatic failure occurred in one patient, and he recovered without specific treatment. In other two patients, total bilirubin increased beyond 10 mg/dl due to

Table 2 Results of Eight Patients Encountering Hepatic Infarction After Surgical and Interventional Treatments

Pt. No.	Possible cause of hepatic infarction	Maximal level			Number	Distribution	Site	Size (cm)	Concomitant complication	Specific treatment	Outcomes	Duration of Stay (days)
		AST (U/L)	ALT (U/L)	T-Bil (mg/dL)								
1	Inadvertent hepatic artery injury during lymph node dissection	8560	6620	4.6	2	Section / Less than section	L / S67	11 / 7.5	None	PGE1	Discharged	47
2	Ligation of RHA	422	504	12.2	1	Less than section	S58	13	HJ stricture, ARDS	None	Discharged	75
3	Long-term clamp of PHA	1289	2075	2.6	1	Less than section	S8	9	Wound sepsis, pleural effusion	PGE1	Discharged	64
4	Inadvertent hepatic artery injury during lymph node dissection	5310	3900	12.4	1	Less than section	S67	7.5	PJ leakage	None	Discharged	36
5	Inadvertent hepatic artery injury during lymph node dissection	5125	4565	2.1	2	Section / Less than section	L / S8	7.5 / 7.5	ARDS, sepsis of unknown origin	CHDF and PE	Died of sepsis of unknown origin after 2 months	57
6	Long-term clamp of PHA	1988	1616	8.2	1	Less than section	S7	6.5	HJ leakage	PGE1	Discharged	45
7	Inadvertent hepatic artery injury during lymph node dissection	5490	5265	1.1	2	Section / Section	A / L	15 / 10	None	None	Discharged	33
8	Inadvertent ligation of RHA	3920	3000	2	2	Less than section / Less than section	S7 / S8	7.5 / 6	None	None	Discharged	111
9	Inadvertent hepatic artery injury during lymph node dissection	1354	1223	4.8	3	Less than section / Less than section / Less than section	S2 / S4 / S5	10 / 5 / 5	Pneumonitis, intraabdiminal hematoma	None	Discharged	40
10	Celiac axis compression syndrome	11810	3825	14.7	1	Section	A	12.5	Hepatic failure	None	Discharged	39
11	Injury of RHA	519	697	5	1	Less than section	S7	5	PJ leakage	None	Discharged	40

AST aspartate amino transferase, *ALT* alanine amino transferase, *T-Bil* total bilirubin, *CHA* common hepatic artery, *RHA* right hepatic artery, *PHA* proper hepatic artery, *PJ* pancreatojejunostomy, *HJ* hepaticojejunostomy, *ARDS* acute respiratory distress syndrome, *PGE1* prostaglandin E1, *CHDF* continuous hemodiafiltration, *PE* plasma exchange

persistent obstructive jaundice or stricture of hepaticojejunostomy. The hepaticojejunostomic stricture, which was identified 7 days after the operation, was successfully treated by percutaneous transhepatic biliary drainage. In no patient was surgical or percutaneous drainage for hepatic infarction required during entire clinical course.

The overall hospital mortality rate was 9.1%. One patient died of sepsis of unknown origin, 2 months after occurrence of hepatic infarction during hospitalization. Sepsis of unknown origin of which the infecting primary causative organisms were acinetobacter baumannii was not definitively correlated with hepatic infarction.

All these 17 infarcts showed diminishment with hepatic parenchymal atrophy or scarring. Six of 10 discharged patients died of cancer recurrence after a mean survival of 11.2 months (range 4 to 20 months). Other four patients have survived uneventfully during a mean follow-up of 35.5 months (range 18 to 59 months).

Discussion

The common causes of hepatic infarction include hepatic artery occlusion due to arteriosclerosis, thrombosis, embolism, hepatic artery aneurysm, and polyarteritis nodosa. It was also reported that infarction occurred without vascular occlusion in the setting of shock, biliary disease, or anesthesia, presumably secondary to diminished hepatic blood flow.[7,8] We speculate that possible causes of the hepatic infarction were inadvertent injury of the hepatic artery during lymph node dissection in five patients. However, there is also a possibility that microthromboemboli from atherosclerotic lesion induced diminished hepatic arterial flow. Miyazaki et al.[9] advocated that one major lobar branch of the hepatic artery could be safely resected without reconstruction when the interlobar hepatic artery running into the Glissonian sheath around the hepatic duct confluence is preserved. In the present study, three patients with ligation or suturing of right hepatic artery encountered hepatic infarction in spite of the fact that the Glissonian sheath around the hepatic duct confluence had been preserved. This result argues against the resection of a major lobar branch of the hepatic artery, without careful consideration, even when the interlobar hepatic artery running into the Glissonian sheath around the hepatic duct confluence is preserved. In the present series, five of the 17 infarcts extended for one whole section of the liver and of the other 12 infarcts were less than one section. The reported frequencies of hepatic infarction extending to two sections are 0%,[10] 0%,[11] 20%,[8] and 35.7%.[12] There are no reports of hepatic infarction being distributed through the entire liver, which must be attributable to the recruitment of other collateral pathways including inferior phrenic arteries, intercostals arteries, and gastric arteries that were presumably not ligated at the initial surgery.[13]

A few clinicians have reported that portal thrombosis was an important cause of liver infarction.[14,15] Saegusa et al.,[14] in a postmortem study, reported that 15 of 20 patients with liver infarction had portal thrombosis, four had hepatic arterial obstruction (three of these four also had portal thrombosis), and 17 had septic, hypovolemic, or cardiogenic shock. In the present study, eight of 11 patients underwent superior mesenteric-portal vein reconstruction. In these eight patients, there is a possibility that a decrease in portal flow or microthrombus of the portal vein as well as a decrease in hepatic arterial flow was responsible for the hepatic infarctions.

Definitive clinical diagnostic criteria of hepatic infarction have not been established. In imaging studies of hepatic infarction, diagnosis was based on a wedge-shaped, low-attenuation area extending to the liver surface on CT.[8,11,12,16] Differential diagnosis based on CT findings includes hepatic abscess, laceration, and rarely tumor such as lymphoma.[8] Laceration and tumor can be distinguished from infarction on the grounds of clinical history. Differentiating hepatic infarction from abscess is more difficult, and we diagnosed hepatic infarction based on the coexistence of contrast-enhanced CT findings and an acute increase in serum aminotransaminase, as Smith et al.[12] previously reported. CT should be performed as soon as possible after the rapid increase of serum aminotransferase was identified for early and definite diagnosis of hepatic infarction.

The therapeutic strategy of hepatic infarction has not been established. In the present series, three patients underwent intravenous administration of PGE1. The intravenous administration of PGE1 has been used for the treatment of fulminant hepatic failure.[17] Prostaglandins have been shown to have a beneficial effect in a variety of animal models of hepatic failure due to toxins, hypoxia, ischemia, and immune mediation.[18,19] PGE1 is known to increase hepatic blood flow and has been shown to improve clinical outcome in various settings like liver dysfunction or ischemia/reperfusion injury.[20,21] Intravenous PGE1 might be considered for hepatic infarction patients with stable blood pressure.

In our series, no patient with hepatic infarction required percutaneous or surgical drainage. Stewart et al.[16] reported that 11 of 12 patients (92%) with infected hepatic infarctions responded to percutaneous drainage such that they survived to discharge from the hospital. However, they did not mention of an acute increase in serum aminotransaminase and frequency of infection of hepatic infarction; infected infarction may be confused with hepatic abscess. However, once hepatic infarction becomes infected, percutaneous or surgical drainage would be necessary.[13]

We performed PE and CHDF in one patient for treatment of septic ARDS and prevention of hepatic failure. In Japan, the first-line treatment for fulminant hepatic failure is PE and CHDF, which are covered by medical insurance.[22]. However, to our knowledge, there has been no randomized study to indicate that the use of PE and CHDF is effective in treating acute liver failure. Partial portal arterialization with an arterioportal shunt should theoretically increase portal flow and portal PO2, which would contribute to preventing massive hepatic necrosis.[23,24] Iseki et al.[24] reported that two of three cases of postoperative hepatic arterial occlusion after hemihepatectomy were successfully treated by mesenteric arterioportal shunt. Partial portal arterialization with an arterioportal shunt would be potentially effective for diffuse hepatic infarction and hepatic infarction after major hepatectomy.

In several reported case series of hepatic infarction of various causes, the mortalities were 0% (none of four cases),[10] 7.7% (one of 13 cases),[16] 25% (one of four cases),[25] 30% (three of 10 cases),[12] 40% (two of five cases),[8] and 50% (four of eight cases).[11] In these reports, the mortalities of hepatic infarction after pancreato-biliary surgery were specifically 0% (none of one case, none of six cases),[16,25] 16.7% (one of six cases),[12] 100% (one of one case),[8] and 50% (one of two cases).[11] The causes of death of these three mortalities after pancreato-biliary surgery were complicated a 3-month course of hepatic failure and sepsis,[12] acute renal failure,[8] and hepatic infarction beyond two sections with infection,[11] respectively. These results indicate the mortalities of hepatic infarction after pancreato-biliary surgery might not be so high without the presence of concomitant complications.

Conclusion

Surgeons should pay attention to inadvertent injury of hepatic artery during lymph node dissection to prevent hepatic infarction. Hepatic infarctions after pancreato-biliary surgery do not frequently extend to the entire liver and are able to be treated with medical treatment unless infection or hepatic failure develops.

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ORIGINAL ARTICLE

Influence of Chemotherapy on Liver Regeneration Induced by Portal Vein Embolization or First Hepatectomy of a Staged Procedure for Colorectal Liver Metastases

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Abstract

Background Although portal vein embolization (PVE) and staged hepatectomy (StHx), as well as prehepatectomy chemotherapy, have improved the resectability rate of patients with multiple bilobar colorectal liver metastases, the impact of prehepatectomy chemotherapy on liver hypertrophy following PVE and/or StHx has remained unclear.

Methods Sixty patients who underwent PVE followed by one-stage hepatectomy and StHx with or without PVE were analyzed. Liver hypertrophy following PVE and/or the first hepatectomy of StHx and the clinical course after final hepatectomy was compared between patients with and without prehepatectomy chemotherapy.

Results No difference of volume of the future liver remnant (FLR) before or after the procedure was seen between the chemotherapy group and the nonchemotherapy group. Even in 38 patients who underwent right PVE prior to a planned right hemihepatectomy, the chemotherapy group ($n=14$) and the nonchemotherapy group ($n=24$) were comparable in terms of volumes of FLR before ($P=0.71$) and after ($P=0.29$) PVE and posthepatectomy courses. However, the liver hypertrophy ratio for patients showing steatosis in adjacent nonmalignant liver parenchyma, which frequently is induced by chemotherapy, was lower than that for patients without steatosis ($P=0.04$).

Conclusions Although prehepatectomy chemotherapy did not impair liver hypertrophy, PVE and/or StHx accompanied by prehepatectomy chemotherapy should be performed with particular care to minimize risk of liver failure after the procedure.

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CA	Carbohydrate antigen
CDDP	Cisplatin
CEA	Carcinoembryonic antigen
CT	Computed tomography
FA	*l*-Folinic acid
FLR	Future liver remnant
5-FU	5-Fluorouracil
PET	Positron emission tomography
PLT	Platelet count
PS	Prediction score
PT-INR	Prothrombin time as the international normalized ratio
PVE	Portal vein embolization
StHx	2-Stage hepatectomy
TB	Total bilirubin

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Introduction

Numerous retrospective and prospective series including large numbers of patients have demonstrated a long-term survival benefit of liver resection for patients with hepatic metastases from colorectal cancer. Presently, using improved

techniques such as prehepatectomy portal vein embolization (PVE), planned two-stage hepatectomy (StHx), and hepatectomy combined with local ablation, liver resections are performed increasingly for metastatic liver cancer. Both preoperative PVE[1,2] and StHx with or without PVE[3–5] are reported to be effective in accomplishing complete removal of diffuse liver metastases in a bilobar distribution, extending indications for resection in these patients. Further, recent developments in chemotherapeutic agents offer hope for many colorectal cancer patients. These drugs can reduce tumor bulk to the extent that some patients with initially unresectable disease become candidates for potentially curative resections. Resectability has become an important end point for chemotherapy, focusing on curative potential of treatment as opposed to classic end points such as response or progression-free survival. Consequently, chemotherapy followed by liver resection is a strategy increasingly chosen for treating colorectal cancer metastases. Unfortunately, such chemotherapy may result in liver damage that compromises liver regeneration and patient recovery.

Although PVE or StHx together with prehepatectomy chemotherapy has improved the resectability rate of patients with multiple bilobar colorectal liver metastases, chemotherapy conventionally is discontinued at least 1 month prior to PVE or the first-stage hepatectomy in StHx and again before the final liver operation, since chemotherapy was believed to impair liver regeneration. Acting according to this theoretical rationale has the important inherent drawback of allowing or favoring tumor progression during that period.[6–8]

In the present study, we assessed the impact of prehepatectomy chemotherapy on volume of the future liver remnant (FLR) following PVE or StHx by retrospectively comparing FLR hypertrophy, operative variables, and postoperative liver function test results in patients who underwent prehepatectomy PVE or StHx with or without PVE between those treated with and without prehepatectomy chemotherapy.

Patients and Methods

Patients

From 1992 to 2008, a total of 311 patients diagnosed with liver metastasis from colorectal cancer underwent liver resection with curative intent at the Department of Gastroenterological Surgery of the Yokohama City University Graduate School of Medicine. Among these patients, 34 underwent PVE prior to one-stage hepatectomy, while 26 were treated with StHx with ($n=19$) or without PVE ($n=7$). These 60 patients included 35 men (58%) and 25 women (42%); their median age was 63 years (range, 37 to 79). Numbers of metastases at diagnosis were one in nine

patients, two in four patients, three in four patients, and four or more in 43 patients; the median number of metastases at diagnosis was 5 (range, 1 to 25). Median maximum size of metastases at diagnosis was 50.5 mm (range, 9 to 185). Prehepatectomy chemotherapy was given to 28 of these 60 patients. Among the 28, chemotherapy was administered before PVE or the first-stage hepatectomy in StHx in 20 and during PVE or the first hepatectomy and final hepatectomy in 20 (12 patients received chemotherapy at both times). Hypertrophy of the FLR determined following PVE with or without a first hepatectomy was compared between patients with and without chemotherapy.

The second phase of analysis assessed hypertrophy of the FLR following right PVE prior to a planned right or extended right hepatectomy; 38 patients (14 with prehepatectomy chemotherapy and 24 without) were selected and analyzed. Patients treated with PVE as well as StHx whose metastases in the FLR were resected before PVE or at the same time as PVE—with the aim of avoiding rapid growth of the metastases in the FLR—were included in this analysis because their first hepatectomy was not based upon compensatory hypertrophy of nonresected liver; all of these patients underwent a small excision of metastases within the FLR. Among the 14 patients with chemotherapy, chemotherapy was performed before PVE or first hepatectomy in 12 and during PVE or first hepatectomy and final hepatectomy in eight (six patients received chemotherapy at both times).

Preoperative staging included physical examination, measurement of serum carcinoembryonic antigen (CEA), and carbohydrate antigen 19–9, colonoscopy, barium enema, abdominal ultrasonography, abdominal computed tomography (CT), and chest imaging by routine chest radiography or CT. Imaging by positron emission tomography was introduced for preoperative staging after 2002.

Hepatectomy Procedures

Unresectability was defined as technical inability to completely remove all metastases according to a prediction score (PS) system introduced by Yamanaka et al.[9] The PS was calculated using the formula $PS=-84.6+0.933a+1.11b+0.999c$, where a is the anticipated resection fraction (%) calculated from computed tomographic volumetry, b is the indocyanine green retention rate (%) at 15 min, and c is the patient age in years. A PS exceeding 50 contraindicated a given hepatectomy. Accordingly, patients with a PS of 50 or more underwent either a two-stage hepatectomy or prehepatectomy PVE.

Intraoperative ultrasonography was used to identify any occult tumors not detected preoperatively and to confirm relationships between tumors and vasculobiliary structures. Parenchymal dissection was performed using ultrasonic

dissectors. When necessary, the liver pedicle was clamped intermittently in cycles including 15 min of clamping and 5 min of reperfusion. The Brisbane 2000 terminology of the International Hepato-Pancreato-Biliary Association was used to categorize operative procedures.[10]

Prehepatectomy Chemotherapy

Chemotherapy for resectable metastases was indicated for marginally resectable disease (more than or equal to four nodules in right and left hemilivers; massive, unfavorably located tumors; or limited concomitant extrahepatic disease; $n=13$). Patients whose metastases would have posed difficulties for safe removal also received prehepatectomy chemotherapy initially ($n=15$). Among these 28 patients treated with prehepatectomy chemotherapy, 19 underwent final prehepatectomy chemotherapy with a combination of 5-fluorouracil (5-FU), l-folinic acid (FA), and cisplatin (CDDP); in 11 of the 19 patients, a chronomodulated regimen was used. Treatment consisted of a 5-day course of infusion via the hepatic artery through an implanted arterial access port (Vital-Port, Cook Vascular, Leechburg, PA, USA). On each of 5 days, 5-FU (500 to 600 mg/m^2/day), FA (100 mg/m^2/day), and CDDP (10 mg/m^2/day) were delivered. In principle, this 5-day course was repeated three or more times at 9-day intervals. In patients who received chronomodulated infusions, the same drugs were administered using a Graseby Model 3000 infusion pump (Graseby Medical, Watford, UK). Peak delivery was scheduled at 4 a. m. for 5-FU and FA and 4 p.m. for CDDP, essentially as described in previous reports.[11] Three patients received systemic chemotherapy consisting of 5-FU and FA alone ($n=2$) or combined with irinotecan ($n=1$). Six patients received both three cycles of chronomodulated hepatic arterial infusion chemotherapy as outlined above and three cycles of systemic infusion of 5-FU and FA with oxaliplatin (FOLFOX-4).

Portal Vein Embolization

In patients without chemotherapy, we usually performed PVE 4 weeks before hepatectomy. In patients with chemotherapy at the time of PVE and final hepatectomy, timing of hepatectomy was decided case by case according to the response to chemotherapy. When response to chemotherapy was insufficient, second- or third-line chemotherapy regimens were introduced. On the other hand, hepatectomy sometimes was performed as early as 4 weeks after PVE when a favorable response to chemotherapy was obtained. Under general anesthesia, a 7-Fr balloon-tip catheter was inserted into a portal vein through an ileocolic vein, and the portal venous branches of the hemiliver, sectors, or segments targeted for resection were embolized. Completeness of occlusion was confirmed by portography at the end of the procedure. In patients for whom StHx with PVE was planned, a catheter was inserted through the reopened obliterated umbilical vein in the round ligament at the time of initial hepatectomy. The embolic material was a mixture of 1 g of gelatin pellets (Gelfoam powder; Upjohn, Kalamazoo, MI, USA), 10 to 20 mL of oleic acid monoethanol amine (Oldamine; Grelan, Tokyo, Japan), and 20 mL of diatrizoate sodium meglumine (76% Urografin; Schering, Berlin, Germany).

CT Volumetry

Helical CT or CT with arterioportography was performed to define hepatic metastases preoperatively. CT was performed with an Asteion scanner (Toshiba Medical, Tokyo, Japan). After administering a contrast agent, serial transverse scans were obtained at 5-mm intervals from the dome of the liver to its most inferior part with the patient suspending respiration in inspiration for each image acquisition. Each slice of the liver was traced with a cursor, and the corresponding area was calculated by computer. The hypertrophy ratio in terms of liver volume corresponding to FLR after PVE with or without first hepatectomy was calculated using the data thus obtained, both prior to PVE or first hepatectomy and also generally 1 week before the final liver resection. The formula was used: [(volume of FLR after procedure−volume of FLR before procedure)/volume of FLR before procedure]×100%. Aggregate volume of liver neoplasms was subtracted from total liver volume to assess of functional liver volume.

Perioperative Factors Included in Calculations

Hypertrophy of the FLR following PVE or first hepatectomy was assessed and compared between patients with and without prehepatectomy chemotherapy. Biochemical liver function parameters and other laboratory data following final liver resection including serum total bilirubin (TB), platelet count (PLT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), as well as prothrombin time as the international normalized ratio (PT-INR), also were compared between the two groups.

Adjacent nonmalignant liver parenchyma was examined pathologically using paraffin-embedded tissue sections stained with hematoxylin and eosin. If adjacent normal liver parenchyma was affected by PVE prior to hepatectomy, biopsy specimens from nonembolized liver were taken for examination whenever possible. Pathologic examination was undertaken in all 28 patients with prehepatectomy chemotherapy and 28 of 32 patients without chemotherapy. Pathologic effects of chemotherapy in normal liver tissue were graded according to the extent of steatosis relative to the estimated total extent of the resected parenchyma. One of

four grades was assigned as follows: no steatosis (grade 0), indicating no steatosis evident throughout the specimen; mild steatosis (grade 1), steatosis in less than 30% of the entire specimen; moderate steatosis (grade 2), steatosis in more than 30% but less than 60% of the entire specimen; and severe steatosis (grade 3), steatosis in 60% or more of the entire specimen.

Among postoperative complications, hyperbilirubinemia was defined as a serum bilirubin concentration on postoperative day 7 of 3 mg/dL or greater. Biliary fistula was diagnosed when bile drainage from the abdominal wound or drain was apparent, with a total bilirubin concentration in the drainage fluid of more than 5 mg/mL or three times the serum concentration. Intra-abdominal abscess or liver stump abscess was confirmed by percutaneous drainage.

Statistical Analysis

Statistical comparisons of baseline data were performed with the Mann–Whitney U test, the χ^2 test, or Fisher's exact test. A difference was considered significant when the two-sided P value was below 0.05.

Results

Liver Hypertrophy Induced by PVE and/or First Hepatectomy of StHx

When characteristics were compared between patients who had chemotherapy prior to hepatectomy and patients who had no prehepatectomy chemotherapy, these two groups were comparable in terms of prehepatectomy variables such as age, gender, primary site and histology of the colorectal neoplasm, Dukes stage, timing of metastasis (synchronous vs. metachronous), number of metastases, maximum liver tumor diameter, and prehepatectomy CEA concentrations (Table 1).

Hepatectomy procedures, segments of FLR, portal branches embolized, and first-stage hepatectomy procedure in patients treated with StHx are presented in Table 2. No significant differences in these variables were seen between groups, except for hepatectomy procedures. StHx with PVE was performed more frequently for patients with prehepatectomy chemotherapy, while PVE following one-stage hepatectomy was more common in patients without chemotherapy (P=0.04).

Table 1 Demographic and Clinical Features of Patients with Portal Vein Embolization and/or Staged Hepatectomy

Variable	CTx (+) (n=28)	CTx (−) (n=32)	P value
Age	60.5±1.9 (62, 37–76)	64.5±1.5 (65, 49–79)	0.14
Gender			
Male	13	22	0.12
Female	15	10	
Primary tumor			
Site			
Colon	17	23	0.42
Rectum	11	9	
Dukes stage			
A/B	8	12	0.59
C	20	20	
Histology			
Well	10	11	>0.99
Moderate/others	18	21	
Liver metastases			
Timing			
Synchronous	21	20	0.41
Metachronous	7	12	
Number			
Before CTx	8.3±1.2 (6, 1–25)	6.5±1.0 (5, 1–21)	0.20
After CTx	7.9±1.1 (6, 1–25)	6.5±1.0 (5, 1–21)	0.25
Maximum size, mm			
Before CTx	64±7 (53, 9–168)	63±8 (51, 16–185)	0.91
After CTx	52±7 (40, 9–140)	63±8 (51, 16–185)	0.10
Serum CEA, ng/mL			
Before CTx	1,889±1,445 (74, 2–40,609)	207±78 (33, 1–2,021)	0.13
After CTx	708±393 (22, 2–10,536)	207±78 (33, 1–2,021)	0.97

Values of age, number of tumor, maximum size, and serum CEA are means±SEM. Medians and ranges are shown in parentheses

CTx chemotherapy, *Well* well-differentiated adenocarcinoma, *Moderate* moderately differentiated adenocarcinoma, *CEA* carcinoembryonic antigen

Table 2 Hepatectomy Procedure and Volume of Future Liver Remnant

	CTx (+) (n=28)	CTx (−) (n=32)	P value
Procedure			
One-stage Hx with PVE	11	23	0.04
StHx	5	2	
StHx with PVE	12	7	
Segments of FLR			
Left hemiliver	14	25	0.15
Lateral section	4	2	
Medial section	3	1	
Posterior section	2	1	
Others	5	3	
Embolized portal vein			
Right trisections	2	0	0.23
Right hemiliver	18	26	
Left trisections	1	0	
Left hemiliver	0	2	
Others	2	2	
First-stage hepatectomy procedure			
Hemihepatectomy	2	1	0.92
Sectionectomy	5	2	
Partial resection	10	6	
FLR volume			
Before the procedure	267±17 (285, 104–458)	303±25 (282, 124–702)	0.39
After the procedure	381±24 (385, 149–743)	390±30 (348, 182–777)	0.76
Hypertrophy volume	114±18 (92, −8–417)	87±12 (76, −8.7–274)	0.27
Hypertrophy ratio	49±9 (37, −4.1–212)	32±5 (32, −2.7–115)	0.27

Values of FLR volume and hypertrophy volume and ratio are means±SEM. Medians and ranges are shown in parentheses

CTx chemotherapy, *Hx* hepatectomy, *PVE* portal vein embolization, *StHx* two-stage hepatectomy, *FLR* future liver remnant

The mean interval between PVE or first hepatectomy and CT imaging used to assess hypertrophy of the FLR was 28±3 in the group without chemotherapy vs. 90±19 days in the group with chemotherapy ($P<0.01$). Volumes of estimated FLR before and after PVE and/or first-stage procedure were comparable between groups with and without chemotherapy ($P=0.39$ and $P=0.76$, respectively). Actual hypertrophy volumes (volume of FLR after the procedure−volume of FLR before the procedure) and hypertrophy ratios also were comparable between groups ($P=0.27$ for each; Table 2).

Pathologic examination of the normal liver disclosed steatosis in 61% (17 of 28) of specimens in the chemotherapy group and 21% (six of 28) in the nonchemotherapy group ($P<0.01$). The grade of steatosis was severe in one patient, moderate in seven, and mild in nine in the chemotherapy group and moderate in one and mild in five in the nonchemotherapy group.

Liver Hypertrophy Induced Following Right PVE

In 38 patients with a right PVE prior to a planned right or extended right hepatectomy, the chemotherapy group ($n=14$) and the nonchemotherapy group ($n=24$) were comparable in terms of prehepatectomy variables except for site of colorectal primary (Table 3). Patients treated with PVE as well as first-stage hepatectomy numbered five in the chemotherapy group and three in the nonchemotherapy group ($P=0.12$).

The FLR in all of the above patients was the left hemiliver. The mean interval between PVE and CT imaging used to assess the hypertrophy of the FLR was 29±5 in the nonchemotherapy group vs. 56±11 days in the chemotherapy group ($P=0.01$). Volumes of estimated FLR before and after PVE were comparable between the chemotherapy group and the nonchemotherapy group ($P=0.71$ and $P=0.29$, respectively). Actual hypertrophy volume and hypertrophy ratio also were comparable between groups ($P=0.64$ and $P=0.72$, respectively; Table 4).

Right PVE was completed successfully without complications in all patients undergoing it in both groups. When intraoperative variables concerning final hepatectomy were compared between the chemotherapy group and the nonchemotherapy group, no significant differences were noted in operative time or total blood loss during hepatectomy. The proportion of patients requiring transfusion tended to be larger

Table 3 Demographic and Clinical Features of Patients with Right Portal Vein Embolization

Variable	CTx (+) (n=14)	CTx (−) (n=24)	P value
Age	60.9±2.7 (65, 37–73)	66.3±1.5 (66, 49–79)	0.19
Gender			
Male	7	16	0.49
Female	7	8	
Primary tumor			
Site			
Colon	5	18	0.04
Rectum	9	6	
Duke's stage			
A/B	3	11	0.18
C	11	13	
Histology			
Well	6	9	>0.99
Moderate/others	8	15	
Liver metastases			
Timing			
Synchronous	10	14	0.50
Metachronous	4	10	
Number			
Before CTx	7.1±1.7 (6, 1–23)	5.5±1.1 (5, 1–21)	0.65
After CTx	6.5±1.4 (6, 1–16)	5.5±1.1 (5, 1–21)	0.70
Maximum size, mm			
Before CTx	66±10 (56, 30–168)	70±10 (55, 16–185)	>0.99
After CTx	48±9 (39, 12–130)	70±10 (55, 16–185)	0.09
Serum CEA, ng/mL			
Before CTx	806±341 (166, 2–3,740)	254±99 (62, 1–2,021)	0.28
After CTx	607±282 (131, 2–3,740)	254±99 (62, 1–2,021)	0.83

Values of age, number of tumor, maximum size, and serum CEA are means±SEM. Medians and ranges are shown in parentheses

CTx chemotherapy, *Well* well-differentiated adenocarcinoma, *Moderate* moderately differentiated adenocarcinoma, *CEA* carcinoembryonic antigen

in the nonchemotherapy group than in the chemotherapy group, but the difference did not reach significance. No patient in either group died within 60 days of hepatectomy. In the chemotherapy group, six patients (43%) had postoperative complications, including biliary fistula, hyperbilirubinemia, intra-abdominal bleeding, and intra-abdominal abscess (in one patient each) and ascites (in two patients). Six complications occurred in four patients in the nonchemotherapy group (17%), including hyperbilirubinemia and portal vein thrombosis, intra-abdominal bleeding and biliary fistula, intra-

abdominal abscess, and biliary fistula. No difference in frequency of postoperative complications was noted (P= 0.13). Complications were resolved with medical or interventional treatment in all patients, except for the two patients with postoperative bleeding (one in each group); both required re-exploration for hemostasis (Table 5).

As for liver function parameters and other laboratory data after final liver resection, no significant differences over time were evident in AST or ALT between groups. However, minimum PLT counts (at 3 days postoperatively)

Table 4 Volume of Future Liver Remnant in Patients with Right Portal Vein Embolization

Variable	CTx (+) (n=14)	CTx (−) (n=24)	P value
FLR volume, mL			
Before right PVE	285±25 (305, 122–458)	275±21 (270, 124–467)	0.71
After right PVE	402±37 (412, 188–743)	366±32 (310, 182–741)	0.29
Hypertrophy volume, mL	117±31 (88, −8–417)	91±14 (76, −8.7–274)	0.64
Hypertrophy ratio, %	49±16 (33, −4.1–212)	33±4 (35, −2.7–63)	0.72

Values are means±SEM. Medians and ranges are shown in parentheses

CTx chemotherapy, *FLR* future liver remnant, *PVE* portal vein embolization

Table 5 Operative Variables in Patients with Right Portal Vein Embolization

Variable	CTx (+) (n=14)	CTx (−) (n=24)	P value
Operative time, mean±SEM, min (median, range)	448±40 (440, 222–845)	468±20 (467, 295–652)	0.38
Total blood loss, mean±SEM, L (median, range)	1.6±0.3 (1.2, 0.3–3.9)	1.9±0.3 (1.7, 0.3–5.6)	0.23
Transfused patients (%)	7 (50)	19 (79)	0.06
Hospital stay, mean±SEM, days (median, range)	25±5 (21, 8–68)	27±4 (20, 8–107)	0.75
Morbidity (%)	6 (43)	4 (17)	0.13
Ascites	2	0	
Biliary leakage	1	2	
Liver failure	1	1	
Portal vein thrombosis	0	1	
Postoperative bleeding	1	1	
Intra-abdominal abscess	1	1	
Postoperative liver function tests, mean±SEM			
Maximum TB, mg/dL	2.1±0.3	2.7±0.3	0.11
Minimum PLT, ×10^4/mL	11.4±1.2	14.7±1.1	0.02
Maximum PT-INR	1.3±0.03	1.2±0.03	0.06
Maximum AST, IU	395±74	441±85	0.88
Maximum ALT, IU	265±43	346±106	0.98

CTx chemotherapy, *TB* total bilirubin, *PLT* platelet count, *PT-INR* prothrombin time as the international normalized ratio, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

were lower in the chemotherapy group (mean±SD, 11.4± 4.5×10^4/mL) than in the nonchemotherapy group (14.7± 4.2, P=0.02; Table 5). Further, PLT counts preoperatively and 1 day postoperatively in the chemotherapy group (19.2± 7.5×10^4/mL and 12.6±4.7) were lower than in the non-chemotherapy group (26.7±4.8×10^4/mL and 16.5±3.3; P<0.01 and P=0.02, respectively). No significant differences in maximum PT-INR and TB (each at 1 day post-operatively) were seen between groups (Table 5). However, PT-INR on postoperative day 5 in the chemotherapy group (1.25±0.08) was significantly prolonged compared with the nonchemotherapy group (1.16±0.10, P=0.02), and serum TB on postoperative day 7 in the nonchemotherapy group (1.3±0.6 mg/dL) was greater than in the chemotherapy group (0.9±0.4 mg/dL, P=0.03).

Pathologic examination of liver parenchyma was undertaken in all 14 patients with chemotherapy and 21 of 24 patients without chemotherapy. Steatosis was seen in 36% (five of 14) of specimens in the chemotherapy group and 14% (three of 21) in the nonchemotherapy group (P=0.22). The grade of steatosis was moderate in one patient and mild in four in the chemotherapy group and was mild in three in the nonchemotherapy group.

Pathologic Findings Caused by Chemotherapy: Impact on Liver Hypertrophy

Among the 56 patients who had pathologic examination of adjacent nonmalignant liver parenchyma, 23 showed steatosis (mild steatosis in 14, moderate in eight, and severe in one). No difference in FLR volume before and after PVE, hypertrophy volume or hypertrophy ratio was seen between patients with and without steatosis in adjacent liver parenchyma. However, in the 35 patients with pathologic evaluation who underwent right PVE following right hemihepatectomy, hypertrophy volume and hypertrophy ratio for patients showing steatosis (n=8) were lower than for patients showing no steatosis (n=27; P=0.02 and P=0.04, respectively; Table 6).

Discussion

The volume gain of the nonembolized FLR has been reported to range from 7% to 27%, averaging 12% of the total liver,[12] or 20% to 46% beyond pre-PVE FLR volume 2 to 8 weeks after PVE.[13–15] We previously found that the ratio of FLR hypertrophy to FLR prior to the first procedure was approximately 28% in StHx and exceeded 50% in StHx plus PVE.[16] Human liver regeneration after hepatectomy is influenced by several factors including extent of liver resection,[17–20] liver function,[17–21] age,[21] and hepatotrophic factors in portal blood.[22,23] As previously reported, the rate of hypertrophy is less rapid in injured or cirrhotic liver[2] than in normal liver. In patients whose extent of hepatectomy was intermediate, normal livers quickly regained or exceeded preoperative initial volumes in 1 month, followed by a gradual return to preoperative size when preoperative

Table 6 Volume of Future Liver Remnant According to Grade of Steatosis in Adjacent Nonmalignant Liver Parenchyma

Variable	Patients with PVE and/or first Hx		P value	Patients with right PVE		P value
	Steatosis (+) (n=23)	Steatosis (−) (n=33)		Steatosis (+) (n=8)	Steatosis (−) (n=27)	
FLR volume, mL						
Before right PVE	264±20 (258, 104–495)	300±21 (300, 122–702)	0.32	273±31 (294, 124–385)	281±19 (299, 122–467)	0.97
After right PVE	341±21 (345, 149–551)	417±27 (402, 182–777)	0.11	318±33 (331, 188–449)	399±29 (382, 182–743)	0.25
Hypertrophy volume, mL	77±12 (83, −8.7–218)	117±15 (90, 2.3–417)	0.13	45±15 (64, −8.7–89)	118±18 (90, 2.3–417)	0.02
Hypertrophy ratio, %	36±7 (28, −4.1–133)	44±7 (35, 0.5–212)	0.31	19±7 (21, −4.1–53)	46±8 (35, 0.5–212)	0.04

Values are means±SEM. Medians and ranges are shown in parentheses

PVE portal vein embolization, *Hx* hepatectomy, *FLR* future liver remnant

volume had been exceeded. In contrast, injured livers regenerated less rapidly than normal liver, with volumes 2 to 3 months after hepatectomy representing only 80% of those evident preoperatively. After a large resection in normal liver, approximately 90% of initial volume was regained within 2 to 3 months, while injured livers were restored only from 70% to 80% of initial volume in 3 to 5 months.[17] Similar changes related to intrahepatic disturbances induced by PVE are likely. In the present study, the interval between PVE or first hepatectomy and CT imaging in the chemotherapy group was approximately 3 months, which was longer than in the nonchemotherapy group (approximately 1 month). According to a previous report using sequential volume calculation based on CT,[17] normal livers as opposed to cirrhotic or injured livers showed regeneration after hepatectomy to reach a plateau phase within 1 to 2 months irrespective of extent of resection. The pattern of regeneration rates after PVE was similar to those found after liver resection. Therefore, the difference in time to CT between groups should not preclude valid comparison of liver volume.

Previous intensive systemic or intra-arterial chemotherapy generally is considered a risk factor for poor postoperative course of liver resection. Also, ongoing chemotherapy could worsen histologic changes within the nontumorous liver, including sinusoidal congestion or portal/sinusoidal fibrosis. In previous reports, 5-fluorouracil has been associated with increased risk of severe steatosis, although not of steatohepatitis[24]; oxaliplatin-based combination regimens, increased risk of vascular lesions in the liver[25,26]; and irinotecan-containing regimens, increased risk of steatosis and steatohepatitis.[25] The impact of such prehepatectomy chemotherapy on estimated FLR volume hypertrophy has not been well defined until now. Previously, prehepatectomy chemotherapy ordinarily was discontinued prior to PVE and subsequently until surgery because various antimitotic agents have been shown

in experimental models to interfere with liver resection-induced regeneration.[27,28] However, a recent report concluded that continuing chemotherapy while PVE was performed did not impair hypertrophy of the FLR.[29]

Our study involved 5-FU, FA, and CDDP, which commonly are used to treat colorectal cancers, so steatosis was expected to be a constant histologic abnormality in exposed nonneoplastic liver. Indeed, histologic examination in the chemotherapy group more frequently demonstrated steatosis than in the nonchemotherapy group. However, the ratio of FLR hypertrophy after the procedure was comparable between the two groups even though 5-FU has been shown to adversely affect liver regeneration after hepatectomy in animal studies.[30] To eliminate the effect of differences in procedure between groups, patients with PVE following right hemihepatectomy were selected for a second analysis. Here also, histologic steatosis was more frequent in the chemotherapy group than in the nonchemotherapy group, but the difference was not statistically significant. The ratio of FLR hypertrophy after the procedure was comparable between the two groups, as in the first analysis. Our results indicated no significant drawback of prehepatectomy chemotherapy on liver regeneration. When day-to-day changes in postoperative liver function tests and other laboratory data were compared after final liver resection, lower PLT counts and prolonged PT-INR were evident in the chemotherapy group. More severe coexisting liver disease would predict delayed functional recovery, particularly concerning protein synthetic capacity.[17] Accordingly, risk of delayed hepatic functional recovery in the chemotherapy group may be reflected by alterations in postoperative PT. A decreased PLT count in the chemotherapy group was observed even before hepatectomy, so the difference in serial PLT counts most likely resulted from differences in baseline PLT. In patients with liver injury, decreased PLT counts, even at 3 months postoperatively and later, reportedly tended to

remain low,[31] so our finding of decreased PLT also may reflect delayed hepatic functional recovery after chemotherapy. However, prehepatectomy chemotherapy was not associated with increased morbidity and mortality, and length of hospital stay also was comparable. TB tended to be greater in the nonchemotherapy group than in the chemotherapy group probably because of a higher proportion of transfused patients in the nonchemotherapy group.

Impaired liver functional recovery after hepatectomy and reduced liver regenerative capacity have been reported to be related to hepatic steatosis,[32,33] so liver hypertrophy was compared between patients whose liver parenchyma showed steatosis and those without steatosis. Although no difference in liver hypertrophy was seen between subjects with presence and absence of steatosis when all patients were analyzed, liver hypertrophy in patients with steatosis was significantly lower than in patients without steatosis among the patients who underwent right PVE following right hemihepatectomy. According to these results, steatosis, which frequently is induced by aggressive chemotherapy, may impede liver regeneration when one compares groups undergoing similar PVE or hepatectomy procedures. The overall lack of a negative effect of chemotherapy on liver regeneration in our study may have resulted at least partly from a relatively equal prevalence of steatosis in the chemotherapy and the nonchemotherapy groups. The difference in regeneration volume between groups with and without chemotherapy did not reach statistical significance, most likely reflecting differences in surgical procedures among all study patients, even though steatosis was more frequent in the chemotherapy group than in the nonchemotherapy group. On the other hand, absence of a difference in liver hypertrophy between these groups could be attributed to equal prevalence of steatosis between groups among patients with PVE following right hemihepatectomy. Considering prevalence and implications of steatosis, we believe that PVE and/or StHx accompanied by prehepatectomy chemotherapy should be performed with particular care.

Covey et al.[34] recently affirmed that hepatic hypertrophy after PVE can occur during chemotherapy. However, those authors focused on patients receiving chemotherapy shortly after PVE, so the impact of chemotherapy before PVE on hepatic hypertrophy was not addressed. Further, the small number of their patients with no prior chemotherapy may limit applicability of their findings. The current study confirmed that liver growth after PVE can occur even in patients who received chemotherapy shortly before PVE. Additionally, 26 patients had no prior chemotherapy and six received only adjuvant chemotherapy for the primary among our 32 patients in the overall nonchemotherapy group, while among 24 patients undergoing right PVE who were in the nonchemotherapy group, 20 had no prior

chemotherapy and four received only adjuvant chemotherapy for the primary (data not shown). Our data therefore indicated that liver regeneration with perioperative chemotherapy was comparable to regeneration in patients with no prior or concurrent chemotherapy.

In conclusion, continuing chemotherapy while PVE and/or first hepatectomy was carried out did not impair hepatic hypertrophy and allowed successful final liver resection without deterioration of postoperative course. However, steatosis induced by chemotherapy still may limit liver regeneration capacity, so such combined treatment should be performed carefully to minimize risk of liver failure after the procedure.

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ORIGINAL ARTICLE

Assessment of Future Remnant Liver Function Using Hepatobiliary Scintigraphy in Patients Undergoing Major Liver Resection

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Abstract

Background ^{99m}Tc-mebrofenin hepatobiliary scintigraphy (HBS) was used as a quantitative method to evaluate liver function. The aim of this study was to compare future remnant liver function assessed by ^{99m}Tc-mebrofenin hepatobiliary scintigraphy with future remnant liver volume in the prediction of liver failure after major liver resection.

Methods Computed tomography (CT) volumetry and ^{99m}Tc-mebrofenin hepatobiliary scintigraphy were performed prior to major resection in 55 high-risk patients, including 30 patients with parenchymal liver disease. Liver volume was expressed as percentage of total liver volume or as standardized future remnant liver volume. Receiver operating characteristic (ROC) curve analysis was performed to identify a cutoff value for future remnant liver function in predicting postoperative liver failure.

Results Postoperative liver failure occurred in nine patients. A liver function cutoff value of 2.69%/min/m^2 was calculated by ROC curve analysis. ^{99m}Tc-mebrofenin hepatobiliary scintigraphy demonstrated better sensitivity, specificity, and positive and negative predictive value compared to future remnant liver volume. Using ^{99m}Tc-mebrofenin hepatobiliary scintigraphy, one cutoff value suffices in both compromised and noncompromised patients.

Conclusion Preoperative ^{99m}Tc-mebrofenin hepatobiliary scintigraphy is a valuable technique to estimate the risk of postoperative liver failure. Especially in patients with uncertain quality of the liver parenchyma, ^{99m}Tc-mebrofenin HBS proved of more value than CT volumetry.

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Introduction

Major liver resection may result in a small postoperative remnant liver, thereby increasing the risk of postoperative liver failure, especially in patients with parenchymal disease.[1] Posthepatectomy liver failure is the most frequent cause of mortality after liver resection. Although the causes of liver failure are multifactorial, insufficient postoperative remnant liver function is one of the main contributing factors.

Preoperative computed tomography (CT) volumetry, in which liver volume is used as an indirect measurement of liver function, is widely used to identify patients who

should be excluded from a planned liver resection or to select patients who will benefit from preoperative portal vein embolization (PVE).[1–5] Future remnant liver (FRL) volume (FRL-V) is expressed as a percentage of total liver volume (%FRL-V),[3] or as standardized FRL (sFRL), in which FRL-V is calculated as percentage of total liver volume based on body surface area (BSA).[4,6] sFRL recognizes patient characteristics (body weight/BSA) but has only been validated in patients with healthy livers. In patients with a normal liver parenchyma, an %FRL-V or sFRL larger than 25–30% of total preoperative liver volume is considered sufficient for a safe resection,[3,4,7–9] whereas in patients with a compromised liver (e.g., fibrosis, steatosis, or cholestasis), a %FRL-V or sFRL of more than 40% is preferred.[10] The separate cutoff values indicate the necessity to asses the quality of the liver parenchyma in order to perform an accurate and safe preoperative risk analysis using CT volumetry. Preoperative liver biopsy is currently the most reliable method to assess the quality of the liver parenchyma. Biopsies are not routinely performed due the potential unequal distribution of parenchymal damage[11] and the risk of complications.[12,13] As a result, the quality of the liver parenchyma frequently remains unknown, rendering preoperative risk analysis by CT volumetry less reliable.

For accurate preoperative risk analysis, additional tests of liver function are required. Dynamic ^{99m}Tc-mebrofenin hepatobiliary scintigraphy (HBS) was developed as a quantitative method for evaluating total and regional liver function, including FRL function.[14,15] The hepatic uptake of ^{99m}Tc-mebrofenin is similar to the uptake of organic anions such as bilirubin.[16] After the hepatic uptake, ^{99m}Tc-mefrofenin is excreted into the bile canaliculi without undergoing biotransformation during its transport through the hepatocytes. Although ^{99m}Tc-mebrofenin is not metabolized, the uptake and intracellular transit are similar to various endogenous and exogenous substances including bilirubin, hormones, drugs, and toxins. In a recent publication, we demonstrated that ^{99m}Tc-mebrofenin HBS has potential to predict postoperative liver failure in a patient population including both minor and major liver resections.[17] The advantage of using ^{99m}Tc-mebrofenin HBS is the fact that the same cutoff value can be used for both patients with a compromised or normal liver parenchyma,

which makes the test applicable in patients with an uncertain quality of the liver parenchyma. However, it remains uncertain if ^{99m}Tc-mebrofenin HBS is sufficiently accurate to predict liver failure in a population containing high-risk patients requiring major hepatic resection. This study compares preoperative FRL function assessed by HBS with FRL-V, expressed as %FRL-V and sFRL, in the prediction of postoperative liver failure after major liver resection in high-risk patients.

Patients and Methods

Patients

Between May 2000 and November 2006, 213 patients underwent a partial hepatectomy. Of all patients undergoing major liver resection (three or more Couinaud segments), both CT volumetry and HBS were preoperatively performed in 71 patients. Sixteen patients were excluded from the study because of preoperative PVE (n=15) or partial portal vein thrombosis (n=1) in the time period between HBS and CT volumetry. Hence, a group of 55 patients was retrospectively analyzed with the approval of our Institutional Review Board with waiver of informed consent. Table 1 summarizes the types of resection performed. Patients with a preoperative suspicion of hilar cholangiocarcinoma underwent an (extended) hemihepatectomy combined with hilar resection and caudate lobe resection. In cholestatic patients, preoperative biliary drainage was performed more than 6 weeks prior to surgery using endoscopic retrograde cholangiopancreatography or percutaneous transhepatic drainage.

Pre- and perioperative factors associated with postoperative morbidity and mortality were analyzed (Table 5). Histopathology of the resection specimen was assessed by an experienced pathologist taking into account features of cholestasis, steatosis, fibrosis, and chronic inflammation.

Postoperative complications were recorded according to the modified classification of surgical complications proposed by Dindo et al.[18] In-hospital complications were recorded as well as complications requiring hospital readmission within 3 months related to the operation. Minor complications included grade 1 and grade 2 complications. Major complications were defined as grade 3 and severe compli-

Table 1 Types of Liver Resection with the Corresponding Weight of the Resection Specimen

procedure	Number of patients	Percentage	Weight resection specimen (g)
Extended right hemihepatectomy	14	25.5	975±247
Right hemihepatectomy	26	47.2	936±396
Extended left hemihepatectomy	1	1.8	443
Left hemihepatectomy	14	25.5	348±120
Total	55	100.0	

cations as grade 4 and grade 5 complications. Liver failure was defined as bilirubin plasma levels >50 µmol/l and/or prothrombin time index <50%,[19] elevated plasma ammonia levels combined with signs of hepatic encephalopathy and/or hepatorenal syndrome, requiring intensive care treatment.

Surgical Technique

Surgery was performed under low central venous pressure (<4 cm H_2O). Liver parenchymal transsection was performed using Cavitron Ultrasonic Surgical Aspirator (Valley Lab, Boulder, CO, USA). Pringle maneuver was applied in 29 patients (54%) to reduce intraoperative blood loss, with a mean ischemic period of 37 ± 13 min. Intermittent clamping was applied in eight patients (15%).

Scintigraphic Imaging and Data Acquisition

HBS was performed using ^{99m}Tc-mebrofenin as previously described.[14,15] Briefly, after injection of 85 MBq of ^{99m}Tc-mebrofenin (Bridatec; GE-Amersham Health), dynamic images were obtained with a γ-camera (Diacam, Siemens, Milwaukee, WI, USA) for 60 min. During the first 10 min, 60 frames of 10 s were acquired (liver uptake phase) followed by 50 frames of 1 min (liver excretion phase). Total hepatic ^{99m}Tc-mebrofenin uptake rate was calculated as described by Ekman et al.[20] On preoperative HBS, regions of interest (ROIs) were drawn around the total liver, the heart (serving as blood pool), and the total field of view. From these ROIs, three time–activity curves were generated (Fig. 1). Total hepatic ^{99m}Tc-mebrofenin uptake rate, representing total liver function (TL-F), was calculated as percent per minute: (%/min) (of the injected dose) based on these three parameters. Calculations of hepatic ^{99m}Tc-mebrofenin uptake rate were performed using measured values obtained between 150 and 350 s postinjection to ensure that hepatic uptake calculations were performed during a phase of homogenous distribution of the agent in the blood pool, before occurrence of the rapid phase of hepatic excretion. To compensate for differences in individual metabolic requirements, TL-F was divided by BSA and expressed as percent per minute per square meter: (%/min/m²). For determination of FRL uptake, a ROI was drawn around the FRL by two independent investigators, blinded for the results, according to the performed resection, and interobserver variation was calculated. The round ligament was used as the border between segment three and four. Cantlie's line, projected on the liver surface as a plane between the middle of the gallbladder fossa (visible in the late phase of the scintigraphy) and the inferior caval vein, was used as a border between the right and left liver lobes. In addition, the anterior projection of the liver on the CT volumetry was used as a guideline for delineating the FRL on the HBS images

(Fig. 1). FRL uptake function (FRL-F) was calculated by dividing counts within the delineated FRL by the total liver counts and multiplying this factor with total liver ^{99m}Tc-mebrofenin uptake (TL-F) and expressed as percent per minute per square meter: (%/min/m²). In 33 patients, a postoperative HBS was performed within 3 days after the operation to measure actual remnant liver function.

CT Volumetry

Contrast-enhanced CT scans were generated with a helical scanner (Philips, Eindhoven, The Netherlands). Manual 3D reconstructions of the liver were made using reconstructed 5-mm-thick axial slices from 2–3-mm original slices. The total liver as well as tumor(s) and the FRL were manually outlined using portal and hepatic veins as landmarks for segmental division. Integrated software (Mx-View 3.52, Philips Medical Systems) was used to calculate total liver volume (TL-V), tumor volume (TV), and FRL-V. All delineations were made by an experienced radiologist. FRL-V was expressed as percentage of TL-V using the formula:

$$\%FRL-V = \frac{FRL-V}{(TLV-TV)} \times 100\%$$

The nontumorous total liver volume (^{NT}TL-V) was calculated by excluding the tumor volume from the TL-V.

Standardized FRL Measurements

FRL-V was determined using CT volumetry, while total liver volume (^{cal}TL-V) was calculated using a formula based on BSA[6]: $^{cal}TL-V = -794.41 + 1,267.28 \times BSA$ $\left(BSA = \sqrt{height(cm) \times weight(kg)/3,600}\right)$.

The sFRL was calculated as the percentage between FRL-V and calculated TL-V.

Preoperative Risk Assessment

Receiver operator characteristics (ROC) curve analysis was used to calculate the optimal cutoff value for FRL-F in predicting postoperative liver failure. Cutoff values were determined based on the following assumptions: Firstly, the chance that liver failure would develop while the test result was above the cutoff value needed to be as low as possible. Secondly, a test result below the cutoff value should accurately select high-risk patient who might benefit from PVE. Based on literature, cutoff values for %FRL-V and sFRL were set at 30% for patients with normal liver parenchyma[9] and 40% for patients with a compromised liver.[10] Positive predictive values (PPV), negative predictive values (NPV), as well as sensitivity and specificity were determined for each method.

Figure 1 An example is shown of summed HBS images from 150–300 s after i.v. injection of ^{99m}Tc-mebrofenin (**a**). A ROI is drawn around the entire liver (*red line*) and around the mediastinum (blood pool; *yellow line*). A third ROI is drawn around the future remnant liver (*green line*). A blood pool corrected liver-uptake time–activity curve is shown in **b**. The hepatic ^{99m}Tc-mebrofenin uptake is calculated as an increase of ^{99m}Tc-mebrofenin uptake (*y*-axis) per minute over a time period of 200 s (*x*-axis). **c** The use of the anterior projection of the liver on the CT volumetry image as a guideline for delineating the FRL on the HBS image (**d**).
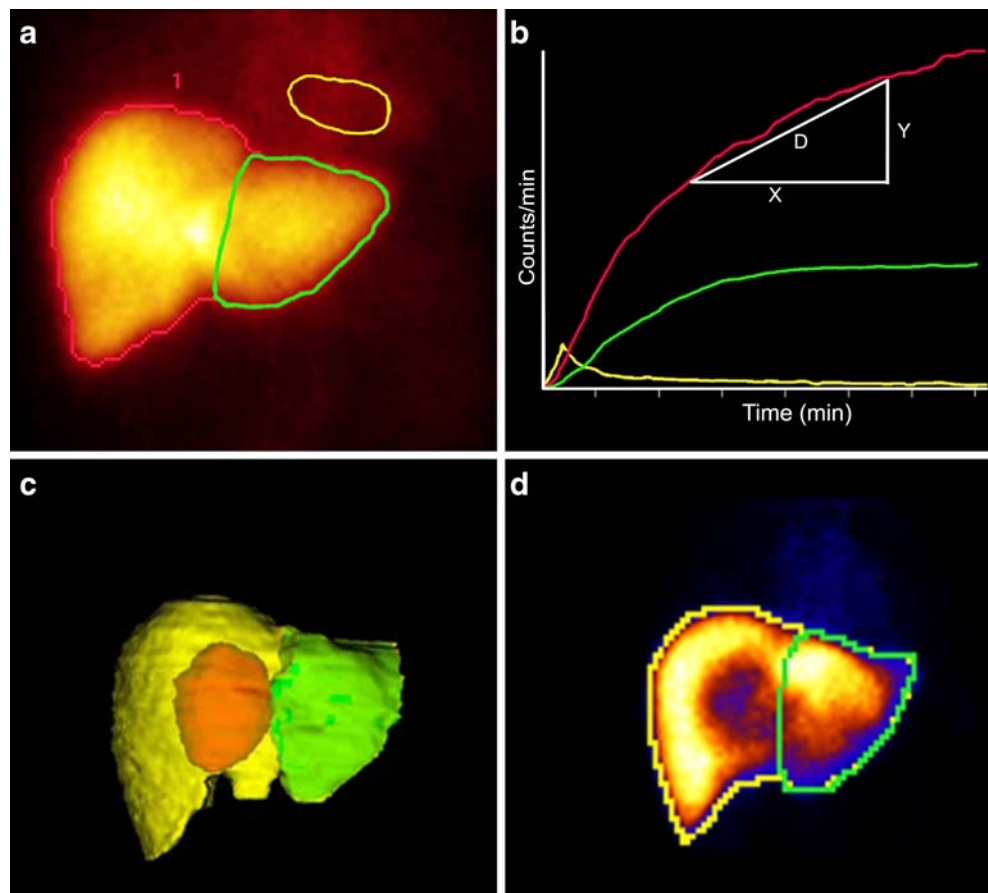
Statistical Analysis

Statistical analysis was performed with GraphPad Prism (GraphPad Software, San Diego, CA, USA) and Statistical Package for Social Sciences (SPSS 12.02, Chicago, IL, USA). ROC curve analysis was used to identify a cutoff value for FRL-F in predicting postoperative liver failure. Univariate analysis of preoperative and intraoperative variables was performed by the independent t test for continuous parameters and by Pearson's ^{2}tests and Fisher's exact test for categorical data. Correlation between variables was tested using the Pearson correlation coefficient r. Continuous data were compared by independent sample t test and expressed as mean±standard deviation. All statistical tests were two-tailed, and differences were considered significant at a P value of ≤0.05.

Results

Patient Characteristics

CT volumetry and ^{99m}Tc-mebrofenin HBS were performed in 55 patients (male 26, female 29, mean age 59±13 years). Indications for liver resection are shown in Table 2. Thirty

patients were diagnosed with a compromised liver parenchyma based on the histopathological evaluation of the resection specimen by an experienced pathologist, including cirrhosis ($n=2$), severe fibrosis ($n=3$), steatosis (>30% of the hepatocytes affected; $n=3$), severe cholestasis ($n=8$), chronic inflammation ($n=3$), or a combination of these diseases ($n=11$).

Liver Function and Liver Volume

TL-F was significantly lower in patients with parenchymal liver disease ($7.4\pm1.4\%/min/m^2$) as compared to patients with healthy liver parenchyma ($8.5\pm1.7\%/min/m^2$, $P=0.007$). ^{NT}TL-V was significantly larger in patients with compromised livers ($1{,}037.1\pm208.0$ vs. 877.0 ± 143.3 mL/m^2, $P=0.001$; Fig. 2).

Table 2 Indications for Liver Resection

Liver metastasis ($n=14$)
Hilar cholangiocarcinoma ($n=19$)
Intrahepatic cholangiocarcinoma ($n=3$)
Hepatocellular carcinoma ($n=6$)
Benign biliary strictures ($n=7$)
Benign lesions ($n=6$)
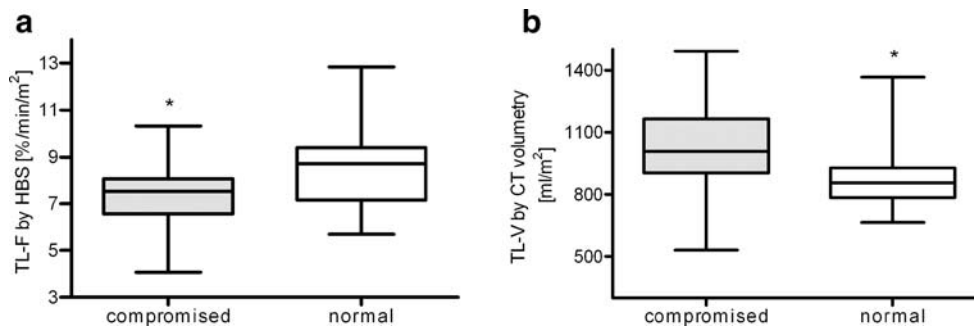
Figure 2 Total hepatic ^{99m}Tc-mebrofenin uptake according to parenchymal status. Patients with parenchymal liver disease had significantly less liver (uptake) function (*gray box*, 7.4±1.4%/min/m^2) as compared to patients with healthy liver parenchyma (*white box*, 8.5±

1.7%/min/m^2, *P*=0.007; **a**). Total liver volume: (^{NT}TL-V) was significantly higher in patients with compromised livers (1,037.1± 208.0 vs. 877.0±143.3 mL/m^2, *P*=0.001; **b**).

According to the type of resection performed, FRL-F was calculated for each individual patient by two independent observers. The interobserver agreement was excellent (Pearson r=0.97), and Bland–Altman analysis revealed almost no bias between the two observers (mean bias of 0.00058 with 95% limit of agreement between −0.835 and 0.836). Preoperative FRL-F correlated strongly with actual postoperative remnant liver function determined within 3 days after surgery (Pearson r=0.83, *P*<0.0001; Fig. 3). Liver weight of the resection specimen revealed a strong correlation (Pearson r=0.91, *P*< 0.0001) with its volume assessed by CT volumetry, confirming the CT measurements.

FRL-V correlated well with FRL-F (Pearson r=0.72, *P*= 0.0001) in patients with normal livers. In contrast, patients with a compromised liver demonstrated only a moderate correlation between FRL-V and FRL-F (Pearson r=0.61, *P*< 0.0003). The slope coefficient of the linear regression curve indicated that FRL-V is associated with significantly (*P*= 0.0015, analysis of covariance test) reduced FRL-F in compromised livers as compared to normal livers (Fig. 4).

Postoperative Complications

In 42 of the 55 (76%) patients, one or more complications occurred following liver resection (Table 3). Minor and major

complications were evident in 14 patients (25%) and 13 patients (24%), respectively. Fifteen patients (27%) developed severe complications requiring ICU treatment, and the mortality rate was 15%. Patients with severe complications had significantly lower FRL-F as compared to patients with no complications (*P*=0.0043), minor complications (*P*= 0.0028), or major complications (*P*=0.0046)

Nine patients (16%) developed postoperative liver failure, of which eight patients died. In four patients, liver failure was evident within 1 week after the operation. Five patients developed liver failure within several weeks after the operation in conjunction with signs of sepsis. Evidence of a compromised liver was seen in eight patients (89%), and in seven patients, an extended hemihepatectomy had been performed. The FRL-F was significantly lower in patients with postoperative liver failure (2.18% vs. 4.32%/min/m^2, *P*=0.0001).

Preoperative and Intraoperative Parameters Associated with Liver Failure

Univariate analysis revealed that elderly patients (*P*=0.043), small %FRL-V (*P*=0.024), small sFRL (*P*=0.012), small
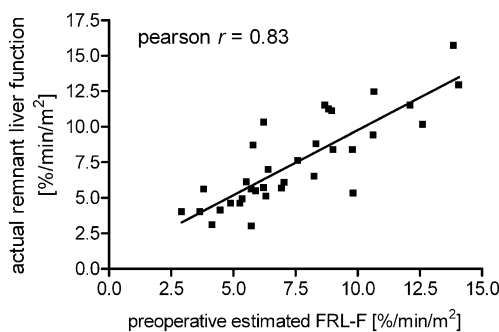
Figure 3 Scatter plot showing the correlation between preoperative FRL-F and actual postoperative remnant liver function measured within 3 days after surgery (33 patients, Pearson r=0.81, *P*<0.0001).
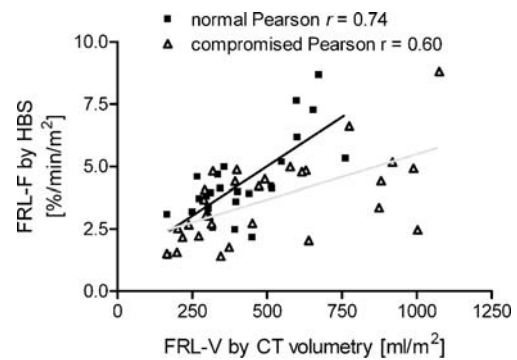
Figure 4 Scatter plot showing the correlation between FRL-F and FRL-V. In patients with normal livers (*black line*), FRL-V correlated well with FRL-F (Pearson r=0.71, *P*=0.0001). Patients with compromised livers (*gray line*) showed a moderate correlation between FRL volume and FRL function (Pearson r=0.61, *P*<0.0003).

Table 3 Postoperative Complications

Grade 0 ($n=13$)	No complications
Grade 1* ($n=5$)	Minor complications
Grade 2* ($n=9$)	Minor complications
Grade 3a ($n=12$)	Major complications
Grade 3b ($n=1$)	Major complications
Grade 4a ($n=5$)	Severe complications
Grade 4b ($n=2$)	Severe complications
Grade 5 ($n=8$)	Severe complications

Grade 1 needed no therapy except analgetics, diuretics, anti-emetics, and physiotherapy. Grade 2 complications required pharmacological treatment. Grade 3 complications required surgical, endoscopic, or radiological intervention (grade 3a under local anesthetics, grade 3b under general anesthetics). Grade 4 complications included life-threatening complications requiring ICU management (grade 4a with single organ dysfunction, grade 4b with multi-organ failure). Grade 5 complications resulted in death

*One patient could have multiple grade 1 or 2 complications

FRL-F ($P=0.001$), resection type ($P=0.001$), prolonged operating time ($P=0.0018$), increased blood loss ($P=0.0018$) during the operation, and the presence of a compromised liver parenchyma ($P=0.024$) were significantly associated with postoperative liver failure (Table 4). Due to a small sample size in the liver failure group ($n=9$), no multivariate analysis was performed.

Preoperative Prediction of Postoperative Liver Failure

ROC analysis revealed that a cutoff value for FRL-F of 2.69%/min/m² was able to identify patients who developed postoperative liver failure with a sensitivity of 89% and a specificity of 87% (Fig. 5). The risk of postoperative liver failure in patients with a FRL-F above 2.69%/min/m² was 2.4% (with a NPV of 97.6% and a likelihood ratio for a negative test result of 0.12). The PPV was 57.1% with a likelihood ratio for a positive test result of 6.8. Table 5 summarizes the sensitivity, specificity, PPV, NPV, and likelihood ratios of the different tests. For an accurate use of %FRL-V and sFRL, two cutoff values were used, and patients were divided in patients with a normal liver parenchyma and patients with a compromised liver parenchyma based on the histopathology of the resection specimen. Using ⁹⁹ᵐTc-mebrofenin HBS, one cutoff value sufficed in both compromised and noncompromised patients. Assuming that, of all the patients, the quality of the liver parenchyma was preoperatively known, sensitivity, specificity, and positive and negative predictive values were still better for FRL-F compared to %FRL-V and sFRL (Table 5).

Discussion

Accurate measurement of liver function before liver resection is crucial in the assessment of resectability,

especially in patients requiring major liver resection. The availability of preoperative PVE to induce hypertrophy of the FRL has further increased the importance of preoperative assessment of regional hepatic function.[7,21–24] In the present study, dynamic planar ⁹⁹ᵐTc-mebrofenin HBS was used to measure liver function. This technique can be implemented in every hospital with a nuclear medicine department, is easy to perform, and has a small interobserver variability. More importantly, preoperative estimated function of the future remnant liver (FRL-F) correlates strongly with actual postoperative liver function,[14] indicating that dynamic planar ⁹⁹ᵐTc-mebrofenin HBS is an accurate method to assess FRL-F.

In this study, we compared FRL-F measured by ⁹⁹ᵐTc-mebrofenin HBS with two parameters based on CT volumetry, which are widely accepted parameters to determine the possible extent of resection.[1–5] Patients with a compromised liver had a significantly lower liver function compared to patients with normal liver parenchyma, whereas their liver volume was significantly larger. FRL-V showed a strong relation with FRL-F in patients with normal liver parenchyma. In contrast, FRL-V and FRL-F only moderately correlated in patients with compromised liver parenchyma in whom FRL-V was associated with reduced FRL-F. The impact of different parenchymal diseases such as steatosis, cholestasis, and fibrosis on liver function and liver volume is unknown and may vary among individuals. In addition, parenchymal damage is often not equally distributed,[11] which can partially explain the moderate correlation between FRL-V and FRL-F in patients with compromised livers. ROC curve analysis yielded an FRL-F cutoff of 2.69%/min/m² for the prediction of postoperative liver failure. This cutoff value is comparable to the cutoff value determined in a patient population including both minor and major resections.[17]

A reliable preoperative test should primarily establish whether patients with a FRL-F above the critical threshold
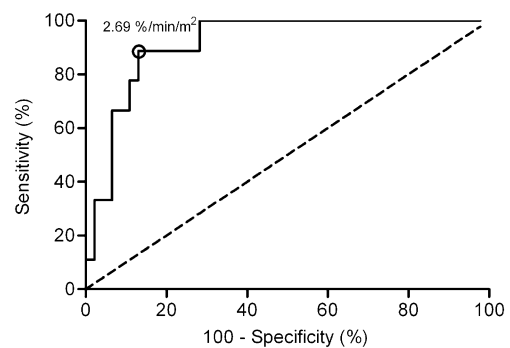
Figure 5 Receiver operator characteristic curve analysis of FRL-F in the prediction of liver failure. A cutoff value for FRL-F of 2.69%/min/m² identified patients with a significant risk of developing postoperative liver failure (area under the curve=0.916; 95% confidence interval 0.837–0.994).

Table 4 Comparison of Pre- and Intraoperative Parameters in Patients with or Without Liver Failure

	Patients with liver failure (n=9)	Patients without liver failure (n=46)	P value
Demographics			
Male/female	7:2	19:27	0.069[a]
Age	67.1±6.0 (58–67)	57.1±13.7 (18–78)	0.027[b]
BMI	25.1±2.1	24.0±3.6	0.33[b]
FRL volume			
%FRL-V (%)	35.0±22.0	49.7%±17.8	0.013[b]
sFRL (%)	35.2±9.2	49.2%±3.6	0.018[b]
FRL-F (%/min/m^2)	2.2±0.6	4.3%±1.6	0.001[b]
Comorbidity			
Diabetes (yes/no)	2:7	5:41	0.32[a]
Chronic hepatitis (yes/no)	2:7	3:43	0.18[a]
Vascular disease(yes/no)	3:6	9:37	0.39[a]
Compromised liver (yes/no)	8:1	22:24	0.024[a]
Resection type			
Left hemihepatectomy	1	13	
Right hemihepatectomy	1	25	0.001[c]
Extended hemihepatectomy	7	8	
Preoperative laboratory values			
AST	51.4±19.1	48.2±32.4	0.24[b]
ALT	57.9±27.2	65.6±65.6	0.55[b]
Bilirubin	19.9±14.9	14.2±12.0	0.20[b]
AF	265.2±204.6	280.1±260.5	0.76[b]
GGT	409.9±272.7	392.7±605.7	0.13[b]
Albumin	39.4±5.8	39.5±5.9	0.84[b]
Prothrombin time	13.0±1.5	13.1±0.90	0.63[b]
Intraoperative parameters			
Blood loss (mL)	5,200 cc±2,673	3,025±2,464	0.021[b]
Operating time (min)	507.4±135.1	382.3±131	0.011[b]
Pringle maneuver yes/intermittent/no	3:2:3	26:6:14	0.62[c]
Pringle time (min)	35.0±5.0	36.71±13.5	0.96[b]
Intermittent total ischemia time (min)	40.0	47.6	0.5[b]

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *AF* alkaline phosphatase, *GGT* gamma-glutamyltransferase, *BMI* body mass index

[a] Fisher's exact test

[b] Mann–Whitney U test

[c] χ^2 test

can be safely resected. One patient developed liver failure despite a FRL-F above 2.69%/min/m^2 (Table 6). This cirrhotic patient developed massive necrosis after left hemihepatectomy, due to an obliterated right hepatic artery and a compromised portal venous system. When CT volumetry would have been used as selection criterion for operation, two patients developed liver failure despite a % FRL-V of more than 40% (Table 6). Standardized FRL wrongly predicted a safe resection in three patients (Table 6). Although the formula generally used to calculate TL-V based on BSA is used for all patients, it is derived from patients with normal liver parenchyma. In our study,

patients with a compromised liver had significantly larger liver volumes resulting in a relatively larger FRL-V in relation to their BSA. As a consequence, there is an overestimation of liver function in these patients.

Secondly, a preoperative test should be accurate in selecting high-risk patients who might benefit from PVE, without treating patients unnecessarily. Despite having a FRL-F below the critical value of 2.69%/min/m^2, 43% of these high-risk patients did not develop liver failure. In literature, a similar percentage was reported when using CT volumetry for the prediction of postoperative hepatic dysfunction.[8] Additional negative predictive factors, in-

Table 5 Overview of the Sensitivity, Specificity, PPV, NPV as well as likelihood ratio's for FRL-F, %FRL-V, and sFRL in the Prediction of Postoperative Liver Failure

Outcome parameter	FRL-F	%FRL-V	sFRL
Cutoff value	2.69%/min/m^2	Normal liver <30%	Normal liver <30%
		Compromised liver <40%	Compromised liver <40%
Sensitivity	89%	78%	67%
Specificity	87%	80%	87%
PPV	57%	44%	50%
NPV	98%	95%	93%
LR+	6.8	4.0	5.1
LR−	0.12	0.19	0.38

FRL future remnant liver, *%FRL-V* future remnant liver/total liver volume percentage, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* likelihood ratio for positive test result, *LR−* likelihood ratio for negative test result

cluding high body mass index, significant intraoperative blood loss, and prolonged operating time, were described in patients with hepatic dysfunction, underlining the multifactorial cause of postoperative liver failure. In our study, univariate analysis revealed that, besides small FRL volume and function, increased intraoperative blood loss, prolonged operating time, a compromised liver parenchyma and older age were associated with liver failure. Unfortunately, a multivariate analysis was not possible in our study due to the small number of patients with postoperative liver failure. Cutoff values for the prediction of postoperative complications and hepatic dysfunction have been reported using CT volumetry,[3,4,7–9] indocyanine green clearance test,[25] galactose elimination capacity,[26] and ^{99m}Tc-GSA scintigraphy.[27–30] These cutoff values were, however, mostly not based on accurate risk calculations and no or inappropriate multivariate analyses had been performed.

Morbidity and mortality rates reported in our study were high, which is explained by the patients selected for this study. We only included patients undergoing major liver resection of which the majority (55%) had parenchymal liver disease. A relatively high proportion (39%) of patients had undergone resection on the suspicion of hilar cholangiocarcinoma, including six patients who had developed

postoperative liver failure. These patients require large resections and biliary anastomoses, with increased risk of postoperative morbidity and mortality, reported up to 10–20%.[31–33] The overall postoperative mortality in patients operated for benign lesions or liver metastasis in our institution is 2%.[34] In addition, none of the patients included in this study had undergone PVE. In some patients who developed postoperative liver failure, PVE would be indicated in retrospect; however, in these patients, the performed resection was larger than anticipated because of unexpected intraoperative findings. Patients included in this study may be different from patient populations in other clinical practices in which most patients have noncompromised livers. However, the fact that postoperative morbidity and mortality were considerable did add necessary power to the study in which risk assessment was the primary goal. Further research is, however, warranted for subgroup analysis of different patient populations.

The main advantage of HBS lies in the fact that liver function is measured, taking into account the presence of underlying parenchymal liver disease. Hence, one cutoff value for the prediction of liver failure suffices in all possible patients regardless of the quality of the liver parenchyma. In contrast, volumetric assessment of the FRL

Table 6 Overview of the Results of the Three Different Preoperative Tests in Patients with Liver Failure

	Liver parenchyma	FRL-F (%/min/m^2)	%FRL-V (%)	sFRL (%)
1	Normal	2.17	**46**	**57**
2	Compromised	2.52	38	24
3	Compromised	2.67	22	38
4	Compromised	1.56	20	23
5	Compromised	2.22	32	31
6	Compromised	1.41	29	**41**
7	Compromised	2.17	24	25
8	Compromised	1.51	16	19
9	Compromised	**3.36**	**88**	**101**

The marked values indicate a false negative result of the test

requires two distinct cutoff values for patients with a compromised or noncompromised liver, assuming that the quality of the liver parenchyma is known. Especially in patients with uncertain quality of liver parenchyma, preoperative HBS is therefore of more value than %FRL-V or sFRL. The results of our study have led us to use HBS routinely, in addition to CT volumetry, in all patients considered for major liver resection. Preoperative PVE is performed when FRL-F is lower than 2.69%/min/m^2 or %FRL-V is less than 30%. Although around 40% of these patients will not develop liver failure, the risk of a potentially lethal complication outweighs the relatively low complication rate observed after PVE.[35]

Conclusion

HBS is a simple technique that can be implemented in every hospital with a nuclear medicine department. It is a valuable technique to estimate the risk of postoperative liver failure in high-risk patients undergoing major liver resection. Especially in patients with uncertain quality of the liver parenchyma, ^{99m}Tc-mebrofenin HBS is of more value than CT volumetry since only one cutoff value can be used in both normal and compromised livers. Therefore, additional HBS can improve risk assessment in patients requiring extensive liver resection.

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ORIGINAL ARTICLE

Clinicopathologic Analysis of Ampullary Neoplasms in 450 Patients: Implications for Surgical Strategy and Long-Term Prognosis

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Abstract

Background Whether ampullary neoplasms are best surgically managed by pancreaticoduodenectomy versus local ampullectomy is controversial. We sought to examine the outcome of patients undergoing pancreaticoduodenectomy versus ampullectomy, as well as to identify factors predictive of lymph node metastasis in patients with ampullary neoplasms.

Methods Between 1970 and 2007, 450 patients who underwent surgical resection of ampullary adenoma or adenocarcinoma were identified from a prospective, single-institution database. Data on clinicopathologic factors, morbidity, mortality, and survival were analyzed.

Results The initial surgical procedure was pancreaticoduodenectomy in 96.7% patients and ampullectomy in 3.3%. Final diagnosis was invasive adenocarcinoma (77.1%) or adenoma (22.9%). Median tumor size was similar for adenomas associated with an adenocarcinoma (2.5 cm) versus adenomas without invasive cancer (2.9 cm; $P=0.71$). Morbidity was comparable with pancreaticoduodenectomy (52.2%) versus ampullectomy (33.3%; $P=0.15$), as was 30-day mortality (pancreaticoduodenectomy, 2.1% versus ampullectomy, 0%; $P=0.6$). Metastatic disease to regional lymph nodes was present in 54.5% patients with adenocarcinoma. Factors associated with presence of lymph node metastasis included tumor size ≥1 cm (OR 2.1), poor histologic grade (OR 4.8), perineural invasion (OR 3.0), microscopic vessel invasion (OR 6.6), and depth of invasion > pT1 (OR 4.3; all $P < 0.05$). Specifically, risk of lymph node metastasis increased with T stage (T1, 28.0%; T2, 50.9%; T3, 71.7%; T4, 77.3%; $P<0.001$).

Conclusion When surgery is indicated, radical resection is required for early invasive adenocarcinoma of the ampulla of Vater, as lymph node metastases are present in nearly 30% of patients with T1 disease. Pancreaticoduodenectomy should be the preferred approach for most ampullary neoplasms that require surgical resection.

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Introduction

The ampulla of Vater refers to the flask-shaped structure formed by the junction of the common bile duct and the main pancreatic duct. The ampulla is usually located in the posteromedial wall of the second portion of the duodenum at the major duodenal papilla. Malignancies can arise from the biliary or pancreatic ductal epithelium, but also within the epithelium of the ampulla itself.

Ampullary neoplasms are uncommon relative to other gastrointestinal neoplasms. In one large autopsy series, occult ampullary adenomas and adenocarcinomas were observed in 0.2% of patients.[1,2] The annual age-adjusted incidence of ampullary adenocarcinoma is 0.3 per 100,000 individuals, with ampullary cancer comprising less than one percent of all digestive cancers.[3]

While endoscopic removal of certain ampullary lesions is feasible and associated with a good outcome,[4,5] other ampullary lesions require surgical management. However, whether ampullary neoplasms are surgically best managed by pancreaticoduodenectomy versus local ampullectomy remains controversial. Information regarding the optimal surgical management of ampullary lesions is particularly important because they are typically diagnosed earlier compared with other peri-ampullary tumors resulting in a higher rate of resectability.[6] Despite this, approximately 50% of ampullary neoplasms will recur.[7] As such, it is important to determine which factors determine clinical outcome in patients with ampullary adenomas and adenocarcinomas. In addition, data on factors predictive of lymph node metastasis in patients with ampullary neoplasms may be important to guide the extent of the operative approach necessary for this disease.

To date, most series on the topic of ampullary neoplasia have been limited by small sample sizes.[6,8–13] In addition, prior studies have not focused on identifying potential risk factors that impact the incidence of lymph node metastasis associated with ampullary lesions. Such information, however, is critical to inform the surgeon whether a more extensive operation with a lymphadenectomy might benefit certain patients rather than a local ampullectomy. In the current study, we sought to determine those factors associated with outcome following surgical resection of ampullary adenomas and adenocarcinomas. Specifically, we examine both peri-operative and long-term outcome of patients with ampullary neoplasms who were managed with ampullectomy versus pancreaticoduodenectomy. In addition, we identify those factors predictive of lymph node metastasis in patients with ampullary neoplasms.

Methods

Between January 1, 1970 and June 30, 2007, 450 patients who underwent surgical resection of an ampullary adenoma or adenocarcinoma at the Johns Hopkins Hospital were identified from a prospectively collected database. Only patients with a documented ampullary neoplasm as defined by gland-forming neoplasm centered on the ampulla were included. If an in situ component was associated with an invasive adenocarcinoma, a portion of the in situ carcinoma had to involve the ampullary epithelium. Carcinomas of the distal bile duct, pancreas, or duodenum, as well as carcinoid tumors of the ampulla, were excluded. As the current study sought to examine outcome following surgical management of ampullary neoplasms, patients who had endoscopic excision of an ampullary neoplasm were also excluded. The Johns Hopkins Institutional Review Board approved this study.

The following data were collected for each patient: demographics; operative details: resection margin status; tumor size; presence or absence of invasive carcinoma (e.g. adenoma versus invasive adenocarcinoma); presence of lymph node metastasis; presence of perineural and/or vascular invasion; peri-operative morbidity and mortality; vital status; and date of death or last follow-up. Peri-operative morbidity and mortality was defined as any complication or death that occurred within 30 days of surgery or during the same admission as the operation. Definitions of specific complications such as pancreatic fistula and delayed gastric emptying are described elsewhere.[14,15] Long-term survival status (e.g. alive versus dead) was determined by review of the medical records as well as through use of the United States social security death index and the national tumor registry.

Operative Approach and Peri-operative Care

The technique of pancreaticoduodenectomy and details regarding peri-operative management has been previously described.[14,16–18] In general, a partial pancreatectomy with pylorus preservation was performed. A distal gastrectomy was performed in instances when a pylorus preserving procedure would have left an inadequate resection margin, the duodenal stump appeared ischemic, or patients were part of a clinical trial comparing standard and radical pancreaticoduodenectomy.[19] Prophylactic octreotide was not typically administered, except in a minority of cases as part of a separate clinical trial.[20] Drains were routinely placed intraoperatively near the pancreatic and biliary anastomoses. A subset of patients underwent an ampullectomy. In general, patients were considered for ampullectomy only if the lesions were smaller than 2 cm and had no evidence of dysplasia. Ampullectomy was performed at the discretion of the operating surgeon and consisted of local resection of the ampulla through a transduodenal approach in conjunction with a pancreaticobiliary sphincteroplasty.[21]

Statistical Analysis

Categorical variables were compared using Chi-squared tests or logistic regression where applicable. Continuous variables were compared using the Mann–Whitney rank

sum test. A one-way analysis of variance was used to test for differences among independent categorical variables with multiple levels. Actuarial survival was estimated using the nonparametric product limit method (e.g. Kaplan–Meier) and differences in survival were examined with the log-rank test. Multivariate Cox proportional hazard models were used to determine which peri-operative risk factors were associated with long-term survival while controlling for competing risk factors. The most parsimonious model was created using a stepwise approach that included factors that were statistically significant (e.g. $P \leq 0.10$) on univariate analysis. Averages were provided as median values and statistical significance was designated as $P < 0.05$. All statistical analyses were performed using Intercooled STATA Version 8.2 (Chicago, IL, USA).

Results

Clinicopathologic Characteristics

Table 1 summarizes the clinicopathologic characteristics of the 450 patients who underwent surgical resection for an ampullary neoplasm. Of those patients who underwent surgical resection, 347 (77.1%) patients had an ampullary adenocarcinoma, whereas 103 (22.9%) had an ampullary adenoma. While the median patient age was comparable in patients diagnosed with invasive adenocarcinoma (68 years) versus adenoma (66 years; $P=0.10$), more patients with invasive adenocarcinoma were male (adenocarcinoma, 57.4% versus adenoma, 45.6%; $P=0.04$). Patients undergoing surgery for invasive adenocarcinoma versus adenoma had comparable medical comorbidities (Table 1).

Table 1 Clinicopathologic Characteristics of Patients with an Ampullary Neoplasia

	Adenocarcinoma ($n=347$)	Adenoma ($n=103$)	P value
Patient demographics	n (%)[a]	n (%)[a]	
Age (years), median (range)	68 (29–90)	66 (31–87)	0.10
Gender, male	199 (57.4)	47 (45.6)	0.04
Race, Caucasian	306 (88.2)	94 (92.2)	0.3
Past medical history			
Hypertension	127 (38.5)	29 (33.7)	0.4
Coronary artery disease	51 (21.1)	15 (23.4)	0.7
History of tobacco use	62 (19.8)	11 (13.3)	0.2
Diabetes mellitus	44 (13.3)	10 (11.6)	0.7
History of alcohol abuse	35 (10.7)	7 (8.2)	0.5
Myocardial infarction	28 (8.5)	8 (9.3)	0.8
COPD	28 (8.5)	5 (5.8)	0.4
Peripheral vascular disease	18 (5.4)	5 (5.8)	0.9
Preoperative tumor markers			
CEA (>3 ng/mL)	33 (28.5)	6 (24.0)	0.7
CA 19–9 (>36 U/mL)	75 (63.0)	6 (23.1)	<0.001
Preoperative signs/symptoms			
Jaundice	237 (72.3)	10 (11.9)	<0.001
Weight loss	138 (42.3)	14 (16.7)	<0.001
Abdominal pain	106 (32.6)	30 (35.7)	0.6
Pruritis	50 (15.4)	1 (1.2)	<0.001
Nausea or vomiting	46 (14.2)	11 (13.1)	0.8
Fevers	24 (7.4)	5 (6.0)	0.7
Gastrointestinal bleeding	11 (3.4)	4 (4.8)	0.6
Asymptomatic (incidentaloma)	10 (3.1)	16 (19.1)	<0.001
Invasive preoperative procedures			
Any procedure	321 (96.1)	77 (89.5)	0.02
Biliary stent[b]	253 (78.1)	35 (43.2)	<0.001
Biopsy	188 (57.1)	57 (68.7)	0.06
ERCP	247 (89.2)	34 (52.3)	<0.001
Endostent	187 (58.1)	16 (19.5)	<0.001
PTC/PBD	100 (31.0)	20 (24.7)	0.3
EUS	24 (14.2)	7 (17.0)	0.7

COPD chronic obstructive pulmonary disease, *ERCP* endoscopic retrograde cholangiopancreatography, *PTC/PBD* percutaneous transhepatic cholangiography/percutaneous biliary drain, *EUS* endoscopic ultrasound

[a] Unless otherwise specified

[b] Includes endostent and transhepatic biliary stent

Patients who harbored an invasive adenocarcinoma were more likely to present with weight loss (42.3% versus 16.7%, $P<0.001$), pruritis (15.4% versus 1.2%, $P<0.001$), and jaundice (72.3% versus 11.9%; $P<0.001$) compared with patients who had an adenoma. Patients with invasive adenocarcinoma were also more likely to require preoperative biliary drainage compared with patients who had an adenoma (78.1% versus 43.2%, respectively; $P<0.001$). Benign ampullary neoplasms were more often discovered as an incidental finding during the workup for unrelated reasons (adenocarcinoma, 19.1% versus adenoma, 3.1%; $P<0.001$). While the tumor marker carcinoembryonic antigen (CEA) was in the normal range in the majority of patients in both groups (adenocarcinoma, 71.5% versus adenoma, 76.0%; $P=0.71$), serum CA19-9 was abnormal more often in patients with invasive adenocarcinoma (adenocarcinoma, 63.0% versus adenoma, 23.1%; $P<0.001$). Of all preoperative symptoms and laboratory values examined, only preoperative serum CA19-9 remained associated with an increased odds of harboring an underlying invasive adenocarcinoma (OR 5.79; $P=0.004$) on multivariate analysis.

Surgical Details and Peri-operative Outcome

Of the 450 patients with an ampullary neoplasm who underwent surgical resection, a pancreaticoduodenectomy was performed in 435 (96.7%) patients while a local ampullectomy was performed in 15 (3.3%) patients. Intraoperative and postoperative data are summarized in Table 2. Median blood loss was higher among patients who underwent pancreaticoduodenectomy (600 mL) compared with ampullectomy (300 mL; $P=<0.001$). As expected, operative time was also longer for pancreaticoduodenectomy (347 min) compared with ampullectomy (201 min; $P\leq$ 0.001). Among those patients who underwent a pancreaticoduodenectomy, a pylorus preserving pancreaticoduodenectomy was more commonly performed in patients with an adenoma (90.0%) versus adenocarcinoma (81.6%; $P=0.04$). One patient with an ampullary adenocarcinoma incurred a vascular injury during a pancreaticoduodenectomy that required a total pancreatectomy.

Morbidity following pancreaticoduodenectomy (52.2%) and ampullectomy (33.3%) was not statistically different ($P=0.15$). Most complications were minor and included wound infection (pancreaticoduodenectomy, 11.1% versus ampullectomy, 20.0%; $P=0.3$), delayed gastric emptying (pancreaticoduodenectomy, 16.0% versus ampullectomy, 0%; $P=0.09$), and pancreatic leak (pancreaticoduodenectomy, 20.7% versus ampullectomy, 0%; $P=0.049$). As expected, delayed gastric emptying and pancreatic leak were noted exclusively in patients who had undergone pancreaticoduodenectomy. Among patients who underwent pancreaticoduodenectomy, the incidence of pancreatic fistula was higher among patients who had an underlying adenoma (30.0%) versus patients who had an adenocarcinoma of the ampulla (18.2%; $P=0.01$). The higher rate of pancreatic fistula among patients with an adenoma corresponded to a higher incidence of a "soft" gland among patients with an adenoma (92.3%) versus invasive adenocarcinoma (59.4%; $P=0.001$).

Median length of stay in the hospital was shorter for patients undergoing an ampullectomy (7 days) versus

Table 2 Intra- and Postoperative Data on Patients Undergoing Resection of Ampullary Neoplasia Stratified by Procedure Type

	PD ($n=435$)	Ampullectomy ($n=15$)	P value
Intraoperative data	n (%)[a]	n (%)[a]	
Blood loss (mL), (median, range)	600 (75–9,000)	300 (100–750)	<0.001
Op. time (min), (median, range)	347 (210–979)	201 (135–356)	<0.001
Resection of major visceral vein	4 (0.95)	0 (0)	0.7
Pylorus preserving	359 (82.5)	N/A	
Total pancreatectomy	1 (0.2)	N/A	
Postoperative mortality	9 (2.1)	0 (0)	0.6
Reoperation rate	16 (3.8)	0 (0)	0.15
Postop LOS (days), (median, range)	11 (5–388)	7 (6–30)	0.02
Postoperative morbidity	222 (52.2)	5 (33.3)	0.15
Specific complications			
Pancreatic fistula	88 (20.7)	0 (0)	0.049
Delayed gastric emptying	68 (16.0)	0 (0)	0.09
Wound infection	47 (11.1)	3 (20)	0.3
Abdominal abscess	36 (8.5)	0 (0)	0.2
Cardiac event	19 (4.5)	0 (0)	0.4
Bile leak	16 (3.8)	1 (6.7)	0.6

PD pancreaticoduodenectomy, *Op.* operative, *min* minutes, *Postop. LOS* postoperative length of stay, *N/A* not applicable

[a] Unless otherwise specified

pancreaticoduodenectomy (11 days; $P=0.02$). Among those patient undergoing a pancreaticoduodenectomy, postoperative length of stay was comparable among patients with an adenoma (11 days) versus invasive adenocarcinoma (13 days; $P=0.07$). The 30-day mortality rate associated with pancreaticoduodenectomy (2.1%) was not different from that with ampullectomy (0%; $P=0.6$).

Pathological Details and Risk of Lymph Node Metastasis

On pathological review, the final diagnosis was invasive adenocarcinoma (77.1%) or adenoma (22.9%). Of those patients with dysplasia on pre-operative endoscopic biopsy, 58.9% had adenocarcinoma on final surgical pathology. Median tumor size was slightly smaller among invasive adenocarcinoma lesions (2.0 cm) versus adenoma lesions (2.9 cm; $P\leq0.001$). Among patients with an invasive adenocarcinoma, 61 (17.6%) had an adenocarcinoma that was associated with an adenoma (Fig. 1). Adenomas associated with an invasive cancer were not different in size (median, 2.5 cm) compared with adenomas without any associated invasive cancer (median, 2.9 cm; $P=0.7$).

Among patients with an invasive adenocarcinoma of the ampulla, roughly half of the patients had T1 (9.2%) or T2 (41.1%) disease. The majority of patients had either a well- (5.1%) or moderately differentiated (56.8%) cancer. Perineural invasion was identified in 99 patients (41.4%), while microscopic vascular invasion was noted in 104 (43.6%) patients (Table 3). Pancreaticoduodenectomy (96.1%) was associated with high incidence of a microscopically negative surgical margin (R0).

Of the 347 patients with invasive carcinoma, metastatic disease to the regional lymph nodes (N1) was present in 189 (54.5%). On univariate analysis, factors associated with an increased risk of lymph node metastasis included tumor size ≥1 cm (OR, 2.1; 95% CI, 1.1–4.2), poor histologic grade (OR, 4.8; 95% CI, 2.9–8.1), perineural invasion (OR, 3.0; 95% CI, 1.7–5.2), microscopic vascular invasion (OR, 6.6; 95% CI, 3.5–12.4), as well as depth of invasion > pT1 (OR, 4.3; 95% CI, 1.7–10.8). Specifically, the risk of lymph

node metastasis increased with T stage (T1, 28.0% versus T2, 50.9% versus T3, 71.7% versus T4, 77.3%; $P<0.001$). On multivariable analysis, after controlling for competing risk factors, both histologic grade (OR, 3.4; 95% CI, 1.7–6.8) and microvascular invasion (OR, 4.0; 95% CI, 1.9–8.3) remained as independent predictors of lymph node metastasis. T stage, while not significant, continued to show a strong trend toward being associated with the risk of lymph node metastasis (OR, 4.4; 95% CI, 0.93–20.4).

Outcome

Following ampullectomy, three out of 15 patients (20.0%) developed a recurrent mass. All three patients had initially been diagnosed with a non-invasive adenomatous neoplasm following the initial ampullectomy. One patient was managed with endoscopic removal of the recurrent mass with subsequent pathology revealing an adenoma with dysplasia. The other two patients underwent salvage pancreaticoduodenectomy. Final pathology revealed ampullary adenocarcinoma in these two patients; one patient with ampullary adenocarcinoma also had nodal metastasis at the time of re-presentation.

The 1-, 2-, and 5-year overall survival for patients undergoing resection of an ampullary adenoma were 99%, 96%, and 86%, respectively, which was no different than age-matched population-based controls ($P=0.62$). Patients who underwent resection of an invasive adenocarcinoma had a worse long-term survival than did patients with an adenoma, with a 1-, 2-, and 5-year survival of 82%, 64%, and 45%, respectively ($P\leq0.001$; Fig. 2). Among those patients with invasive adenocarcinoma of the ampulla, a number of clinicopathologic factors were associated with survival. Specifically, on univariate analysis receipt of blood transfusion, the presence of perineural invasion, and lymph node metastasis were all significant predictors of poor survival. Patients who received an intraoperative blood transfusion had a median survival of 25.7 months compared with 55.4 months for patients who did not ($P=0.005$). The presence of perineural vascular invasion was

Figure 1 a Gross appearance of a polypoid ampullary lesion with an associated adenocarcinoma. **b** Microscopic appearance of an ampullary adenocarcinoma (×64 magnification).
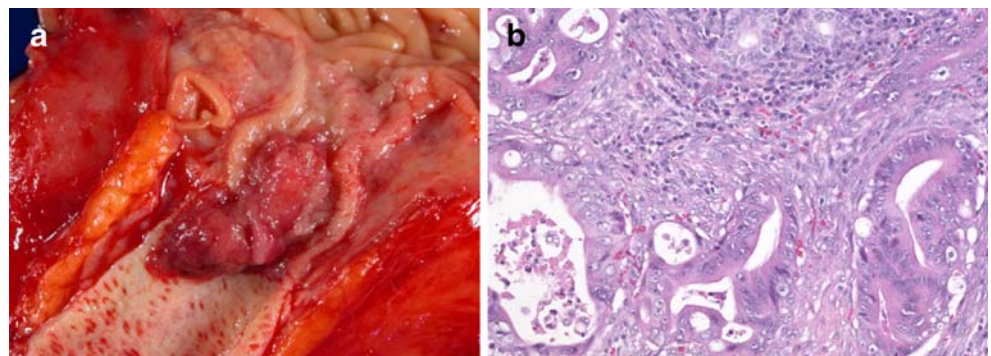
Table 3 Pathologic Characteristics of Ampullary Adenocarcinoma Lesions

	Adenocarcinoma (n=345) n (%)[a]
Pathology	
Primary tumor diameter (cm), median (range)	2 (0.1–6.6)[b]
Positive resection margin	13 (3.9)
Positive lymph nodes	189 (55.8)
pT1 only	7 (28.0)
T2 only	57 (50.9)
T3 only	81 (71.7)
T4 only	77 (77.3)
Diameter <1 cm	16 (40.0)
Grade of differentiation	
Well	16 (5.1)
Moderate	180 (56.8)
Poor	119 (37.5)
Undifferentiated/anaplastic	2 (0.6)
Vascular (small vessel) invasion	99 (43.6)
Perineural invasion	99 (41.4)

[a] Unless otherwise specified

[b] For instances when there is an associated adenoma, the diameter refers only to the size of the invasive cancer

also associated with a poor prognosis. Whereas the median survival had not been reached for patients with no perineural invasion, those with perineural invasion had a median survival of 20.3 months ($P<0.001$). Similarly, the presence of lymph node metastasis was associated with a significantly worse long-term outcome. Patients with lymph node metastasis had a median survival of 23.4 months compared with 79.1 months for patients without node metastasis ($P<0.001$; Fig. 3). On univariate analysis, receipt of adjuvant chemoradiation (5-flurouracil plus 50.4 Gy) was not associated with a survival benefit ($P=0.4$). However, among patients with invasive adenocarcinoma who had perineural invasion, receipt of adjuvant chemoradiation tended to have an improved survival (median survival: no adjuvant chemoradiation, 12.5 months versus adjuvant chemoradiation, 30.4 months; $P=0.08$). On multivariate analysis, receipt of intraoperative blood transfusion (hazard ratio [HR] 1.7, $P=0.01$), presence of perineural invasion (HR 2.2, $p<0.001$), and regional lymph node metastasis (HR 2.0, $P=0.002$) each remained independently associated with an increased risk of disease-specific death.

Discussion

William Stewart Halsted is credited with performing the first local resection of an ampullary carcinoma in 1898.[22] Two German surgeons, Walter Kausch and George Hirschel, later performed a pancreaticoduodenectomy for ampullary cancer in 1912 and 1914, respectively.[23–25] Allen O. Whipple was the first American surgeon to perform a

pancreaticoduodenectomy for ampullary cancer, and presented his first three operations for ampullary adenocarcinoma to the American Surgical Association in 1935.[26] Since these initial reports, management of lesions of the ampulla has remained somewhat controversial. While endoscopic removal of certain ampullary lesions is feasible and associated with a good outcome,[4,5] other ampullary lesions require surgical management. When surgery is indicated, some surgeons have proposed local resection of select ampullary neoplasms,[27,28] while other investigators have advocated for routine pancreaticoduodenectomy.[6,29] Because of the relative scarcity of the disease, data on the surgical management of neoplasms of the ampulla of Vater have been lacking. Most studies suffer from small sample size[28,30,31] or provide aggregate data on "peri-ampullary"
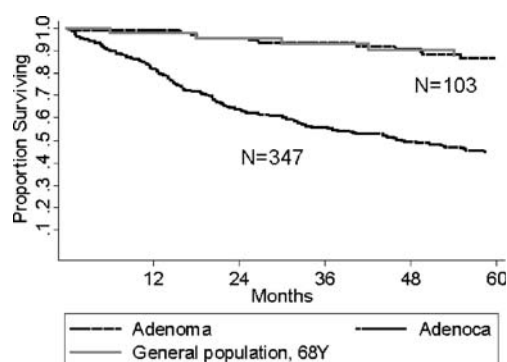
Figure 2 Overall survival for patients undergoing resection of an ampullary adenoma was no different than age-matched population-based controls. Patients who underwent resection of an adenocarcinoma had a significantly worse long-term survival ($P<0.001$). *adenoca* adenocarcinoma.
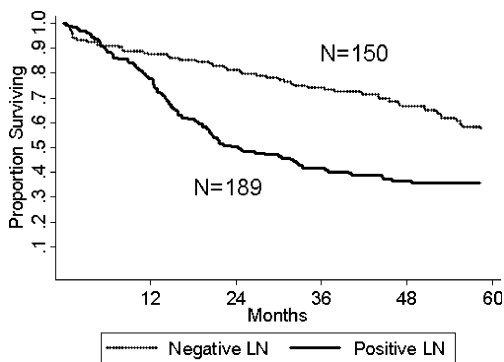
Figure 3 Patients with lymph node metastasis had a significantly worse median and 5-year survival (23.4 months and 35.0%, respectively) compared to patients without nodal metastasis (79.1 months and 56.4%, respectively; $P<0.001$). *LN* lymph node.

neoplasms.[18,32] The current study is important because it provides the largest single-institutional series on the surgical management of patients with neoplasms of the ampulla of Vater. More importantly, data from the current study provide important information on the prognosis of patients following resection of ampullary neoplasms. In addition, our data help inform the operative approach to patients with lesions of the ampulla. Specifically, our data show that even in well-selected patients, ampullectomy can often result in suboptimal outcomes and the need for salvage pancreaticoduodenectomy.

Several investigators have proposed specific criteria to identify which patients may be appropriate for local resection.[27,28,33] In particular, ampullectomy has been suggested to be the procedure of choice in ampullary lesions that measure less than 2 to 3 cm in size.[28,33] In the current study, tumor size was not associated with an underlying adenocarcinoma. In fact, among adenomatous lesions, tumor size was near identical between those lesions harboring an associated invasive carcinoma (2.5 cm) and those that did not (2.9 cm; $P=0.7$). In addition, of the adenomas resected, 40.9% had an associated underlying invasive adenocarcinoma. Presenting symptoms and most laboratory values were also not different between patients with an underlying adenocarcinoma versus an adenoma. As such, preoperative diagnosis and differentiation of benign versus malignant ampullary lesions cannot be reliably determined by size or symptoms. All ampullary lesions referred for surgical management should therefore be considered potentially malignant.

Among patients with invasive carcinoma, one of the most important clinicopathologic variables to influence survival is the presence of lymph node metastasis.[30,34] In fact, the presence of lymph node metastasis was associated with a median survival less than one-third that of patients without nodal metastasis (Fig. 3). An adequate lymph node dissection at the time of surgery for ampullary neoplasms not only

provides important prognostic information, but also may decrease the risk of local recurrence.[35] In addition, lymph node involvement may have implications on adjuvant therapy. Data that inform the surgeon whether a lymphadenectomy might benefit certain patients rather than a local ampullectomy is therefore critical. In the current study, factors associated with an increased risk of lymph node metastasis included tumor size, poor histologic grade, perineural invasion, microscopic vascular invasion, as well as depth of invasion/T stage. Some authors have suggested that ampullectomy without a lymphadenectomy is an adequate therapy for patients with early or T1 lesions due to the low incidence of lymph node metastasis.[28,31,36–38] However, data from the current study found this decidedly not to be the case. Although the risk of lymph node metastasis increased with T stage, the incidence of lymph node metastasis was still clinically significant in patients with T1 disease (28.0%). Our findings are consistent with data from smaller series that have noted an incidence of lymph node metastasis of 20% to 25% for patients with T1–T2 disease.[30] In aggregate, these data strongly suggest that even patients with early invasive adenocarcinoma of the ampulla have a high risk of lymph node metastasis and are best served with an operation that includes lymph node dissection and clearance.

In the current series, only 15 (3.3%) patients underwent local resection of their ampullary lesion. However, even in this extremely select cohort of patients the incidence of recurrence was high. Specifically, three out of 15 patients (20.0%) developed a recurrent mass, and two of them required salvage pancreaticoduodenectomy. Out of these three patients, one patient had metastasis to the regional nodal basin at the time of re-presentation. Other authors have reported a high recurrence rate after local resection. Branum et al.[21] reported that six of eight patients developed a recurrence following local ampullectomy. In a separate study, Sperti et al.[39] noted three tumor recurrences in five patients. Qiao et al.[34] reported a series of 127 patients, three of whom underwent a local resection. Despite obtaining negative margins in each case, all three patients developed a recurrence. Two patients died of tumor recurrence, while the third patient was salvaged by pancreaticoduodenectomy for local recurrence 48 months after the initial ampullectomy. Our group, as well as others,[30,34] therefore caution that local resection of ampullary lesions has a very limited role for gland-forming neoplasms.

We favor pancreaticoduodenectomy rather than local ampullectomy not only based on oncologic principles, but also because the morbidity and mortality associated with pancreaticoduodenectomy are low. Pancreaticoduodenectomy can now be performed with a low mortality rate and short recovery time at high-volume centers.[40] Early

experience with pancreaticoduodenectomy was associated with mortality rates up to 25%. More recently, hospital mortality after major pancreatic resections has dramatically decreased.[18,40,41] At the Johns Hopkins Hospital, the mortality rate associated with pancreaticoduodenectomy has been reported to be less than 5% with an average postoperative length of stay of just 8 days.[42] In the current study, there was no significant difference in the morbidity or mortality associated with local ampullectomy compared with pancreaticoduodenectomy. Pancreaticoduodenectomy can therefore be performed safely with low peri-operative mortality and morbidity for lesions of the ampulla.

Survival following pancreaticoduodenectomy for invasive adenocarcinoma of the ampulla was associated with a 5-year survival of 45%. Other published data have reported 5-year survival around 50%.[34,39,43] Compared with invasive adenocarcinoma of the pancreas which has a 5-year survival in the range of 20%,[42] ampullary adenocarcinoma has a much better overall survival following resection. The improved survival for ampullary adenocarcinoma may be related in part to the higher rate of resection of ampullary lesions.[6,44] Perhaps more importantly, ampullary carcinoma probably has a different biologic behavior from pancreatic cancer.[30,45] Specifically, carcinoma of the ampulla has been noted to exhibit differences in macroscopic growth patterns with a lower frequency of local infiltration and lower rates of vascular and perineural invasion compared with pancreatic carcinoma.[30,46] There may also be molecular genetic differences between ampullary and pancreatic malignancies.[47] When ampullary adenocarcinoma is associated with vascular or perineural invasion the prognosis, however, is considerably worse. In fact, vascular invasion (OR 6.6) and perineural invasion (OR 3.0) both strongly predicted an increased risk of lymph node metastasis and a worse long-term survival.

The current study had several limitations. Despite having the largest pancreaticobiliary surgical experience in the country, only a relatively small number of patients underwent local ampullectomy. As such, direct comparisons between patients who underwent pancreaticoduodenectomy and local ampullectomy have limited statistical power. These comparisons were, however, not the main focus of the current study. Rather, data and statistical analyses on peri-operative outcomes, risk of lymph node metastasis, and overall outcome for all patients with ampullary neoplasms are robust and are based on the largest series reported to date. Another limitation of the current study is that it was restricted to patients who underwent surgical management only. While endoscopic removal may have a role in treating a sub-set of ampullary lesions, our dataset was limited to patients who were managed surgically. As such, data presented herein cannot necessarily inform which ampullary lesions are best managed endoscopically. Rather, our data are best utilized to inform how best to manage surgically patients with ampullary neoplams when they are in need of an operative procedure.

In conclusion, pancreaticoduodenectomy is required for early ampullary adenocarcinoma, as lymph node metastases are present in 28.0% of patients with T1 disease. Benign versus malignant ampullary lesions cannot be routinely distinguished based on preoperative symptoms or lesion size. Even in highly select patients, ampullectomy often can result in suboptimal outcomes with the need for salvage pancreaticoduodenectomy. Pancreaticoduodenectomy should be the preferred approach for most ampullary neoplasms that require surgical resection.

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ORIGINAL ARTICLE

Beyond Epithelial to Mesenchymal Transition: A Novel Role for the Transcription Factor Snail in Inflammation and Wound Healing

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Heinz-Johannes Buhr · Hubert Georg Hotz

Abstract

Introduction Snail, a transcription factor linked to epithelial to mesenchymal transition (EMT) during embryonic development and tumor progression, is associated with migration of cells. During inflammation and tissue injury, cell movement is also observed to provide the first line of defense against bacteria and to promote wound healing. Therefore, we studied the function of Snail in activated macrophages in a variety of inflammatory processes.

Materials and Methods In this study, we examined the expression and localization of Snail during inflammation and tissue injury in rats and human tissue specimens, by immunohistochemistry, Western blot, and real-time PCR. We investigated Snail expression after stimulation of macrophages with TGF-β1, LPS, Interleukin-8, and MMP-3 in vitro. To further understand the role of Snail in activated macrophages, we used Stealth siRNA against Snail, transfected the human macrophage cell line THP-1, and measured migration of cells in an in vitro invasion assay.

Results and Discussion We found a strong, transient, and time-dependent activation of Snail in migrating macrophages at the sites of injury in vivo and in vitro, as well as in patients with inflammatory bowel disease. Furthermore, we showed that induction of Snail in macrophages is dependent on TGF-β1 signaling pathway. Downregulation of Snail by Stealth siRNA led to impaired migration of THP-1 cells in an invasion assay after stimulation with TGF-β1.

Conclusion We conclude that TGF-β1 induced migration of activated macrophages during inflammation and wound healing is mediated by snail. These results give insights in a novel EMT-like mechanism present in immune cell movement during tissue injury.

Introduction

The transcription factor Snail, a zinc finger protein, has been shown to be implicated in triggering epithelial to mesenchymal transition (EMT) which convert epithelial cells into mesenchymal cells with migratory properties.[1] The central event during the loss of epithelial phenotype is the repression of the adhesion molecule E-cadherin by Snail.[2] Snail-induced EMT is believed to play a crucial role in both embryonic development and pathological circumstances such as tumor progression and fibrosis.[3,4] In addition, several lines of evidence point to a role for Snail in the cell survival contributing to the protection of cell death. Interestingly, recent evidence shows that the members of the Snail family (including Snail itself) participate in the regulation of cell adhesion and migration independently of the induction of EMT such as mesoderm formation in *Drosophila*.[5] The idea that Snail is involved in cell movement that does not require a full EMT arises the question whether triggering of EMT would be just one of the mechanisms used by this transcription factor to induce loss of cell adhesion and to increase cell migration.[5]

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Tissue injury triggers an organized and complex cascade of cellular and biochemical events that result in a healed wound. This wound healing response can be divided into three distinct but overlapping phases: (1) hemostasis and inflammation, (2) proliferation, and (3) remodeling.[6] The inflammatory phase is an essential phase of healing, characterized by increased vascular permeability, chemotaxis of neutrophils from the circulation into the wound milieu, local release of cytokines and growth factors, and activation of migrating cells, especially macrophages.[6]

Here, we report for the first time the transient and time-dependent activation of Snail in migrating macrophages during inflammation and wound healing. We have investigated gastrointestinal anastomoses as well as wound healing of the skin in rats, and have found an induction of Snail expression mostly in macrophages, and to a less amount in neutrophils at the wound site. In order to assign these results to human specimens we examined sites of wound healing in the human skin and in addition in acute appendicitis, and found an increased expression of Snail in macrophages only at the wound site.

Inflammation occurs also during chronic inflammatory bowel disease (IBD) like Crohn's disease (CD) and ulcerative colitis (UC)[7,8] and therefore we examined tissue samples of patients with these diseases. Snail expression was also found in macrophages in affected colon tissues in contrast to healthy ones.

Although the mechanisms that modulate the function of Snail in migrating macrophages remain incomplete, we examined four pathways which are activated during inflammation, transforming growth factor beta (TGF-β1),[9–12] lipopolysaccharide (LPS),[8] interleukin-8 (IL-8),[13] and matrix metalloproteinase-3 (MMP-3),[14,15] and found that only TGF-β1 and to a less extent LPS are able to increase the induction of Snail. The other examined pathways seem to play minor roles in the regulation of Snail. To verify the role of Snail in the mechanism of TGF-beta-1-induced migration, we generated siRNA-Snail transiently transfected human macrophages (THP-1), measured cell migration in vitro in the presence of TGF-β1, and found an impaired movement of transfected THP-1 cells. Based on these findings, we propose a novel function of Snail in macrophage movement that is an unexpected and EMT-independent mechanism of this transcription factor.

Material and Methods

Patients and Paraffin-Embedded Tissue Sample

Ten tissue samples of inflamed wounds, acute appendicitis, 20 intestinal tissue samples of both Crohn's disease and ulcerative colitis, and 15 tissue samples of healthy intestine were obtained from patients, who underwent surgery at the Charité, CBF. Tissues were divided in two parts and either fixed in 4% formalin and embedded in paraffin, or shock frozen and stored at −80°C.

Animal Model

Male Wistar rats were anesthetized with isoflurane, followed by intraperitoneal injection of xylazinhydrochloride (Rompun, 12 mg/kg BW; Bayer, Leverkusen, Germany) and esketaminhydrochloride (Ketanest S, 40 mg/kg BW; Parke-Davis/Pfizer, Karlsruhe, Germany). A 3-cm median laparotomy was made and the descendent colon carefully mobilized. The colon was divided by a scissor and subsequently reanastomosed using 6–0 prolene by one layer continuous sutures. The laparotomy was closed in two layers with 3–0 absorbable suture (Vicryl, Ethicon, Germany). For pain relief, a subcutaneous injection of Carprofen (Rimadyl, 4 mg/kg BW; Pfizer) was given after surgery and the animals had food and water ad libitum; 6, 24, 48, and 96 h and 2 weeks after surgery, the animals were sacrificed by a lethal dose of isoflurane and the anastomoses was excised. The edge 5-mm oral and aboral the suture line was considered "anastomoses" and was harvested for the following experiments. The anastomoses were divided longitudinal in two parts and either fixed in 4% formalin and embedded in paraffin, or shock frozen and stored at −80°C.

Immunohistochemistry

Immunohistochemical staining was performed on paraffin-embedded tissue. Three-micrometer-thick sections were cut, using a rotation microtom (Leica, RM2125RT). The sections were deparaffinized in xylene (2×5 min) and rehydrated in graded alcohols (100–70%, 5 min each) and distilled water. After antigen retrieval with 0.01% EDTA pH 8.0 (10 min boiling in a microwave), endogenous peroxidase activity was blocked with 1% hydrogen peroxide in distilled water for 25 min followed by washing with distilled water and finally phosphate-buffered saline (PBS) +0.1% Tween for 5 min. To bind nonspecific antigens, the sections were incubated with 1× Power Block (BioGenex, San Ramon, Ca) for 5 min. The primary antibodies for Snail, and CD68 were either polyclonal rabbit anti-Snail (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or polyclonal mouse anti-CD68 (Thermo Scientific, Fremont, CA, USA). Antibody dilution ranges from 1:50 to 1:150 in antibody diluent (DCS, Hamburg, Germany) for 30 min at 37°C. As negative control, sections were incubated with antibody diluent instead of the primary antibody. This was followed by incubation with biotinylated anti-rabbit/anti-mouse immunoglobulin G (1:200, Santa Cruz) for 30 min at 37°C and after washing with PBS+Tween by peroxidase-

conjugated avidin–biotin complexes (KPL, Gaithersburg, MD, USA) and 3,3`-diaminobenzidine (Sigma, DE, USA). The sections were then counterstained with Mayer's hematoxylin for 2 min, upgraded alcohols (70–100%, 2 min each), mounted and analyzed by standard light microscopy. Cells stained positive for Snail were counted in ten random fields of view at ×100 magnification and expressed as the average number of cells/field of view.

Cell Lines and Stimulation with TGF-β1, LPS, Interleukin-8, and MMP-3

The human monocyte lymphoma cell lines THP-1 as well as Jurkat T cells were obtained from the American Tissue Culture Collection (ATCC, Rockville, USA). The cells were cultured in RPMI-1640 medium (PAA Laboratories, Cölbe, Germany) supplemented with 10% fetal calf serum (FCS-Gold, PAA), Penicillin G (100 U/ml), Streptomycin (100 μg/ml), and Amphotericin B (0.25 mg/ml). The cells were incubated at 37°C in humidified air with 5% CO_2. Macrophages were generated from undifferentiated THP-1 using 100 nM phorbol 12-myristate 13-acetate (PMA)/ml medium (Sigma Aldrich). After incubation for 14 h with PMA, media was replaced by fresh RPMI medium and cells were maintained in medium for further 72 h. Differentiation was determined by increased cell attachment to the flasks and by changes in cell morphology. Recombinant TGF-β1 (10 ng/ml), LPS (1 μg/ml), interleukin-8 (10 nM/ml), and MMP-3 (40 nM/ml; Sigma Aldrich) were added to the culture medium. At 24 h and 48 h of stimulation, cells were harvested for western blotting and real-time polymerase chain reaction (PCR).

siRNA

After overnight culture of differentiated THP-1 cells in a six-well dish, the medium was replaced with 2 ml new medium (RPMI-1640 without FBS and antibiotics) and the cells were transiently transfected with Stealth/siRNA-Snail (100 pmol, Invitrogen, Carlsbad, CA, USA) or control-siRNA using Lipofectamine 2000 (Invitrogen) for an additional 48 h (using the manufacturer's protocol). Cells were then treated with TGF-β1 as described above and were harvested for Western blotting and reverse transcriptase (RT)-PCR. Stealth/siRNA-Snail Primer 1: 5′-UGGCACUGGUACUU CUUGACAUCUG-3′; Primer 2: 5′-CAGAUGUCAAGAA GUACCAGUGCCA-3′. Control-Stealth/siRNA Primer 1: 5′-UACCGUCAUGAUACAGUCACCGAGG-3′; Primer 2: 5′-CCUCGGUGACUGUAUCAUGACCGUA-3′.

Reverse Transcriptase Polymerase Chain Reaction

Total cellular RNA was extracted from cell cultures using the NucleoSpin RNA II-Kit (Macherey & Nagel, Düren,

Germany) according to the manufacturer's instructions and resuspended in 50 μl of DMPC-treated distilled water. RNA concentration was determined using a BioPhotometer (Eppendorf Scientific, Hamburg, Germany). Total RNA (2 μg) was primed with an oligo(dT) oligonucleotide and reverse-transcribed with M-MLV reverse transcriptase (Promega, Mannheim, Germany) and dNTPs (Sigma-Aldrich, Seelze, Germany) according to the manufacturer's instructions. First-strand cDNA was amplified with tran-script-specific oligonucleotides using Ready-Mix Taq PCR Reaction Mix (Sigma-Aldrich, Seelze, Germany).

Quantitative Real-Time PCR

Real-time quantitative RT-PCR analyses for Snail (NM_005985.2), and PMM-1 (NM_002676) were performed by using the Light cycler[R] with the Light cycler software 3.5[R] (Roche, Mannheim, Germany). Primers were designed using the Light cycler Probe Design Software 2.0[R] (Roche), each amplifying an approximately 150 bp product. Primers were manufactured by TIBMolbiol (Berlin, Germany). PMM-1 was included as a housekeeping gene control to correct for equal RNA amounts. We then calculated relative amounts of mRNA with the Relative Quantification Software[R] (Roche). The Light cycler runs were done in duplicates. The principle of real-time RT-PCR has been described in detail before. Briefly, real-time RT-PCR is based on fluorescence emission by a sequence-specific primer pair. PCR was performed using the Light cycler Faststart DNA SYBR Green I-Kit (Roche, Mannheim, Germany) according to manufacturer's instructions. SYBR Green I binds to the minor groove of the DNA double helix. In solution, the unbound dye exhibits very little fluorescence; however, fluorescence (wavelength, 530 nm) is greatly enhanced upon DNA binding. Therefore, during PCR, the increase in SYBR Green I fluorescence is directly proportional to the amount of double-stranded DNA generated. Five microliters of diluted cDNA (1:5 with PCR-grade water), 0.5 μl primers (10 pmol/μl), and 0.8 μl $MgCl_2$ (25 mM) in a 10 μl final reaction mixture were used. After a 15-min incubation at 95°C for activation of the polymerase, each of the 35 cycles consisted of 15 s of denaturation at 95°C and amplification of primers for 30 s at 64°C. The melting curve analysis was performed in one cycle of 95°C for 1 s and 65°C for 10 s, each with a temperature transition rate of 20°C/s and then ramping to 95°C at 0.1°C/s.

Western Blotting

For isolation of total protein, the tissue was homogenized in lysis buffer containing 10 mM Tris (pH 6.8), 2 mM EDTA (pH 8.0), 0.15 M NaCL, 0.1% Brij 96, 0.1% NP-40, 2 mM PMSF, and 1× Protease inhibitor cocktail (Sigma-Aldrich).

Protein was estimated using QuantiPro BCA assay kit (Sigma-Aldrich) according to manufacturer's instructions. Thirty micrograms proteins were denatured at 95°C with sample buffer (0.125 M Tris (pH 6.8), 4% SDS, 20% glycerol, 2% mercaptoethanol, and 0.03 mM bromphenol blue) for 5 min and were separated by electrophoresis in 12% SDS-PAGE gels according to their molecular weight. Proteins were transferred onto a PVDF membrane (PerkinElmer, Zaventem, Belgium), blocked 2 h in blocking solution (5% non-fat dry milk in TBS) at room temperature on a rotating plate for 2 h. The membrane was then exposed to the primary antibody overnight at 4°C. The primary antibodies were Snail (anti-rabbit, Biozol, Eching, Germany), and β-actin (anti-mouse, LabVision, Fremont, USA), and the dilution range was from 1:1,000 to 1:10,000. After washing with TBS, the membranes were incubated for 1.5 h at room temperature with peroxidase-linked secondary antibody (Roche, Mannheim, Germany), and signals were detected using Lumilight Plus Western Blotting Kit reagents (Roche, Mannheim, Germany) according to the manufacturer's instructions and luminescence imaging (LAS-1000, Fujifilm).

In Vitro Invasion Assay

For in vitro invasion assay, 1.5×10^5 cells of differentiated macrophages were plated in the top chamber of Matrigel-coated PET membranes (24-well insert, pore size 8 μm; Becton Dickinson). Medium with 20% FCS and 1% BSA was used as a chemoattractant in the lower chamber. The cells were incubated for 48 h in a humified tissue culture incubator, at 37°C and 5% CO_2 atmosphere. Those cells that did not migrate through the pores in the membrane were removed by scraping the membrane with a cotton swab. Cells transversing the membrane were fixed with methanol and stained with hematoxylin. Cells in ten random fields of view at ×100 magnification were counted and expressed as the average number of cells/field of view. All experiments were performed in triplicates. The data were represented as the average of the three experiments with the SD of the average indicated.

Results

Expression of Snail in Activated Macrophages

Recent findings in our group revealed that Snail is highly induced in the invasive front of ductal adenocarcinomas of the pancreas, where tumor cells begin their migration to distant tissues due to EMT.[16] During inflammation a comparable migrating process occurs in immune cells which move to the affected region.[17–19] It is well known that macrophages exhibit similar behavior by infiltrating inflamed tissues.[20–22] Based on this evidence our idea was to look for a possible role of Snail in these migrating macrophages which may be not related to EMT. We used inflamed tissue derived from an intestinal anastomotic model and wounded skin of rats and performed immunohistochemistry. As shown in Fig. 1, Snail was highly induced in inflamed areas of colon, whereas low expression of this transcription factor in normal tissue was observed. To prove which cells express Snail, we performed double immunostaining with a marker for macrophages (CD68). Interestingly, induction of Snail expression was identified in macrophages (Fig. 2c), only at sites of inflammation and injury.

Expression of Snail is Time- and Distance-Dependent in Inflamed Tissues

In inflammation and injury neutrophils are recruited from the blood followed by monocytes which locally differentiate into macrophages.[23] During the resolution of inflammation and wound healing, active macrophages are downregulated by specific signals, promoting apoptosis.[24] We hypothesized that Snail positive macrophages are recruited cells that migrate to the wound. Therefore, we examined the time-course of intestinal wound healing and several tissue samples of increasing distance to the local wound site, by cell count, immunohistochemistry, and real-time PCR in rats. Our results from the cell count at the anastomotic site at different time points showed an increase of Snail positive macrophages mostly in the lamina propria (Fig. 2c). The expression of Snail was elevated up to 18-fold at 48 h after surgery and then to 7.3-fold at 2 weeks after surgery (Fig. 2a). Real-time PCR results showed the increased expression of Snail at the m-RNA level in a similar way (Fig. 2b). In a second experiment, we examined the expression of Snail depending on the distance to the wound site. With increasing distance to the wound, the expression of Snail decreased on the mRNA and protein level (Fig. 3c). Cell counting revealed a 21.7-fold increased level of Snail positive macrophages at the anastomoses compared to normal colon tissue whereas the amount of Snail positive cells decreased to 14.3-fold at 1 cm distant to the wound and to 4.8-fold at 4 cm to the wound (Fig. 3a). Real-time PCR data confirmed these results (Fig. 3b).

Based on these results, we concluded that Snail positive macrophages, which are only located at the inflamed wound site, are recruited macrophages activated to defend inflammation.

Induction of Snail in Macrophages During Acute and Chronic Inflammation in Humans

Having shown activated macrophages expressing Snail in acute inflammation in rat tissues, we further examined a

Figure 1 Immunofluorescence staining of control colon (*left column*) and inflamed colonic tissue (*right column*) from rats. Tissue was stained for CD68 (marker for macrophages, *green*) and Snail (*red*). Overlay of CD68 and Snail show that macrophages display positive Snail expression. DAPI was used to visualize the nucleus (*blue*).
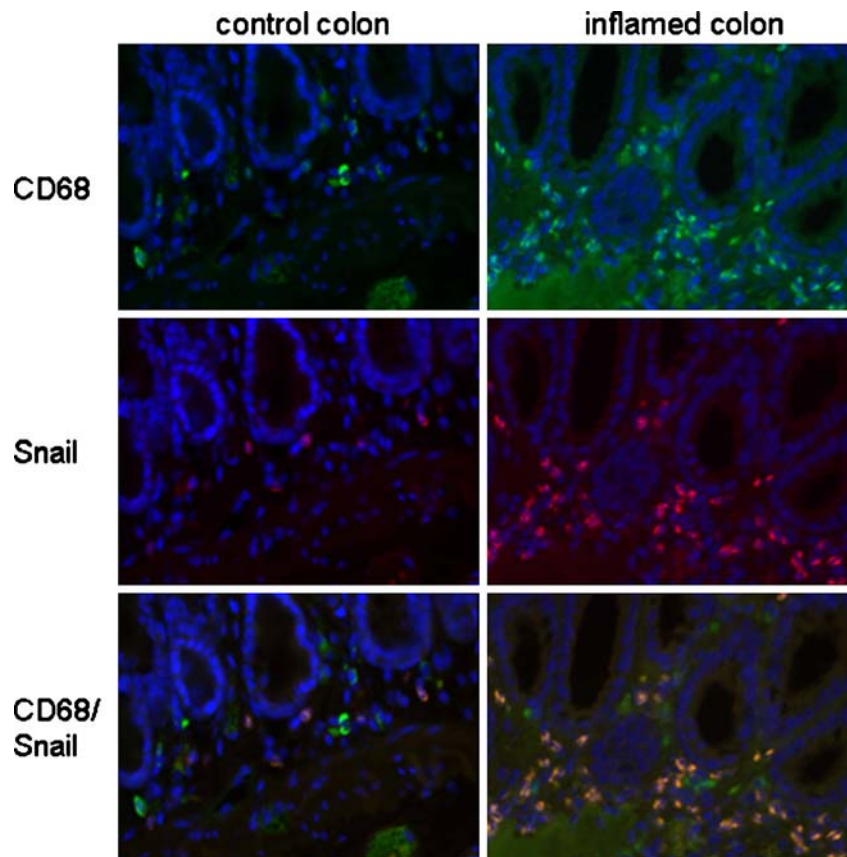
possible correlation in human tissues. Therefore we immunostained acute inflamed tissue from patients with wound infects after surgery and acute appendicitis and found an increased number of Snail positive macrophages at the wound site (Fig. 4).

Beside acute inflammation which occurs after injury, chronic inflammation, characterized by a permanent activation of immune cells, is present in some diseases like inflammatory bowel disease.[7] Two main forms of IBD are Crohn's disease and ulcerative colitis. To determine the expression of Snail during these chronic gut inflammations, we examined Snail mRNA levels in inflamed intestinal tissue of CD and UC patients as well as in normal gut by using real-time PCR. Snail mRNA expression was found to be 12- and fivefold elevated in inflamed intestinal tissue of CD and UC patients as compared to the normal colon, respectively (Fig. 5a). Immunohistological analysis was used for detection of protein expression of Snail and its localization in the colon. Whereas normal colonic tissue does not seem to express Snail constitutively (Fig. 5b), the amount of this transcription factor was markedly elevated in the mucosal lamina propria of CD and UC (Fig. 5c, d). Thus, the protein abundance of Snail in inflamed gut is related to its mRNA expression. Immunohistological staining revealed that the infiltrated macrophages were the predominant producers of Snail. This finding correlates

with our results obtained in the model of anastomotic wound healing in rats and human acute inflammation.

Induction of Snail by TGF-β1 in the Human Macrophage Cell Line THP-1

TGF-β1 has been shown to induce Snail in an EMT-dependent process during various stages of embryonic development.[2] Based on this observation, we next asked whether stimulation of THP-1 macrophages with this cytokine can affect the expression of Snail. In addition, we determined whether other signaling pathways might play a role in the regulation of this transcription factor. We therefore analyzed the induction of Snail in differentiated THP-1 cells after stimulation with TGF-β1, LPS, IL-8, and MMP-3 using real time-PCR and western blotting (Fig. 6). Only stimulation with TGF-β1 led to the induction of Snail in a time-dependent manner. Real-time PCR analysis revealed a threefold induction of Snail within 48 h after stimulation of THP-1 cells with TGF-β1. For LPS and interleukin-8 we observed a slight increase in Snail expression after 48 h. Stimulation of MMP-3 showed no increase in Snail expression (Fig. 6a). Similarly, Western blotting data showed a significant increase of Snail abundance in THP-1 macrophages during stimulation with TGF-β1, but not with LPS, IL-8, and MMP-3 (Fig. 6b).

Figure 2 Expression of Snail in a time-dependent manner during anastomotic wound healing in rats. **a** Cell count of Snail positive immune cells at different time points showed an increase of invading macrophages up to 48 h after surgery. **b** The analysis of the relative mRNA intensity of Snail from the anastomotic site confirmed the results from the cell counts. **c** Immunostaining of tissue from the anastomotic site revealed expression levels of Snail protein (brown color reaction) in macrophages peaking at 48 h. Magnification ×400. All sections were additionally stained with CD68 to confirm the specific expression of Snail in macrophages. (*$P<0.05$ was considered as significant).
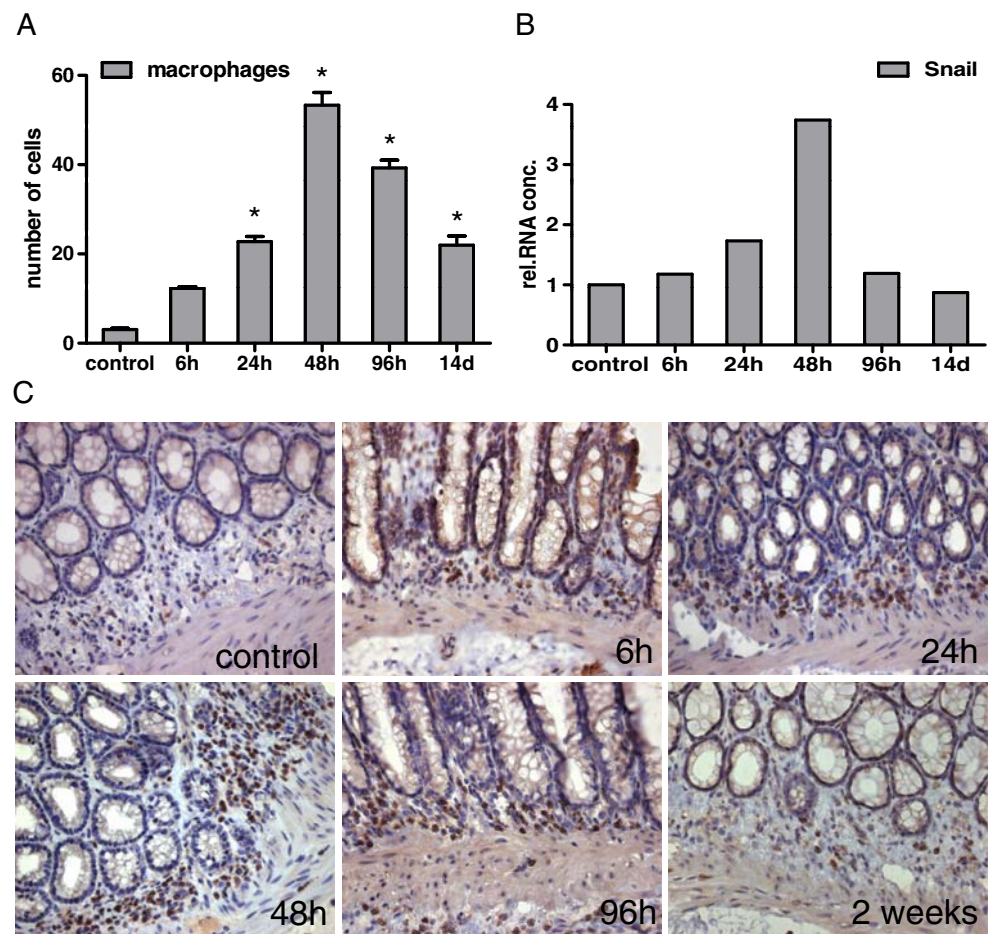
Jurkat T cells were used as a control showing no influence on induction of Snail by all four mediators (Fig. 6c). These results demonstrated that only the TGF-β1 pathway can activate Snail in macrophages during inflammation.

Snail is Necessary for TGF-β-Induced Movement of Macrophages In Vitro

To investigate the invasion-promoting effect of Snail in human macrophages, differentiated THP-1 cells were stimulated with TGF-β1 (10 ng/ml) for 48 h and loaded into artificial basement matrix coated Transwell™ chambers. Numbers of invasive cells in TGF-β1-stimulated macrophages were markedly increased up to 50% compared to untreated cells (Fig. 7b). Jurkat T cells were used as a control for the specific regulation of Snail and showed no increased invasion of cells through the Transwell chambers after stimulation with TGF-β1 (data not shown). To prove the relevance of Snail during cell movement we treated transiently transfected THP-1 cells (Stealth/siRNA-Snail and control-siRNA) with TGF-β1 and observed no significant change in migrating activity in THP-1 cells silenced for Snail in contrast to control siRNA transfected THP-1 cells (Fig. 7b). Therefore, we

conclude that Snail mediates the migrating properties of activated macrophages.

Induction of Snail During Inflammation is not Accompanied by Other EMT-Regulators

Snail, the related transcription factor Slug and the basic helix-loop-helix transcription factor twist are known to be master regulators of EMT, which initiate a complex program of gene regulation controlling this process.[25] Our findings that Snail is upregulated in activated macrophages led us to investigate whether Slug and Twist are also involved in this process. Therefore, we examined the expression of Slug and Twist in tissue specimens of acute and chronic inflammation in rats and humans by immuno-histochemistry and Western blot as well as real-time PCR and found no constitutive expression of these transcription factors (Data not shown).

Discussion

The transcription factor Snail is involved in processes that imply pronounced cell movement, both during embryonic

Figure 3 Expression of Snail in a distance-dependent manner during anastomotic wound healing in rats. **a** Cell count as well as **b** real-time PCR showed a decrease of Snail positive macrophages and Snail mRNA depending on the distance to the anastomotic wound. **c** Immunostaining of different tissue regions 48 h after surgery revealed highest expression levels of Snail protein (*brown*) in macrophages of the anastomotic region (in comparison to 1 and 4 cm distance). All sections were additionally stained with CD68 to confirm the specific expression of Snail in macrophages. Magnification ×400. (*$P < 0.05$ was considered as significant).
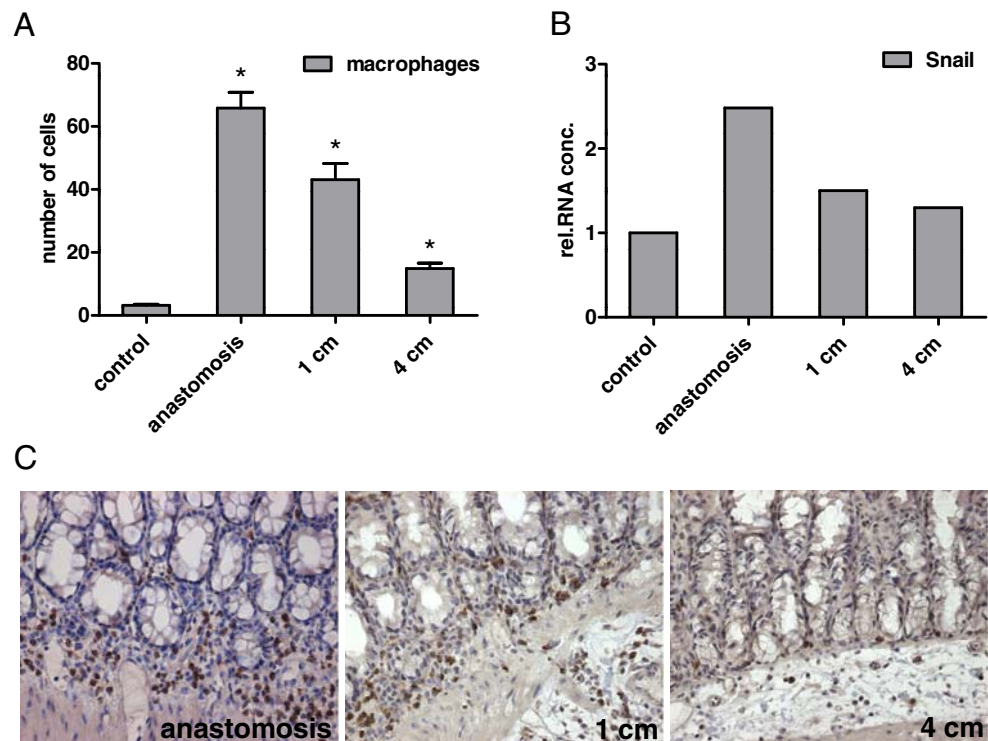
development and in the acquisition of invasive and migratory properties during tumor progression.[2,4,5] This role in promoting cell movement has been extended and one of the best studied mechanisms is the induction of EMT.[26,27] Snail-induced EMT converts epithelial cells into mesenchymal cells by direct repression of E-cadherin.[1] Although Snail seems to be required for all processes of EMT that have been examined, this does not necessarily

mean that the induction of EMT is the prevalent role of Snail genes. They also have additional cellular functions that may occur independently of the induction of EMT such as protection of cells from apoptosis.[28]
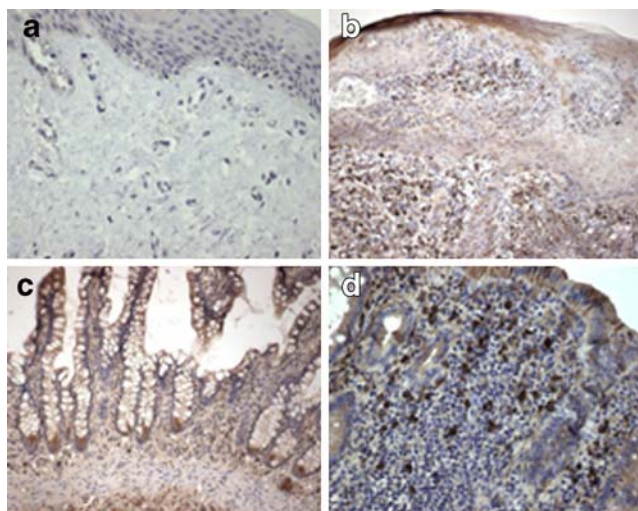
Figure 4 Immunodetection of Snail (brown color reaction) in human tissues. Snail is highly expressed in macrophages during wound healing, the difference demonstrated between normal (**a**) and inflamed skin (**b**). Sections of normal terminal ileum (**c**), and appendicitis (**d**) also depict the induction of Snail during acute inflammation. Magnification ×200.
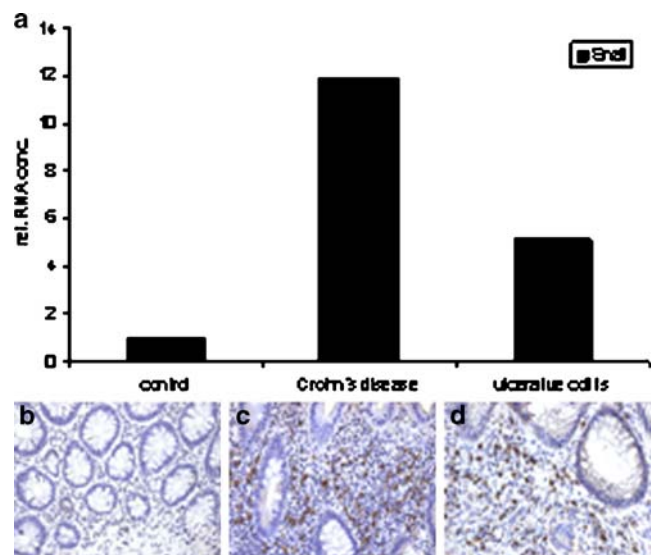
Figure 5 Expression of Snail in chronic inflammatory bowel disease (IBD). **a** Snail mRNA in normal colon, CD, and UC from pooled human tissue material. Immunohistochemistry of normal colonic tissue (**b**), Crohn's disease (**c**), and ulcerative colitis (**d**) from representative patient tissues shows increased expression levels of Snail (*brown*) in macrophages during IBD. Comparison of the mRNA and protein levels of Snail in CD and UC revealed significantly higher expression of Snail in CD than UC. Staining of the macrophage marker CD68 served as a control. Magnification ×400.
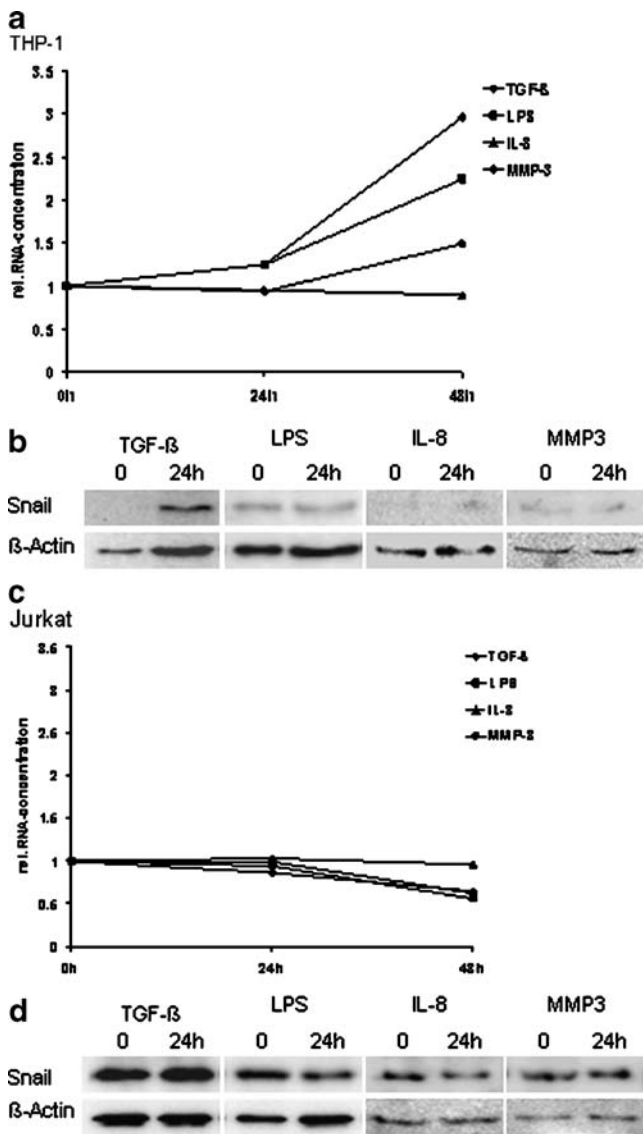
Figure 6 Induction of Snail in human macrophage cell line THP-1. **a** Stimulation of THP-1 cells with TGF-β resulted in an increase of Snail mRNA expression up to threefold by real-time PCR. **b** Western blot analysis confirmed the real-time PCR data indicating TGF-β-mediated induction of Snail protein in macrophages. **c** Stimulation of the control T cell line Jurkat with TGF-β showed no significant differences of Snail expression by real time PCR. **d** Western blot of the control T cell line Jurkat confirmed the real-time PCR data showing no differences of Snail protein expression after treatment with all four stimuli.

Figure 7 a Western blot analysis of THP-1 cells and control-siRNA transfected THP-1 cells compared to Snail gene silenced THP-1 cells (siRNA-Snail THP-1) after stimulation with TGF-β 1. β-actin was used as a loading control. **b** Invasion assay of unstimulated THP-1, control-siRNA THP-1, and siRNA-Snail transfected THP-1 cells versus stimulated with TGF-β 1 (10 ng/ml). After 48 h of stimulation with TGF-β, 50% more THP-1 cells (*blue*) invaded through the membrane of the Transwell chambers compared to untreated cells. The same effect was observed in control-siRNA transfected THP-1 cells after stimulation. In contrast, siRNA-Snail THP-1 cells showed no significant changes in migration after stimulation with TGF-β 1. Magnification ×100. (*$P<0.05$ was considered as significant).

Another mechanism in the human organism that requires directed cell movement without involving EMT is the recruitment of neutrophils and macrophages during injury and inflammation.[23,29] Macrophage differentiation from monocytes recruited to inflammatory foci plays a critical role in defense mechanisms against pathogens and in inflammatory diseases.[30]

Here, we demonstrate for the first time, that Snail expression is induced in invading macrophages at sites of

injury in a time- and distance-dependent manner. We examined different sites of wound healing like skin and anastomoses in the colon of rats and compared the results with specimens from patients with wound healing sites and acute inflammation. Snail expression was only detectable in macrophages at the affected wound site.

Although both processes, EMT and the recruitment of macrophages, share some molecular and cellular characteristics like motile cell behavior, invasion of distinct tissues, and transmigration across endothelial cells, the classical definition of EMT is the transformation of epithelial to mesenchymal and motile cells.[27] Macrophages have no epithelial characteristics like adhesive junctions between cells which enables even undifferentiated monocytes to migrate. During differentiation of macrophages, they have the ability to modulate an inflammatory phenotype and several molecular changes contribute to a coordinated transmigration towards injured tissue.[22]

Our experiments in an anastomotic wound healing model in rats revealed that the number of Snail positive macrophages increased during 48 h after surgery and decreased within the observation period of 2 weeks. We also examined the number of Snail positive macrophages dependent on the distance to the anastomotic site and found a decrease of these cells with increasing distance to the wound. In control tissue, we found only a few Snail-positive macrophages probably due to occasional invasion of bacteria through the mucosa of the colon. These results imply that the activated macrophages express high levels of Snail, whereas the same cell type, more distant to the wound and not involved in wound healing or inflammation, exhibits no expression of Snail.

Thus, we postulate that expression of Snail is a potent marker for migrating macrophages during acute inflammation and early wound healing.

Another question of interest is whether this transcription factor is also involved in chronic inflammation. The impact of Snail on immunologically mediated chronic inflammatory disorders such as Crohn's disease and ulcerative colitis remains poorly understood. Both conditions are heterogeneous diseases with an increased risk for developing colon cancer.[7,31] CD and UC are characterized by an abnormal mucosal immune response.[32] In this study we found a strong induction of Snail in the inflamed gut of both CD and UC patients. The constitutive expression of Snail was rarely detectable in control colonic tissue, whereas the abundance of this transcription factor was markedly elevated in infiltrating macrophages in CD and UC patients. These rare Snail positive macrophages in the normal colon tissue might result from some invading bacteria bearing pathogen-associated molecular patterns which are physiologically present in the normal gastrointestinal tract.[33,34] Thus, we show here for the first time that migrating

macrophages of the mucosal immune system in chronically inflamed tissue exhibit high expression of Snail, which is normally not expressed in adult organs.

Based on these findings, the next step to understand the role of Snail was to investigate regulatory pathways activated during inflammation, especially TGF-β, LPS, IL-8, and MMP-3. TGF-β1 is an important cytokine involved in the mucosal immune response displaying a broad spectrum of activities during inflammation and production of this cytokine is increased in patients with Crohn's disease and ulcerative colitis.[7] Since TGF-β1 is important in regulating lymphocyte infiltration in the intestine as well as in tumor progression, this pathway was the first candidate for a possible regulation of Snail. After exposure to TGF-β1, the differentiated human macrophage cell line THP-1 revealed increasing levels of Snail on the mRNA and protein level in a time-dependent manner.

In contrast, exposure of the differentiated human macrophage cell line THP-1 to LPS did not increase expression of Snail. A key chemokine involved in immune cell recruitment is interleukin-8, which is a strong chemoattractant for monocytes, albeit less potent than TGF-β1.[13] Stimulation of differentiated human macrophages with IL-8 led to no change of Snail expression. Matrix metalloproteinases contribute to tissue injury and inflammation,[14] and stimulate the production of cytokines in macrophages at the site of the developing inflammation. It has been shown that MMP-3 can also trigger EMT[35] and induce Snail expression in murine mammary cells.[1] Treatment of differentiated THP-1 macrophages with MMP-3 showed no increased Snail expression on the mRNA and protein level.

Based on our observation that TGF-β1 induces the expression of Snail in differentiated macrophages, we performed invasion assays to study possible changes of migrating behavior in human macrophages after stimulation with TGF-β1. Interestingly, we observed an increased migration of macrophages through a matrigel-coated membrane compared to untreated cells, suggesting that activation of Snail is regulated through this specific pathway and that Snail might be involved in the recruitment of macrophages at sites of tissue injury and inflammation.

To prove this hypothesis we generated human macrophages with Snail gene silencing using siRNA technique. After stimulation of these cells with TGF-β1 we observed no induction of Snail at the RNA and protein level and notably no significant increase in migratory properties in performed invasion assays.

Since Snail is not the only potent regulator of EMT, we further asked whether other important EMT-inducers like Slug and Twist are also expressed in activated macrophages, but we found no expression of these transcription

factors in macrophages during acute inflammation and wound healing (data not shown).

In conclusion, our results provide a novel mechanism in the recruitment of macrophages by induction of Snail and offer a basis for investigating a new role of this transcription factor, which may be involved in directed cell movement beyond EMT.

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CASE REPORT

GIST with a Twist—Upregulation of PDGF-B Resulting in Metachronous Gastrointestinal Stromal Tumor and Dermatofibrosarcoma Protuberans

Colin J. McCarthy · Gavin C. O'Brien · Robert J. Cummins · Elaine W. Kay · Patrick J. Broe

Abstract

Case Report A 61-year-old male was referred following an incidental radiological discovery of an intra-abdominal mass. His medical history included excision of a lumbar dermatofibrosarcoma protuberans (DFSP) 5 years previously. A CT scan of the abdomen revealed a mass arising from the greater curvature of the stomach. Upper GI endoscopy was normal. He underwent successful laparoscopic resection of this mass.
Materials and Methods The histology of the abdominal mass revealed a gastrointestinal stromal tumor (GIST) with poor prognostic indicators. Immunohistochemical analysis of the GIST and his previous DFSP was performed.
Results Immunohistochemistry suggested a link between the GIST and his previous DFSP involving the PDGF signalling system.
Discussion Both GIST and DFSP are extremely rare tumors. A mutation in the platelet-derived growth factor receptor alpha (PDGFR-α) has been described in 5–15% of GISTs. It has been shown that DFSP is frequently associated with a translocation between PDGF-B (Chr 22) and COL1A1 (Chr 17), causing continuous activation of PDGFR-β. Literature review confirms that there are no previously reported cases of both of these tumors occurring in the same patient.
Conclusion We hypothesize that this patient may have a previously undescribed genetic mutation involving the PDGF signalling system, resulting in these two very rare malignancies. Immunohistochemistry studies confirmed the link on this occasion. Improvements in our understanding of the molecular biology of the PDGF system may novel therapeutic targets in the future.

Keywords Gastrointestinal stromal tumor ·
Dermatofibrosarcoma protuberans · Metachronous · Glivec ·
Imatinib · PDGFR · GIST · DFSP · PDGF-B

Case Report

A 61-year-old male was referred to the outpatient clinic, with a mass arising from the greater curvature of the stomach, found incidentally on a CT scan for abdominal pain. It measured $8 \times 5 \times 5$ cm and extended towards the splenic hilum. Upper GI endoscopy revealed no mucosal abnormality. His past medical history included two excisions of a dermatofibrosarcoma protuberans in the lumbar region 5 years earlier. The margins of the second resection were widely clear. He had a 40-pack year smoking history and a moderate alcohol intake. His mother died of an upper gastrointestinal hemorrhage, of unknown etiology.

Clinical examination was unremarkable, and of note, no masses were appreciated on abdominal examination. Pre-operative investigations including complete blood count, renal panel, and liver function tests were normal. The differential diagnosis included gastrointestinal stromal tumor (GIST), gastric adenocarcinoma, lymphoma, or a metastasis from his previous DFSP. The stomach nodule was successfully removed using a laparoscopic technique.

The histology revealed a completely excised GIST, which stained strongly positive for C-KIT (CD117) (Figs. 1, 2). Although the margins were clear, it had histological

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Figure 1 The gross specimen reveals a cream-colored, heterogenous nodule.

features suggestive of a poor prognosis including a large size (6.9 cm) and high number of mitoses per high-power field. Following multidisciplinary review, he was commenced on Imatinib (Glivec). In view of these two unusual tumors, additional immunohistochemical studies were performed on both the GIST and the DFSP.

Materials and Methods

The original DFSP specimen had been preserved and was retrieved for analysis. Both tumor specimens were processed into blocks. These were then sectioned at 4 μm and stained with hematoxylin and eosin on a Cot-20 automated linear stainer (Midite). Stained sections were then cover slipped and allowed to dry. With regard to the immunohistochemistry, sections 4 μm in thickness were cut from array blocks and floated onto adhesive slides. Sections were then baked at 55°C overnight. All staining was carried out on a BondMax automated immunostainer from Vision BioSystems. Sections were loaded onto the system, and the relevant program was started. The BondMax system dewaxed slides and then carried out the appropriate antigen retrieval technique that was previously optimized for that antibody (Table 1). The diluted primary antibody was added to the sections for 20 min. Following detection, DAB was used as the chromagen, and sections were then counterstained lightly with hematoxylin and processed to coverslip.

Results

As would be expected, the GIST stained positive for c-KIT (CD 117), whilst the DFSP that had been excised a number

of years previously was negative for c-KIT. In addition, both tumors were stained for PDGFR-α and PDGFR-β. The GIST stained positive for CD34 and PDGFR-α, while the DFSP stained positive for CD34, PDGFR-α, and PDGFR-β. Finally, both tumors stained positive for the PDGF-B antibody—confirming our hypothesis that these two unusual tumors may indeed be related by an abnormality in PDGF-B regulation (Figs. 3, 4, Table 2). Although it is widely accepted that the $t(17;22)$ translocation results in the upregulation of PDGF-B resulting in DFSP, we have shown, for the first time, that the overexpression of the PDGF-B molecule may also upregulate the PDGFR-α receptor, thereby causing a metachronous GIST tumor in the same patient.

Discussion

Gastrointestinal stromal tumors are relatively rare mesenchymal tumors, accounting for 1% of all primary gastrointestinal tumors. The incidence is approximately 10–20 per million population per year, most commonly in the 5th and 6th decades of life.[1,2] They can occur anywhere in the alimentary canal where there is smooth muscle; however, they are most commonly (60%) seen in the stomach.[3] They are usually solitary and can be intraluminal or extraluminal. The most common presentation is that of an upper gastrointestinal bleed, due to ulceration of the overlying mucosa.[4] Other symptoms may include abdominal pain and early satiety, although many are discovered incidentally.[1,4]

The gold standard management of GIST has traditionally been surgical excision when possible, providing a 5-year survival of approximately 50%.[5] Chemotherapy and radiotherapy are not useful in the treatment of GIST; however,
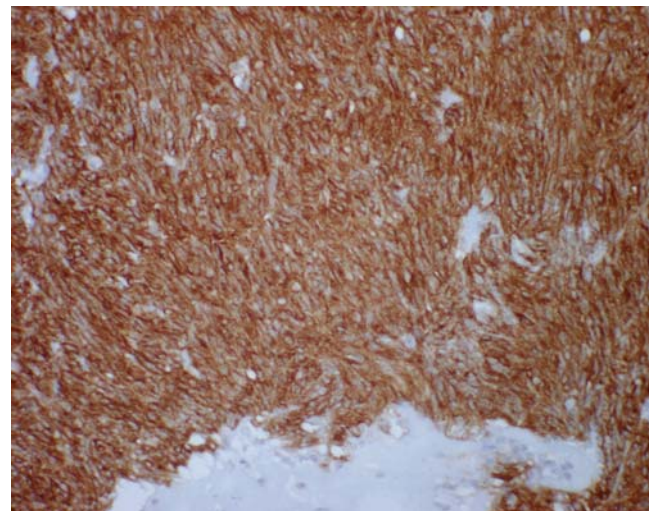
Figure 2 The stomach nodule stained strongly positive for c-KIT (CD117), confirming a gastrointestinal stromal tumor.

Table 1 Antigen Retrieval Technique

Antibody	Manufacturer	Cat. no	Clone	Pretreatment	Dilution
CD34	Dako	M7165	QBEnd 10	ER1 20 min	1 in 60
c-kit	Dako	A4502	Polyclonal	ER1 20 min	1 in 100
PDGFR-α	Cell signaling	#3174	D1E1E	ER1 20 min	1 in 300
PDGFR-β	Cell signaling	#3169	28E1	ER2 20 min	1 in 50
PDGF beta	Novus biologicals	NB200–633	Polyclonal	ER1 20 min	1 in 100

there is increasing interest in tyrosine kinase (TK) inhibitors such as imatinib mesylate (Glivec).[6] This has increased the 5-year survival of patients when used in an adjuvant setting.[5]

GISTs express c-KIT (CD117) in the vast majority of cases.[7] Activation of the c-kit receptor, a product of the c-kit proto-oncogene, results in downstream tyrosine kinase production. GISTs are caused by a gain-of-function KIT mutation in approximately 80% of cases.[8] This mutation causes ligand-independent activation of c-KIT-initiated TK activity, thereby resulting in tumorigenesis.[9]

A distinct subset of GISTs are caused by a mutation in the PDGFR-α gene.[10] Although seemingly mutually exclusive, these two mutations (KIT and PDGFR-α) have identical downstream consequences, namely, the expression of c-kit on the cell membrane.

Dermatofibrosarcoma protuberans is another rare tumor, with an annual incidence of approximately one per million population.[11] The pathogenesis involves a translocation between chromosomes 17 and 22, which leads to a fusion of the PDGF-B gene to the COL1A1 gene. This in turn leads to continuous activation of the PDGFR-β receptor. In
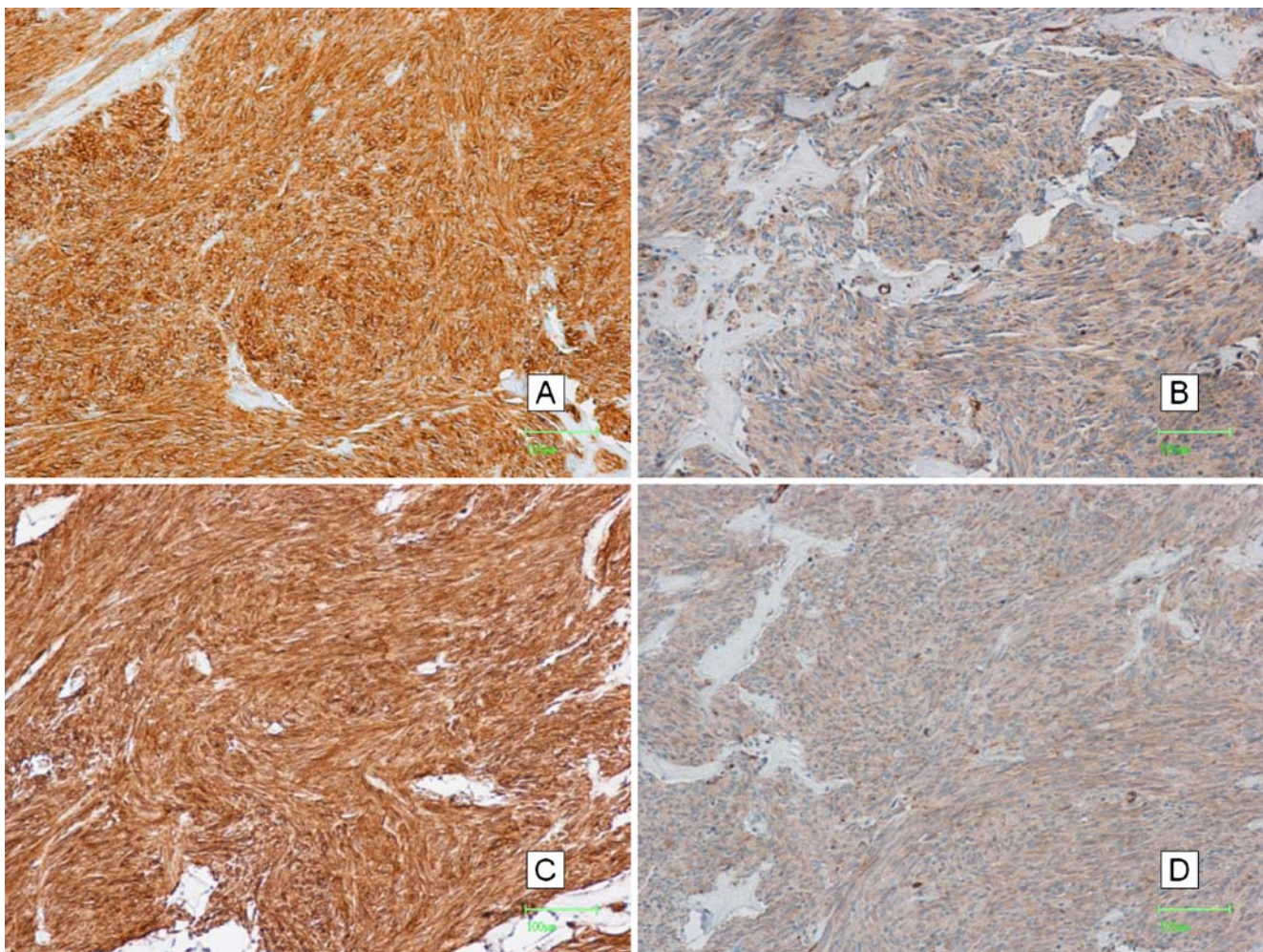
Figure 3 The GIST tumor was stained for c-kit (**a**), PDGF-B (**b**), PDGFR-α (**c**), and PDGFR-β (**d**).
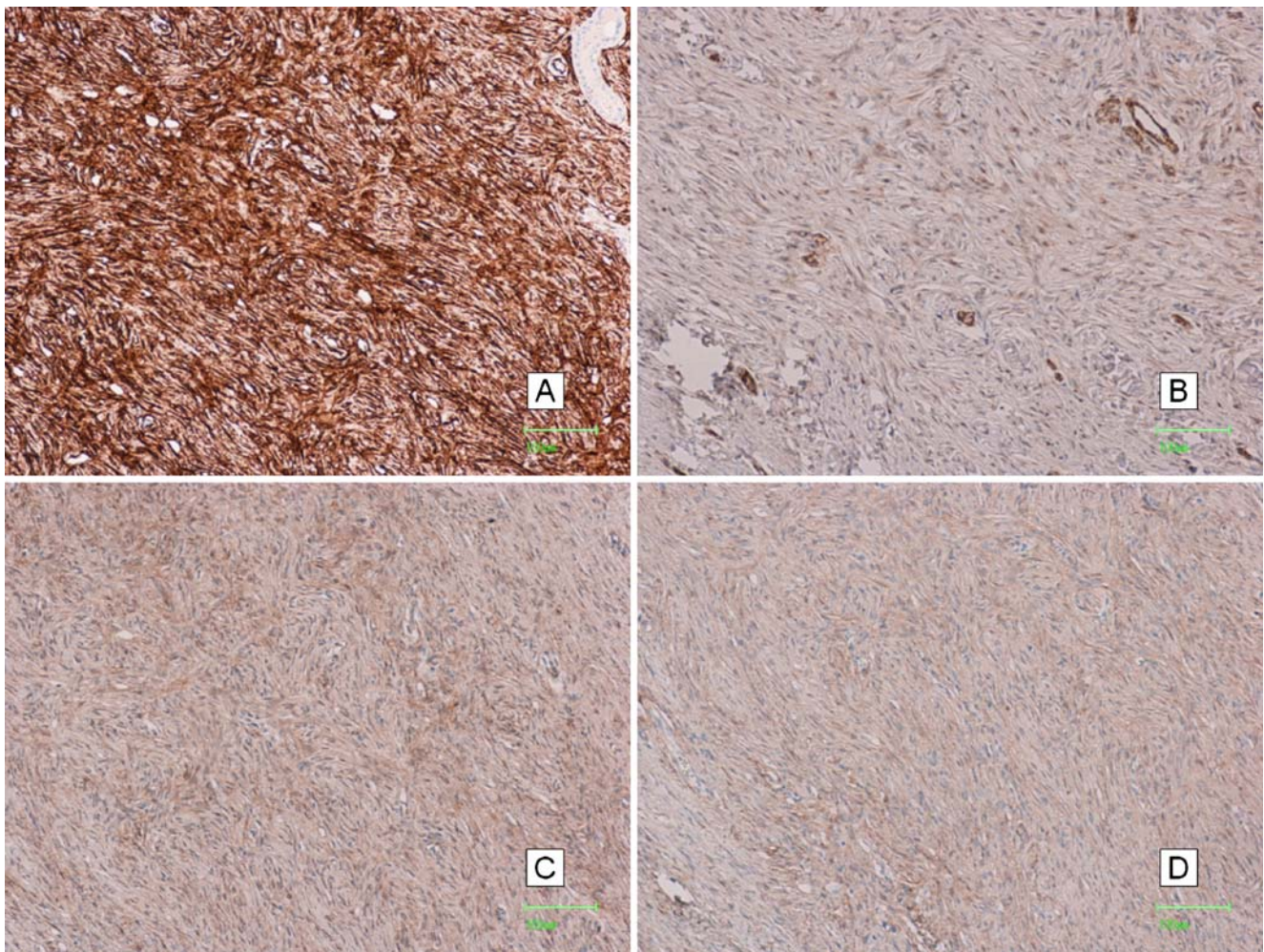
Figure 4 The DFSP tumor was stained for CD34 (a), PDGF-B (b), PDGFR-α (c), and PDGFR-β (d).

a small number of cases, this translocation is not found, suggesting a role for an as-yet undiscovered, potential mutation.[12] Both PDGFR and the PDGF ligands have been shown to play important roles in both normal and pathological cell proliferation.[13] PDGF-B, in particular, has been shown to induce tumorigenesis in a number of animal models.[14]

In view of the fact that our patient had two very rare metachronous tumors, we investigated the possibility of a link between the two. GISTs are associated with a second tumor in approximately 13–27% of patients. These were most commonly colorectal, gastric, and pancreatic adeno-carcinoma.[15,16] In addition, GIST is also seen with

lymphoma, prostatic carcinoma, together with lung and kidney malignancies.[16] However, following an extensive literature review, no previously reported case exists of metachronous GIST and DFSP.

For the first time, we reveal a link between these two tumors involving the PDGF pathway. As well as GISTs, mutations in, and upregulations of, PDGFR-α have been linked to other tumors, including intestinal neurofibroma-tosis, endometrial stromal sarcomas, and brain tumors.[17–19]

The PDGF ligand is composed of two peptide chains, which form a dimer molecule incorporating A, B, C, or D chains. The ligand may be a homodimer (e.g., AA) or a heterodimer (e.g., AB). PDGFR-α binds preferentially to the A, B, and C chains, while PDGFR-β binds only the B and D chains (see Fig. 5).[20] B chains are, therefore, the only chains that bind to both the α and β receptors. Given that DFSP is associated with an overexpression of PDGF-B, and subsequent upregulation of PDGFR-β, it is possible that PDGF-B ligand overexpression may also upregulate the alpha receptor, thereby leading to a GIST in the same patient.

Table 2 Immunohistochemistry Results

Tumor	c-KIT (CD 117)	CD 34	PDGFR-α	PDGFR-β	PDGF-B
GIST	+	+	+	−	+
DFSP	−	+	+	+	+
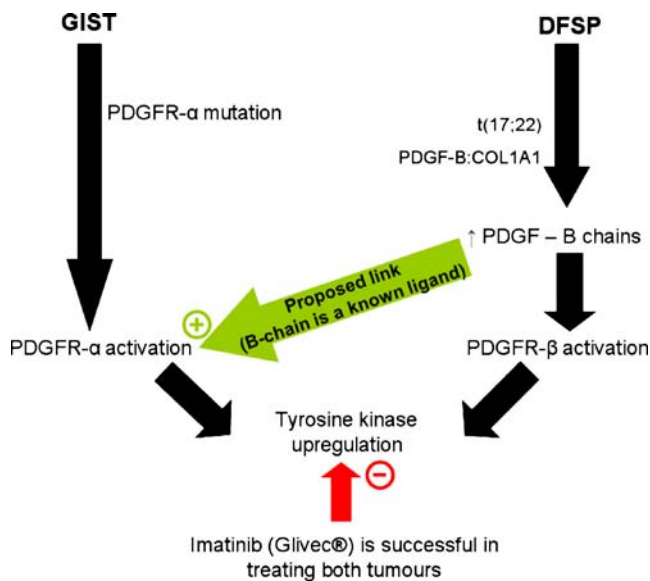
Figure 5 The pathogenesis of both GIST and DFSP are closely related, however a direct link has not previously been described. The proposed mechanism for metachronous tumor development in our specific patient is described above. As this theory is limited by its application to an individual patient, further research on a larger population is needed to validate such a relationship.

In view of the fact that there remains a subset of both GISTs and DFSP in which the exact mutation cannot be identified, the possibility of a germline mutation in the PDGF signaling pathway may be proposed. Such germline mutations have been described in the past. For example, the combination of familial GIST and paragangliomas (Carney–Stratakis syndrome) is associated with germline mutations in the succinate dehydrogenase subunits SDHB, SDHC, and SDHD.[21] Similarly, the combination of GISTs and intestinal neurofibromatosis has been linked to a germline PDGFR-α V561D gene mutation.[17]

Although a minority of GISTs are caused by an activating mutation of PDGFR-α, we propose that in fact, the t(17;22) translocation responsible for development of DFSP has, on this occasion, led to the development of a metachronous GIST in the same patient. The subsequent upregulation of PDGF-B chains has activated both the PDGFR-α and PDGFR-β receptors, leading to the tumor susceptibility seen in this patient, with metachronous development of these two unusual tumors (Fig. 5). Immunohistochemistry played a valuable role in the investigation of this case. However, it should be noted that such techniques do not identify a specific gene mutation. Further research is, therefore, needed to identify the molecular abnormality involved in the pathogenesis.

Conclusion

Whilst GIST has long been associated with presence of synchronous tumors, the exact mechanisms for this remain elusive in the majority of cases. Based on the known pathogenesis of both DFSP and GIST, together with the results of our immunohistochemistry, the role of PDGF-B as an inducer of tumorigenesis is highlighted. The pathogenic link is further strengthened by the common final pathway of TK production and interestingly by the response of both tumors to the tyrosine kinase inhibitor, Imatinib.[22] Further studies detailing the molecular biology of the PDGF molecule may lead to advances in the treatment of these tumors.

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MULTIMEDIA ARTICLE

Supra-Pubic Single Incision Cholecystectomy

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Abstract

Introduction Surgery is moving towards less invasive and cosmetically superior approaches such as single incision laparoscopy (SIL). While trans-umbilical SIL is gaining popularity, incisions may lead to post-operative deformations of the umbilicus and the possibility of an increased rate of incisional hernias. Access within the pubic hairline allows preservation of the umbilicus and results in a scar which is concealed within the pubic hair.

Methods Supra-pubic single incision cholecystectomy was performed in a 30-year-old patient with symptomatic gallstones. A 2.5-cm transverse incision was placed within the pubic hairline and a subcutaneous tunnel was formed. Three 5-mm ports were introduced into the tunnel and perforated the anterior rectus sheath superior to the skin incision. The surgical procedure was then undertaken with conventional laparoscopic instrumentation. The adjacent 5-mm incisions were merged for gallbladder removal. The entry site was closed under direct vision.

Results The above procedure was technically feasible and without complication. Operative time was 45 min, and the patient was discharged 5 h post-operatively.

Conclusions Supra-pubic single incision laparoscopic cholecystectomy may offer a more cosmetically appealing result than standard umbilical access. The operation can be performed by surgeons skilled in single incision techniques with good result.

Introduction

Laparoscopic cholecystectomy is currently the gold standard for the treatment of symptomatic gallstones.[1,2] Besides

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the numerous clinical advantages of laparoscopy over an open approach, recent surveys have demonstrated that patients clearly favor cosmetically superior approaches to the abdominal cavity.[3] Less invasive and cosmetically superior approaches such as single incision laparoscopy (SIL) and natural orifice translumenal endoscopic surgery (NOTES) have been gaining popularity. While pure NOTES will result in cosmetically ideal outcomes, this method is still under development and is currently only performed at specialized centers.[4,5] While we at UCSD have been actively involved in leading the surgical community towards the safe and proficient development of natural orifice techniques, we realize that there is a need to investigate all approaches which may improve the outcomes of surgical patients. Within the past year, single incision laparoscopic surgery has been described for a great variety of procedures with rapidly growing numbers.[6–12] Single incision laparoscopic surgery is performed with slightly modified but still conventional rigid laparoscopic instruments, and results in less scaring when compared to

conventional laparoscopy. Still, single incision laparoscopic surgery requires an abdominal wall incision, usually in the umbilical area, and can lead to all the common problems of conventional laparoscopy particularly pain and hernia formation.[1] Because of the increased size of the umbilical incision during single incision laparoscopy, the possibility of a growing rate of incisional hernias may be anticipated. Additionally, placing a 15 mm or larger incision in the umbilical area might lead to an umbilical deformation depending on technique, original shape, size, and other factors. Finally, many publications in the plastic surgery literature indicate the importance of the integrity of the umbilicus for the overall physical appearance.[13–15] Suprapubic single incision surgery is a novel method for minimal invasive access procedures that preserves the native umbilicus, results in a more discrete scar, and has the potential to reduce the incidence of postoperative hernias.

Material and Methods

Extensive experience with single incision laparoscopic surgery prior to the first human case was gathered in the porcine model and human cadavers using different methods for access (trocars, open approach, and rigid and flexible instrumentation). The initial patient was listed under the institute's Institutional Review Board protocol for single incision laparoscopy. Possible advantages and complications of this new investigational method, and the possibility of conversion to conventional (laparoscopic or open) surgery were discussed with the patient and appropriate informed consent was given.

The patient was placed in reversed Trendelenburg-position and the bladder catheterized. A 2.5-cm transverse incision was placed within the pubic hairline medially. A subcutaneous tunnel was formed in an open fashion in a cephalad direction of about 5 cm in length and the anterior rectus sheath visualized. Next a 5-mm optiview optical bladeless trocar (Endopath Xcel, Ethicon Endo-Surgery Inc., Cincinnati, OH, USA) with an introduced utilizing a 0-degree, 5-mm camera was guided through the subcutaneous tunnel followed by a stepwise passage through the abdominal wall under constant direct visualization. Pneumoperitoneum was then obtained and trocar position was verified. Next, two additional 5-mm low profile trocars (Ethicon Endo-Surgery Inc., Cincinnati, OH, USA), were inserted in a similar fashion, one on each side of the first trocar (Fig. 1). A conventional 5-mm rigid laparoscopic grasper was inserted through the left low profile trocar to retract the gallbladder at the fundus. Dissection of Calot's triangle was achieved with a harmonic scalpel (Ethicon Endo-Surgery Inc., Cincinnati, OH, USA) introduced through the right-sided trocar. Flexible laparoscopic instru-

Figure 1 Setup of trocars.

ments were used for dissection when necessary (Real Hand, Novare Systems Inc., Cupertino, CA, USA). After obtaining a clear critical view, the cystic duct was secured using two distal Hem-o-lock clips (Weck Closure Systems, Research Triangle Park, NC, USA) and one proximal titanium clip (Ethicon Endo-Surgery Inc., Cincinnati, OH, USA). The cystic duct was divided with laparoscopic scissors. Then, the gallbladder was dissected free of the liver bed using alternating retraction from the left-sided grasper and the harmonic scalpel. An Endo-loop was then introduced through the right trocar and placed around the infundibulum of the gallbladder. All trocars were removed and the three adjacent trocar incisions were joined to facilitate removal of the gallbladder. The abdominal wall and the subcutaneous tunnel were closed in layers under direct visualization with interrupted 2-0 Vicryl sutures. The skin incision was closed in a subcuticular fashion (Fig. 2).

Surgical technique: Please see corresponding video.

Results

The patient was a 30-year-old female (BMI=24) with symptomatic uncomplicated gallstone disease and no previous abdominal surgery.

The above described procedure was feasible using conventional rigid and articulating laparoscopic instruments. Time to create the tunnel and achieve abdominal access was approximately 4 min.

Gallbladder retraction was sufficient, and could provide adequate exposure by alternating the grasping site between the infundibulum and fundus of the gallbladder as needed. Visualization was as good as conventional laparoscopic view, and all critical structures were visualized without difficulty. Operative time was 45 min. No complications occurred during the operation, and the immediate postop-

Figure 2 Subcutaneous tunnel before closure.

erative course was uncomplicated. The patient was discharged 5 h after the procedure. Evaluation 3 weeks after the operation showed an uncomplicated course and a clean scar within the re-growing pubic hair (Fig. 3).

Discussion

We describe a new surgical technique for single incision laparoscopic surgery applied in a patient. Supra-pubic single incision cholecystectomy was feasible in this case using a combination of conventional rigid and articulated laparoscopic instruments. This new method delivers a linear scar that can be hidden within the supra-pubic hairline and might therefore appear cosmetically superior when compared to scars after conventional laparoscopy and single incision transumbilical laparoscopy. In addition, the rate of incisional hernias for port sites which avoid the midline is significantly smaller when compared with those placed in the midline.[16] After skin incision access through the abdominal wall can be chosen independent of the location of the skin incision, by creating a subcutaneous tunnel. Therefore, perforation through the rectal muscle instead of the linea alba can be performed easily in order to potentially avoid incisional hernias.

A limitation of this technique seems to lie in the entrapment of trocars in the subcutaneous tunnel and the entry into the abdominal cavity. Parallelism of trocars and limited range of motion of trocars lead to a loss of triangulation, a reduced field of surgery and intermittent conflict of instruments and camera on the outside. However, the use of articulated instruments was helpful to overcome some of these limitations by creating additional degrees of freedom. Still, the access to the abdominal cavity seems to represent the most critical challenge during this new method. Inserting a trocar through a preformed tunnel

to enter the abdominal cavity in an angle smaller than 90° is more difficult than entering in the usual vertical fashion. Incidental injuries might occur easier despite the visual control obtained by the camera inside the port. Additionally, while using optical trocars for laparoscopic access has been reported in great numbers in the upper abdomen[17–19], only few reports can be found about their use in the lower abdomen and safety in that anatomical region might be reduced.[19] Despite this uncertainty, a Foley catheter should be positioned during any supra-pubic access case to minimize the risks of any inadvertent bladder injuries.

New ports with extendable tips might offer solutions for this access. Also, flexible endoscopes might facilitate the passage through the subcutaneous tunnel and could be applied especially for longer and/or curved tunnels or be used in combination with laparoscopic instruments. In that sense, multiple tunnels can be placed to enter the abdominal cavity through a single skin incision. Different types of instruments could potentially be inserted to cover various aspects of abdominal surgery such as retraction, vision, dissection, and assistance. In that sense, this initial case only represents the feasible variation of a new surgical method and further research will be conducted to advance this technique.

Conclusions

Supra-pubic single incision laparoscopic cholecystectomy appeared feasible in our initial case. This new technique potentially offers less visible scaring (concealable in pubic hair) while maintaining an access through a single incision at a stable part the abdominal wall in order to reduce the risks for incisional hernias.

Whether this approach turns out to be superior to conventional laparoscopy, single incision laparoscopy and

Figure 3 Scar 3 weeks postoperatively.

NOTES regarding clinical parameters remains subject to more substantial research.

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HOW I DO IT

Reconstruction Following the Pylorus Preserving Whipple Resection: PJ, HJ, and DJ

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Abstract
Introduction Pancreaticoduodenectomy is one of the most challenging procedures performed by general surgeons.
Discussion Many studies have been performed looking at the technical aspects of reconstruction after pancreaticoduodenal resection. Multiple randomized trials have failed to convincingly demonstrate the superiority of any single approach or technique. Here, we illustrate our approach to reconstruction, with an emphasis on technical aspects and details.
Conclusion The fine points can help avoid technical errors that result in anastomotic failure.

Introduction

The definitive surgical management of periampullary pathology has long been a challenging endeavor. The basic concepts of safe dissection and reconstruction around the head of the pancreas have been well described for greater than 70 years.[1] However, it was not until the last 20 to 30 years that the rates of perioperative morbidity and mortality declined to the point that pancreaticoduodenectomy (PD) became a standard and accepted treatment for periampullary tumors. Currently, at high-volume centers, the rates of perioperative mortality and morbidity are

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typically reported at 1–3%, and 30–40%, respectively.[2,3] Multiple factors have contributed to this decline in morbidity and mortality. These factors include advances in cross-sectional imaging that allow accurate preoperative staging and patient selection,[3,4] advances in perioperative patient management and ICU care including the development of critical pathways,[5] and the development of specialized expertise at high-volume pancreatic surgery centers.[6]

High-quality cross-sectional imaging and the development of specialized centers have done much to assure safe and careful dissection in experienced hands. This leaves anastomotic leakage, particularly at the pancreaticojejunostomy, as the primary cause of morbidity after PD. Contemporary series report varying rates of pancreatic fistula after PD. Published rates vary between 2% and greater than 20%, with several large series reporting fistula rates of approximately 10%. An international group has proposed a clear definition of pancreatic fistula.[7] Much effort has gone into attempts to identify technical or pharmacologic approaches that would reliably reduce the rate of pancreatic fistula after PD. These approaches include variations in anastomotic technique such as pancreaticogastrostomy vs. pancreaticojejunostomy,[8] pancreatic duct stenting,[9] the use of octreotide,[10] and the use of fibrin sealant.[11] None of these approaches were able to demonstrate a reproducible decrease in the pancreatic fistula rate in the setting of randomized clinical trials. Our recent prospective randomized dual-institution study suggested that

an invagination pancreaticojejunostomy may have benefits as compared to a duct-to-mucosa pancreaticojejunostomy.[12]

These studies as well as a large number of retrospective case series have reached essentially the same conclusion, that underlying pancreatic texture (i.e., soft gland) is the primary determinant of pancreatic fistula formation.[2,3,8–11] Multiple permutations of similar and dissimilar techniques failed to alter the fact that soft pancreatic texture predisposes to anastomotic leakage. Perhaps overlooked in all these studies is the important role of meticulous surgical technique. Surgical technique cannot change underlying pancreatic morphology or physiology, but technical precision and the avoidance of any technical errors maximizes the possibility of a good outcome. Additionally, the same concept applies to the performance of the other two anastomoses performed during reconstruction of the GI tract after pylorus-preserving PD (PPPD), namely, hepaticojejunostomy and the duodenojejunostomy. Here, we present our standard approach to reconstruction after PPPD with special emphasis on technical details that we believe are critical for achieving good outcomes. The senior author (C.J.Y.) has evolved to these techniques over 20 years, with input from various colleagues, mentors, and publications and personal experience (as surgeon or assistant) with over 1,000 pancreaticoduodenal resections.

Surgical Technique

Specimen Removal to Pancreaticojejunostomy

After the specimen has been removed, the reconstruction following PPPD begins. If the operative procedure has been performed for a malignant neoplasm, titanium clips may be placed to mark the tumor bed, allowing for targeted postoperative radiation therapy.

We commence the reconstruction by closing the rent at the level of the ligament of Treitz with interrupted 3–0 silk sutures. The retained jejunum is then brought up through a separate small rent in the right side of the transverse mesocolon. This can often be identified as a thin "bare" space to the right of the middle colic vessels. It is critical to ensure that the retained proximal jejunum is not twisted on its mesentery when it is brought through this mesocolic rent and that it reaches to the pancreatic remnant and bile duct without tension.

The pancreatic remnant is then mobilized out of the retroperitoneum for a distance of at least 2 cm posteriorly, by dividing the connective tissue located superiorly and inferiorly along the pancreatic body and elevating the remnant ventrally, away from the splenic vein. A lacrimal duct probe or Baake's dilator of appropriate caliber may be placed in the lumen of the remnant pancreatic duct and used as an atraumatic handle to help elevate the pancreatic

remnant during this dissection. Superiorly, it is uncommon to encounter a substantial-sized blood vessel; however, there may be adjacent lymph nodes present. Our mobilization typically does not go leftward as far as the splenic artery. Inferiorly, one must be careful to avoid injury to small vessels, which may either drain into the inferior mesenteric, splenic, or superior mesenteric vein. Additionally, small arteries originating from the superior mesenteric artery may be found along the inferior border of the gland or posterior to the pancreatic remnant.

Invagination Pancreaticojejunostomy

Following mobilization of the pancreatic remnant, we commence our reconstruction with the pancreaticojejunostomy. This is typically performed in end-to-side fashion, to the proximal-most portion of the available jejunum just distal to the oversewn staple line. Suture placement begins at the superior aspect of the remnant pancreas (Fig. 1), placing an interrupted 3–0 silk corner suture first through the superior edge of the pancreatic remnant and subsequently as a seromuscular bite on the jejunum. This posterior outer row is then performed in horizontal mattress fashion, taking substantial bites of the posterior pancreatic capsule and parenchyma and seromuscular bites of the jejunum. The previously placed lacrimal duct probe or Baake's dilator can be maintained in the lumen of the remnant pancreatic duct, to minimize the chance that any of these posterior stitches will catch and occlude the pancreatic duct. On the average, five to seven of these posterior outer 3–0 silk sutures are first placed and then tied without tension. The tied sutures are then placed on tension retracting leftwards, and a jejunotomy is created with the electrocautery 2–3 mm from the suture line, typically
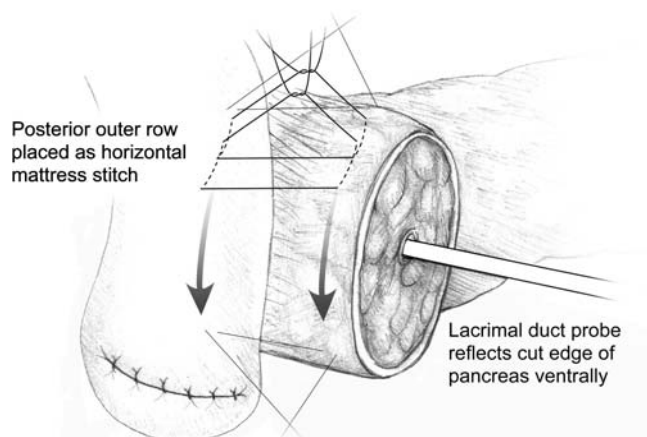
Figure 1 End to side pancreaticojejunostomy: The posterior outer row is performed with 3–0 silk, in horizontal mattress fashion. The *arrows* indicate the direction of consecutive suture placement, starting superiorly and moving inferiorly. A lacrimal duct probe is used to reflect the cut edge of the pancreas ventrally.
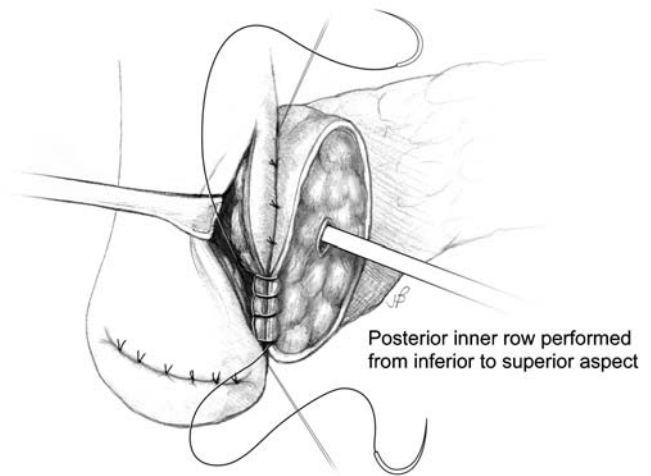
extending from the penultimate superior silk suture to the penultimate inferior silk suture, opening the jejunum full thickness (Fig. 2). All but the most superior and inferior silk sutures are then cut, and a vein retractor is placed to hold the jejunal lumen open. In performing the invagination type of pancreaticojejunostomy, the inner posterior layer is performed using 3–0 polysorb suture in continuous fashion. Two sutures are placed at essentially the same spot at the inferior most aspect of the jejunal opening. A running, locking posterior row is placed, taking good bites of the pancreatic parenchyma and capsule on the pancreas side and full thickness bites of the bowel wall on the jejunal side (Fig. 3). The posterior suture is continued up and around the superior corner of the inner layer of the anastomosis. At the completion of the inner posterior row, the vein retractor and probe or dilator in the pancreas duct are removed, and the anterior inner row is completed by running the 3–0 polysorb suture from superior to inferior along the anterior aspect of the pancreas. These bites typically contain an ample amount of pancreatic capsule and parenchyma as well as full thickness jejunum. The goal is to invaginate or "dunk" all of the cut edge of the pancreas into the jejunal lumen, allowing apposition of the pancreatic capsule to the jejunal serosa (Fig. 4).

The anterior outer layer is then performed using interrupted 3–0 silk sutures, taking ample bites of the pancreatic capsule and parenchyma, and then seromuscular bites of jejunum well away from the anastomosis, allowing the jejunum to roll up over the anterior inner layer, to complete the two-layer invagination end-to-side pancreaticojejunostomy (Fig. 5 and inset). The anterior outer layer sutures are all first placed and then tied down sequentially. When dealing with a gland of soft texture, the first assistant

Figure 3 Invagination pancreaticojejunostomy: A vein retractor holds the jejunum open. The posterior inner layer is performed from inferior to superior with running locking 3–0 polysorb, taking good bites of the pancreas (parenchyma and capsule) and full thickness bites of the jejunum.

crosses the next untied suture adjacent to the one being tied by the operating surgeon, to reduce tension and minimize the chance of the suture cutting through the pancreatic parenchyma.

Duct-to-Mucosa Pancreaticojejunostomy

The posterior outer row of 3–0 silk suture is placed as described above (see "Invagination Pancreaticojejunostomy", Fig. 1). The tied silk sutures are then held on tension, and the pancreatic duct is identified and probed. A small, full-thickness jejunotomy is then created in the jejunum, using electrocautery, in line with the pancreatic duct (Fig. 6). The

Figure 2 Invagination pancreaticojejunostomy: The jejunum is opened with the electrocautery from the penultimate (next to last) superior silk stitch, to the penultimate inferior silk stitch.
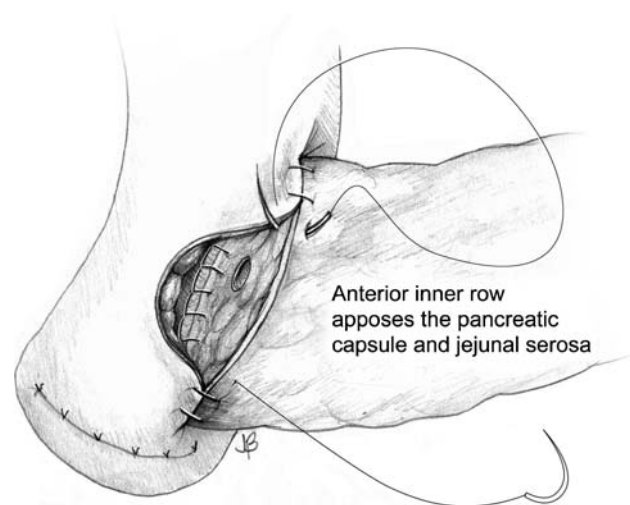
Figure 4 Invagination pancreaticojejunostomy: The vein retractor and lacrimal duct probe have been removed. The anterior inner layer is performed with running 3–0 polysorb, achieving apposition of the pancreatic capsule and the jejunal serosa.
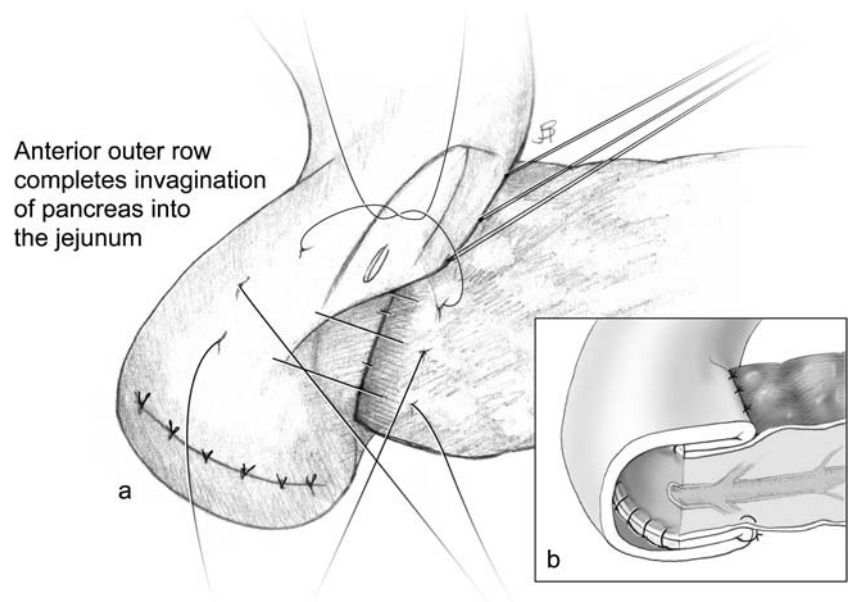
posterior inner layer is then performed using 5–0 PDS suture
and loupe magnification if necessary, taking ample bites of
the pancreatic parenchyma and pancreatic duct and full
thickness bites of the jejunum. If a small pancreas duct is
encountered (1–2 mm in diameter), a total of three to four 5–
0 PDS sutures may be used on the posterior row. For a larger
pancreatic duct, the sutures are spaced no more than 1.5 mm
apart, and up to ten 5–0 PDS sutures may be required
(Fig. 7).

A nice maneuver at this point is to use a sterile pediatric
feeding tube (3.5, 5.0, or 8.0 Fr and sized appropriately to
fit into the pancreas duct) and place one end up into the
pancreatic duct extending 5 cm into the pancreatic body
and the other end through the jejunotomy and downstream

into the jejunum. We typically cut the pediatric feeding tube
to a length of 20 cm, allowing there to be approximately
5 cm within the pancreatic parenchyma and roughly 15 cm
in the downstream jejunum (Fig. 8). The pediatric feeding
tube is not intended as a permanent anastomotic stent but
rather as a temporary guide for the placement of the anterior
inner and outer rows of sutures. Its presence in the lumen of
the pancreas duct minimizes the chance that any of the
anterior inner row sutures will catch the back wall of the
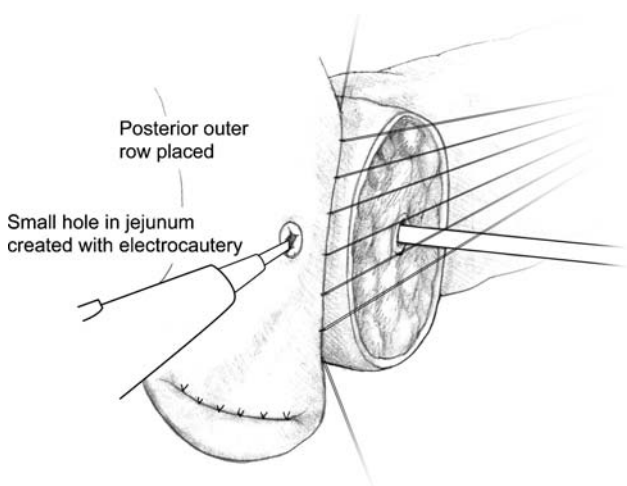
Figure 6 Duct-to-mucosa pancreaticojejunostomy: After the posterior
outer row of 3–0 silk sutures is placed (as per Fig. 1), a small hole is
created in the jejunum using the electrocautery, at the level of the
pancreatic duct.
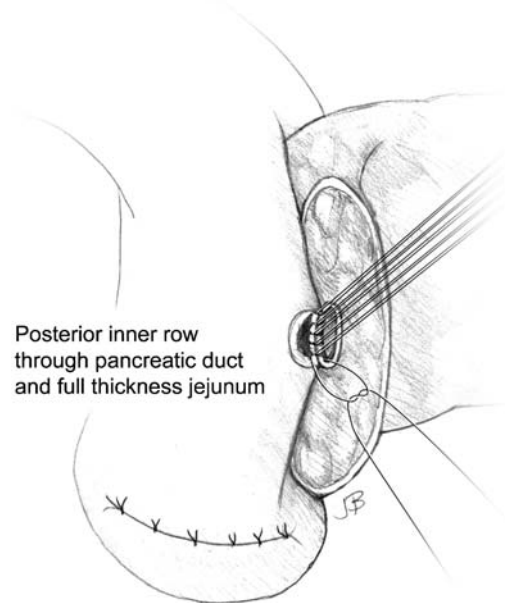
Figure 7 Duct-to-mucosa pancreaticojejunostomy: The posterior
inner row of 5–0 PDS sutures has been placed into a 5-mm pancreatic
duct. The bites on the jejunal side take all layers of the jejunal wall,
while the bites on the pancreas side include the pancreatic duct and a
small amount of pancreatic parenchyma surrounding the duct.
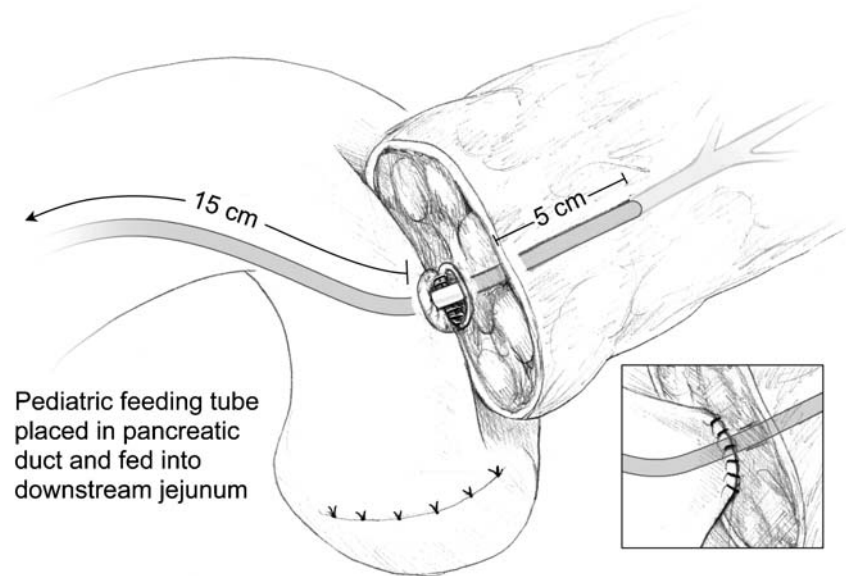
pancreatic duct and occlude the lumen. The anterior inner row is then performed using 5–0 PDS sutures, taking care to avoid snagging the pediatric feeding tube. The pediatric feeding tube is temporarily left in the lumen through the anastomosis, to be removed through the downstream jejunotomy made for the hepaticojejunostomy. Once these anterior inner row of sutures are tied and cut, then the anterior outer row of 3–0 silk sutures are placed, taking ample bites of the pancreatic parenchyma and capsule, and ample bites of the jejunum well away from the anastomosis, allowing the jejunum to be pulled up and over the anterior inner suture line (Fig. 9). Careful technique is used to avoid tearing the parenchyma of the pancreas when these anterior outer layer sutures are tied.

End-to-Side Hepaticojejunostomy

Approximately 10 cm downstream from the pancreaticojejunostomy, we perform a standard biliary-enteric reconstruction as an end-to-side hepaticojejunostomy. We have learned that this hepaticojejunostomy should be sufficiently downstream from the pancreaticojejunostomy to allow a bit of redundancy in the jejunal limb between the pancreaticojejunostomy and the hepaticojejunostomy. This is intentionally done in the rare chance that reoperation is necessary for failure of healing of the pancreaticojejunostomy or revision of the hepaticojejunostomy. Having the pancreaticojejunostomy and the hepaticojejunostomy at least 10 cm apart allows the surgeon to work on either the pancreaticojejunostomy or the hepaticojejunostomy separately and not have to disrupt both anastomoses at the time of reoperation.

We perform our hepaticojejunostomy using a single layer of 5–0 PDS sutures, first opening the jejunum with

the cautery, sizing it appropriately to the internal diameter of the common hepatic duct (or common bile duct). At this point, the pediatric feeding tube used as a temporary pancreas duct stent for the duct-to-mucosa pancreaticojejunostomy is removed. We place the corner stitches at the 3 and 9 o'clock positions of the duct first, with the knots on the outside. We then place the remainder of our posterior sutures with the knots on the inside (Fig. 10), and we tie all the sutures. After checking for patency of the lumen of the common hepatic duct and the jejunum, we use an 8- to 12-French T-tube (cut to yield an "I-tube") to temporarily stent the internal aspect of the anastomosis during the time that we are placing the anterior row of sutures. The anterior row of 5–0 PDS sutures is then placed and subsequently tied down with the knots on the outside to
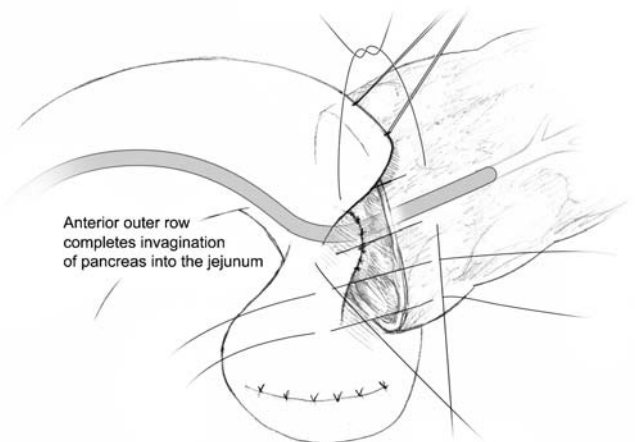
Figure 9 Duct-to-mucosa pancreaticojejunostomy: The anterior outer row of 3–0 silk sutures is placed, pulling the mobile jejunum over the immobile suture line, allowing apposition of the jejunal serosa to the pancreatic capsule.
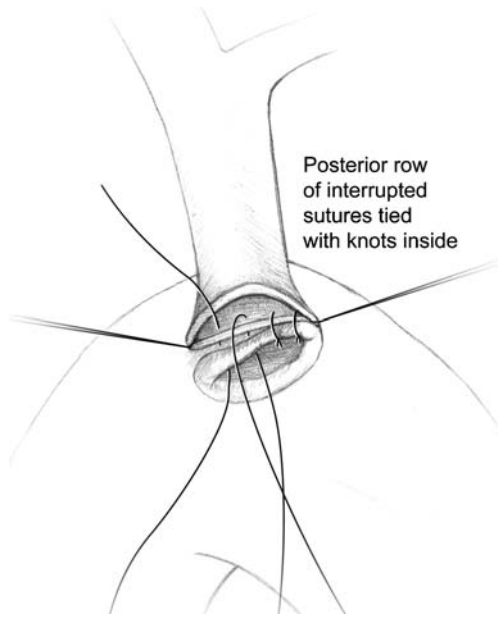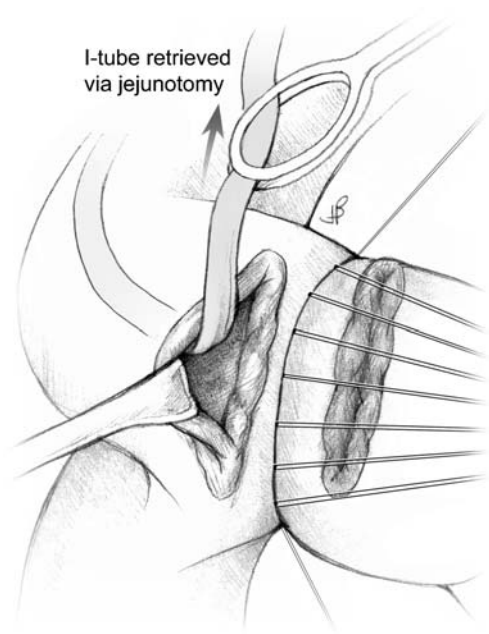
Figure 10 End-to-side hepaticojejunostomy: The posterior row is performed with interrupted 5–0 PDS sutures. For the corner stitches at 3 and 9 o'clock on the duct, the knots are tied on the outside, while for the posterior sutures, the knots are tied on the inside. If a pediatric feeding tube was used for the duct-to-mucosa pancreaticojejunostomy, it is removed via this jejunotomy.

Figure 12 End-to-side duodenojejunostomy: After the posterior outer row of 3–0 silk sutures are placed and tied, the jejunum is opened, the duodenal staple line is excised, and the "I-tube" is retrieved via the jejunotomy.

yield a water-tight anastomosis without tension (Fig. 11). The temporary "I-tube" stent is left in place and removed through the downstream jejunotomy made for the subsequent duodenojejunostomy.

End-to-Side Duodenojejunostomy

About 15 cm downstream from the hepaticojejunostomy, we perform a standard end-to-side duodenojejunostomy in retrocolic fashion. We first place an outer layer of interrupted 3–0 silk sutures, then excise the duodenal staple line and open the jejunum with the electrocautery. At this point, the I-tube used to "stent" the hepaticojejunostomy is removed through the jejunotomy (Fig. 12). The inner layer

Figure 11 End-to-side hepaticojejunostomy: The anterior row is performed with interrupted 5–0 PDS sutures, over an 8- to 12-French "I-tube." The anterior row knots are tied on the outside.
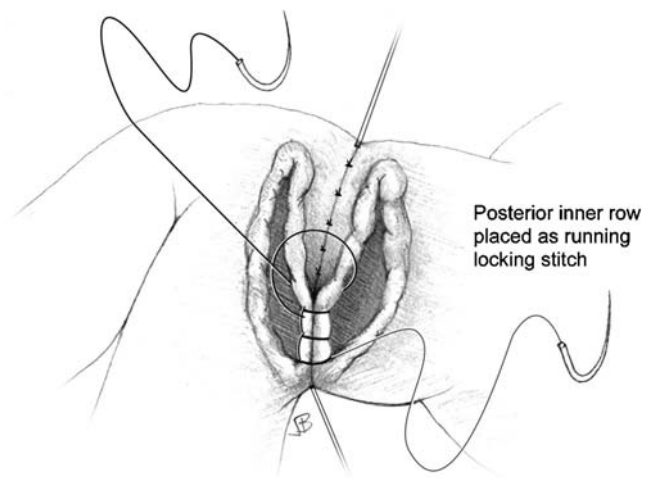
Figure 13 End-to-side duodenojejunostomy: The posterior inner layer is performed with running 3–0 polysorb, using a running locking technique.
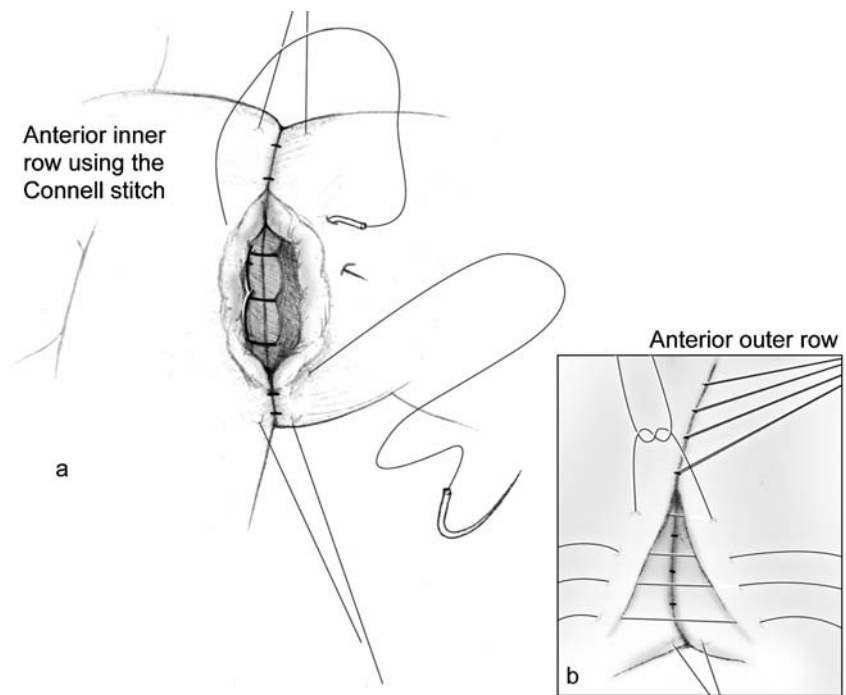
of the duodenojejunostomy is performed using running 3–0
polysorb, with locking sutures on the posterior aspect
(Fig. 13), and the Connell suture anteriorly (Fig. 14). The
anastomosis is completed with an outer anterior layer of
interrupted 3–0 silk (Fig. 14, inset). About 6–8 cm
downstream from the duodenojejunostomy, the efferent
limb of the duodenojejunostomy is secured to the transverse
mesocolon with interrupted 3–0 silk stitches, closing the
mesenteric defect and leaving the pancreaticojejunostomy,
hepaticojejunostomy, and duodenojejunostomy all cephalad
to the transverse mesocolon (Fig. 15). We have observed a
very low incidence of delayed gastric emptying with this
reconstruction and have not felt the necessity to perform the
duodenojejunostomy in antecolic fashion.

We do not place gastrostomy or jejunostomy tubes for
venting or enteral feeding purposes. We do not leave the
temporary pancreatic duct stent or I-tube in place. Our
current practice is to place two 3/16″ round silastic drains
through left- and right-sided flank stab incisions. The
right drain is designed to drain the right subhepatic
space and the retroperitoneal area posterior to the neo-
duodenum and adjacent to the superior mesenteric artery
and vein. The left drain is brought through the gastrocolic
ligament laterally and into the lesser sac, and it rests in the
left subhepatic space, near but not touching the pancrea-
ticojejunostomy. Our critical pathway for PD[5] targets
removal of the nasogastric tube on postoperative day
(POD) 1, a clear liquid diet on POD 2, solid food on POD
3, and hospital discharge on POD 6 or 7. Over 70% of our
patients are able to adhere to this hospital discharge
target.

Discussion

PD is one of the most challenging procedures performed by
general surgeons. In addition to an often difficult dissection, the
continuity of the GI tract is violated in four places and must be
reconstructed with at least three separate anastomoses. Each of
these anastomoses has a potential for leakage, with subsequent
associated morbidity. Many approaches, particularly the ones
focused on the pancreatic anastomosis, have been evaluated,
and many have failed to show a consistent reduction in
anastomotic leakage. One conclusion that is easily drawn from
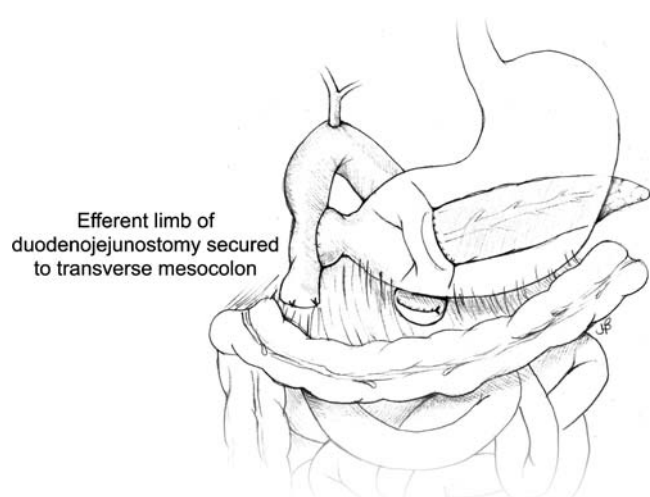
Figure 15 End-to-side duodenojejunostomy: The efferent limb of the
duodenojejun-ostomy is secured to the transverse mesocolon using 3–
0 silk sutures, closing the mesocolic rent, and leaving all anastomoses
cephalad to the transverse mesocolon.

the compilation of many well-done studies is that underlying patient factors and surgery-related technical factors are important determinants of outcomes.

One common theme that can be identified running through the body of scientific literature surrounding reconstruction after PD is that many techniques often have exceptional results in the hands of the individual or small group of surgeons promoting that technique. When a certain technique is generalized in a large study, however, the results achieved tend to regress toward the mean in the literature. This leads one to question whether or not it is truly a certain approach that is critical or if it is the relationship between a particular surgeon and a particular approach that makes a difference. Perhaps, it is the precision employed and the lack of seemingly trivial technical missteps that occur when a surgeon has significant comfort and experience with a particular technique that results in superior outcomes. Development of that comfort level and emphasis on fine points of technique, such as those described in his article, should allow for excellent outcomes with many reconstructive approaches. The reconstruction after PPPD is a technically challenging endeavor, which, if it can be performed safely, frequently allows for a relatively short postoperative hospital stay and return toward optimal quality of life.

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REVIEW ARTICLE

Autonomic Nerve Preservation During Rectal Cancer Resection

José G. Guillem · Steven A. Lee-Kong

Introduction

Identification and preservation of pelvic autonomic nerves is important[1,2] during radical resection of a rectal cancer in order to reduce the risk of genitourinary dysfunction. Detailed anatomic dissections have highlighted the relationship between the pelvic autonomic nervous system (PANS) and other pelvic organs.[3] The superior hypogastric plexus (SHP) receives sympathetic contributions directly from the sympathetic trunk or via the inferior mesenteric ganglion, while the inferior hypogastric plexus (IHP) receives its major parasympathetic contribution (nervi erigentes) from the third sacral nerve root, with lesser contributions from the second and fourth.[3] Physiologic studies in animals and humans have demonstrated the importance of the parasympathetic nervous system in achieving and maintaining erection, while the sympathetic nervous system is important for ejaculation.[4] Both sympathetic and parasympathetic innervation of the urinary bladder influences continence via coordination of detrusor contraction and tone at the bladder neck.[5]

The introduction of total mesorectal excision (TME) in the operative treatment of rectal cancer has resulted in a decrease in local recurrence and improved survival.[6] TME, in conjunction with autonomic nerve preservation (ANP), has improved rates of postoperative genitourinary dysfunction.[1,7–9] In a report from our institution, rectal resection, incorporating the principles of TME and ANP, preserved the ability to have intercourse in 86% and 57% of men undergoing low anterior resection (LAR) and abdominoperineal resection (APR), respectively. The ability to achieve orgasm was maintained in 88% of men after LAR and 85% after APR. In women, postoperative sexual activity was continued in 86% of patients, while the ability to achieve orgasm was maintained in 91%.[10]

Because of the noted improvement in preserving bladder and sexual function following properly performed TME and ANP, surgical training programs have begun to incorporate these techniques into their curriculum. However, widespread implementation is still in its early stages. The purpose of this paper is, therefore, to present a detailed, step-by-step approach for TME and ANP during rectal cancer resection.

Material and Methods

After thoroughly inspecting the liver, peritoneum, and retroperitoneum for evidence of metastatic disease, attention is directed to the rectum and sigmoid colon. The sigmoid is rendered a midline structure by lysing congenital adhesions along the left pelvic sidewall. The redundancy of the sigmoid colon is assessed to determine the degree of left colon mobilization required if a primary anastomosis is to be performed. The retroperitoneum is entered sharply along the white line of Toldt and the retroperitoneal structures identified. At the level of the aortic bifurcation, the SHP lies posterior to the inferior mesenteric artery (IMA). Careful dissection between these two structures, while

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Figure 1 The distal sigmoid/ proximal rectum is elevated anteriorly, exposing the aortic bifurcation and sacral promontory, with identification of the left ureter, left iliac vein, and superior hypogastric plexus. The hypogastric nerves may appear as an obvious discrete band of tissue or as multiple smaller bands.
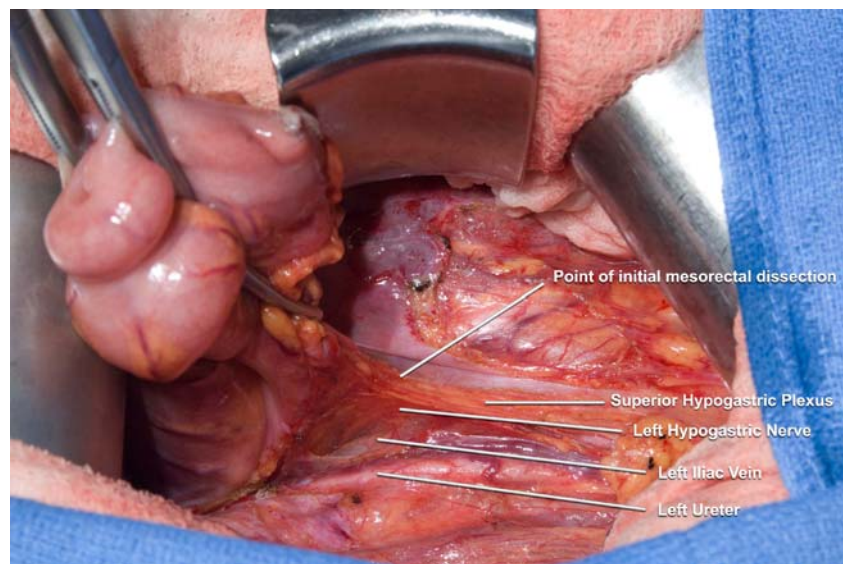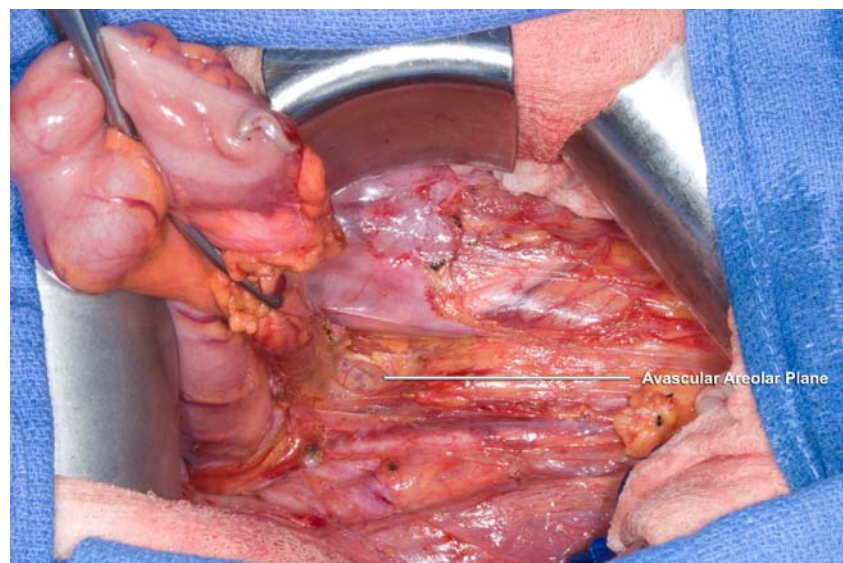

retracting the rectosigmoid "toward the ceiling," allows the plexus to be "dropped down" to its normal anatomic position. The IMA and inferior mesenteric vein distal to the left colic vessels are skeletonized and ligated separately, when possible. The distal sigmoid colon is then transected using a GIA stapling device.

The distal sigmoid/proximal rectum is elevated anteriorly "toward the ceiling" exposing the aortic bifurcation and sacral promontory (Fig. 1), with early identification of the left ureter, left iliac vein, and SHP. The hypogastric nerves may appear as an obvious discrete band of tissue or as multiple smaller bands. Careful dissection of the sigmoid mesentery distally will lead to an avascular, areolar plane separating the mesorectal fascia propria from the presacral fascia (Fig. 2).

Insertion of a closed Mayo scissors into the space between the mesorectal fascia propria and presacral fascia aids in the identification of the paired hypogastric nerves

(Fig. 3). Development of this plane is critical for a successful TME and ANP. The hypogastric nerves, which form a "wishbone-like" pattern as they exit the inferior aspect of the SHP in the midline, descend into the pelvis along the mesorectal fascia 1 to 2 cm medial to the ureters (Fig. 4). Further elevation of the specimen toward the patient's left may cause the hypogastric nerves to be "tented up" (Fig. 5), as they are often adherent to the mesorectal fascia. Careful dissection along the leading edge of the nerves will allow them to be peeled off of the specimen, similar to the peeling of onion skin. Caudal dissection in the posterior midline, while lifting the rectum "toward the ceiling," further develops the avascular areolar plane essential for identification of the sacral nerves (nervi erigentes; Fig. 6).

Anterior dissection begins with incision of Denonvellier's fascia. With the use of the St. Mark's retractor, this plane is developed until the seminal vesicles (in men; Fig. 7) or the

Figure 2 Careful dissection of the sigmoid mesentery distally results in an avascular, areolar plane separating the mesorectal fascia propria from the presacral fascia.
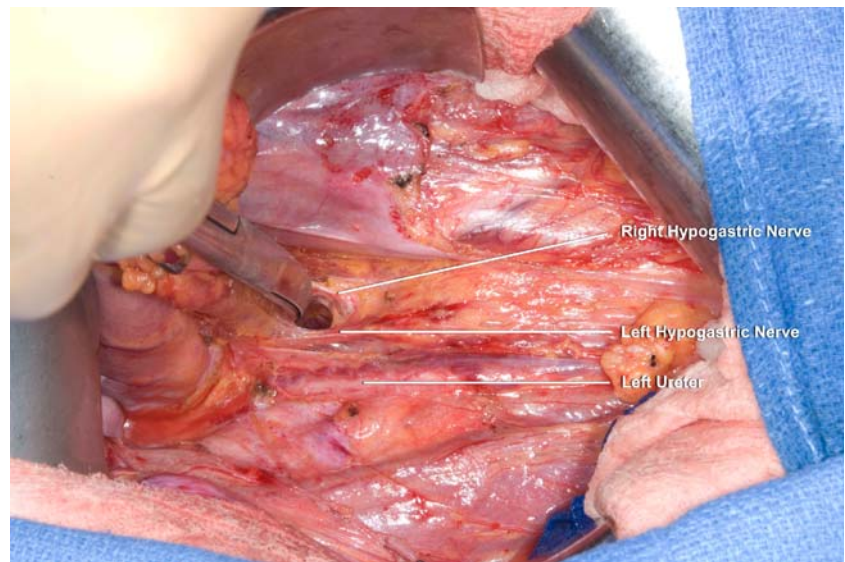
Figure 3 Insertion of closed Mayo scissors into the space between the mesorectal fascia propria and presacral fascia aids in identifying the paired hypogastric nerves.


Figure 4 The hypogastric nerves form a wishbone-like pattern as they exit the inferior aspect of the superior hypogastric plexus in the midline. These nerves descend into the pelvis along the mesorectal fascia, 1 to 2 cm medial to the ureters.
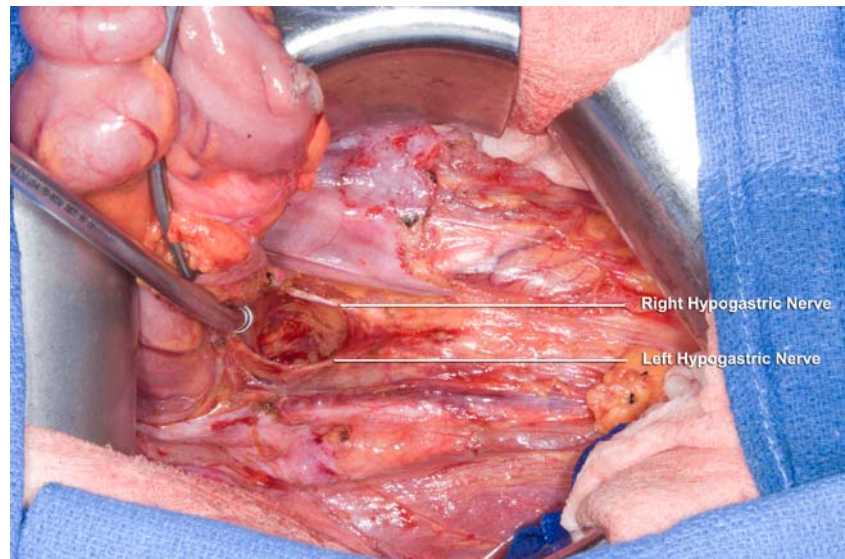
Figure 5 Further elevation of the specimen towards the patient's left may cause the hypogastric nerves to "tent up," as they often adhere to the mesorectal fascia.
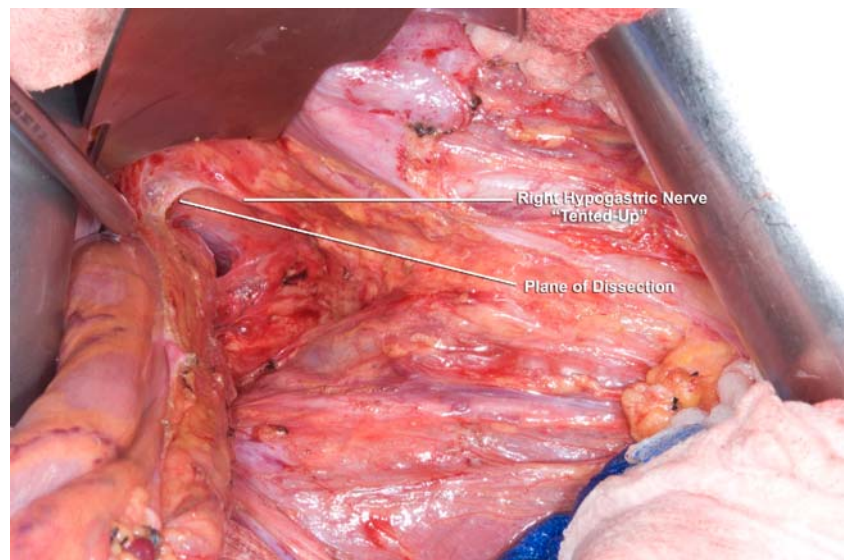
Figure 6 Careful dissection along the leading edge of the nerves permits them to be peeled off of the specimen, similar to peeling of onion skin. Caudal dissection in the posterior midline, while lifting the rectum "toward the ceiling," further develops the avascular areolar plane, which is essential for the identification of the sacral nerves (nervi erigentes).
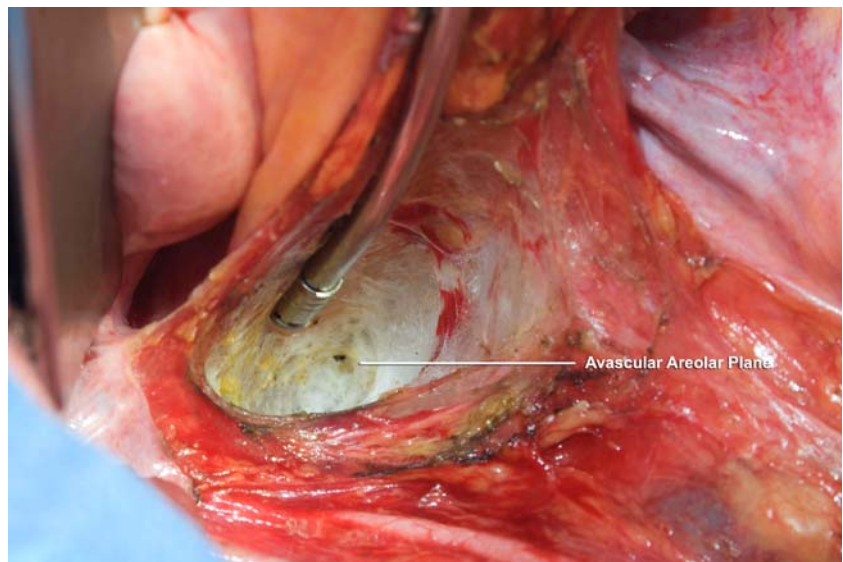

rectovaginal septum (in women) is encountered. In men, care is taken not to injure the vascular capsule of the seminal vesicles, as the plane of dissection between it and the mesorectal fascia may not be clear initially.

The IHP is formed from the interdigitating fibers of the hypogastric (sympathetic) nerves and the sacral (parasympathetic) nerves. This structure appears as a fenestrated, rhomboid-like plate on the pelvic sidewall and is located anterolateral to the rectum and posterolateral to the seminal vesicles in men and corresponding zone in women.

As the mesorectal fascia is developed posterolaterally, the nervi erigentes are encountered, often adherent to the mesorectal fascia. These nerve fibers arise from the second,

third, and fourth sacral nerve roots. Careful retraction of the specimen and dissection along the mesorectal fascia will facilitate release of the nerves and return them to their normal anatomic position along the piriformis muscle. This separation also mimics the peeling of onion skin (Fig. 8).

As distal dissection continues to the levator ani fascia, the mesorectum begins to taper and generally becomes absent 1–2 cm above the uppermost portion of the anorectal ring. For a LAR, transection of the rectum can then be performed at the appropriate level relative to the tumor and the specimen removed. A Veidenheimer non-crushing bowel clamp is placed distal to the lesion (as shown), and following a rectal washout, a linear stapler is used to transect the rectum at a point proximal enough to the vagina to avoid incorporation of vaginal tissue into the stapled anastomosis (Fig. 9). For patients treated with preoperative combined modality therapy, a distal margin of 1 cm may be adequate for complete tumor removal.[11] An APR can be performed at this point if a clear distal margin
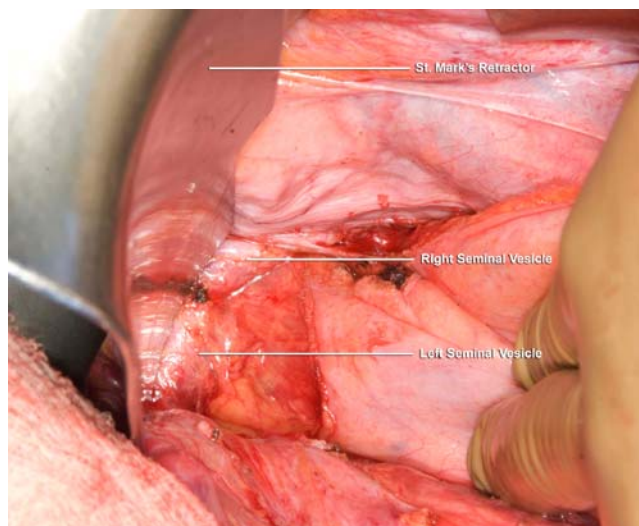

Figure 7 As the mesorectal fascia is developed posterolaterally, the nervi erigentes are encountered (often adherent to the mesorectal fascia). Meticulous retraction of the specimen and dissection along the mesorectal fascia facilitates release of the nerves, returning them to their normal anatomic position along the piriformis muscle. This separation mimics the peeling of onion skin.
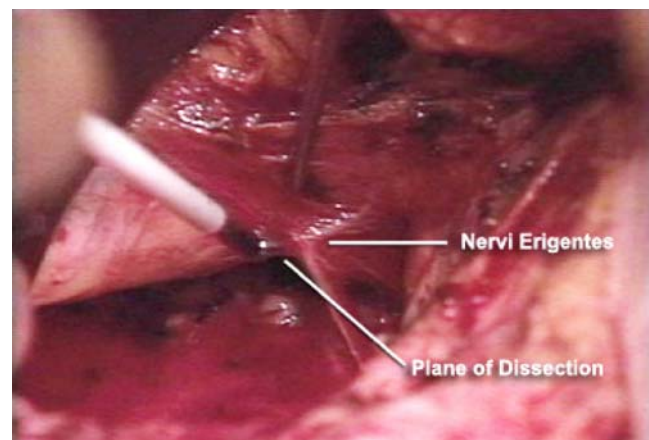
Figure 8 Anterior dissection begins with incision of Denonvellier's fascia. Using the St. Mark's retractor, this plane is developed until the seminal vesicles (in men) or rectovaginal septum (in women) is encountered.

Figure 9 A Veidenheimer non-crushing bowel clamp is placed distal to the lesion. Following rectal washout, a linear stapler is used to transect the rectum at a point proximal enough to the vagina to avoid incorporation of vaginal tissue into the stapled anastomosis.
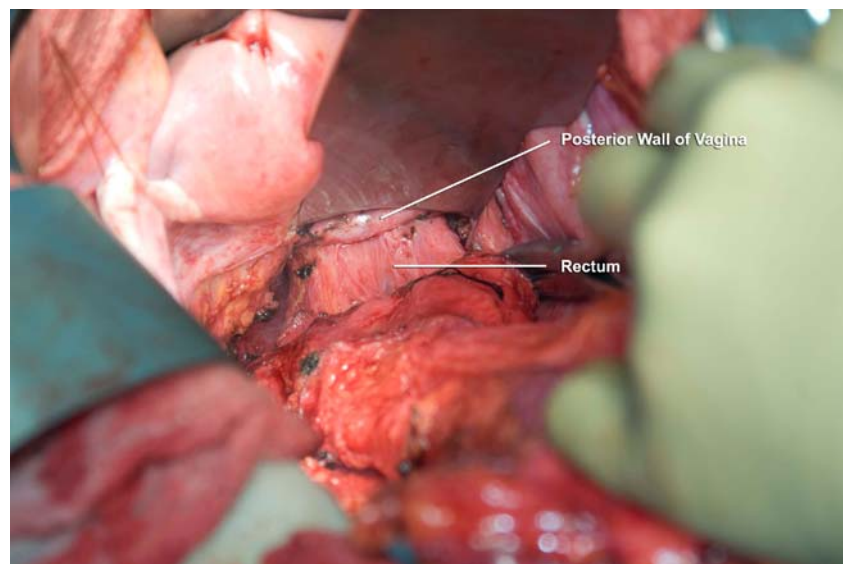
cannot be obtained or if involvement of the anal sphincter mechanism is suspected. The preserved autonomic nervous system can be visualized in its entirety upon removal of the specimen (Figs. 10 and 11). Adequacy of the TME can be judged by appreciation of an intact mesorectal fascia, which should be smooth and glistening upon gross inspection of the resected specimen (Fig. 12).

Discussion

Intraoperative Nerve Identification

Intraoperative identification and preservation of the PANS may reduce rates of postoperative genitourinary dysfunction.[8,12,13] In a study of 150 consecutive patients undergoing TME for rectal cancer, complete identification (by intraoperative visual inspection) of the PANS was achieved 72% of the time. In this study, risk factors contributing to incomplete nerve identification included previous pelvic surgery and intra-operative blood loss >1,000 mL. Patients who had complete identification of the PANS experienced a significant reduction in postoperative urinary dysfunction (4.5% versus 38.5%, $p<0.001$).[8]

Given the importance of nerve identification, some surgeons advocate the use of intraoperative nerve stimulation (INS) to aid in autonomic nerve identification and preservation. A recent study suggests that the use of INS with bladder manometry to intraoperatively identify parasympathetic nerves can improve rates of postoperative urinary dysfunction. Patients with evidence of unilateral or bilateral parasympathetic nerve damage had higher rates of long-term bladder catheterization after hospital discharge than those with confirmed bilateral preservation (33%

Figure 10 Upon removal of the specimen, the preserved autonomic nervous system can be visualized in its entirety.
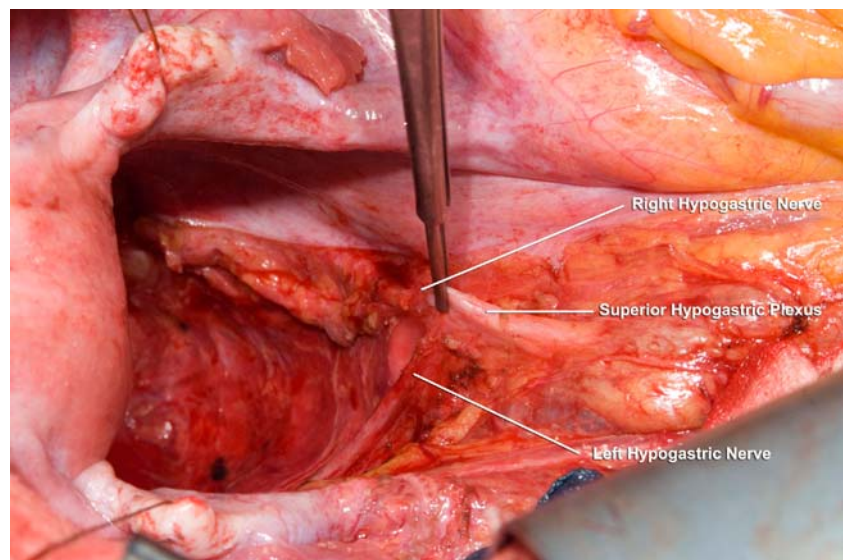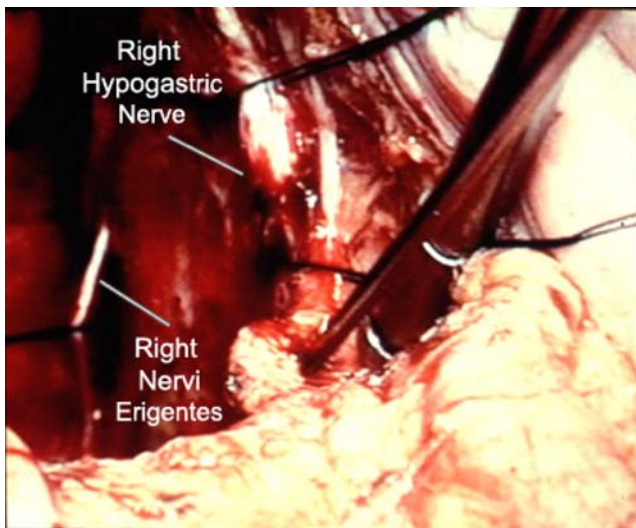
Figure 11 Upon removal of the specimen, the preserved autonomic nervous system can be visualized in its entirety.

versus 0%, $p=0.001$) and reported deterioration of postoperative urinary function (60% versus 4%, $p<0.001$). They also suggest that INS results are more sensitive than macroscopic assessment by the surgeon for identification of nerve damage.[12]

The CaverMap (Uromed Corp., Norwood, MA) nerve stimulator device, used to demonstrate penile tumescence in response to intraoperative parasympathetic nerve stimulation, may also enhance autonomic nerve identification and preservation.[14] In the Memorial Sloan–Kettering Cancer Center experience with this device, all 21 male study patients undergoing rectal cancer resection had at least unilateral response to INS. Sexual function (erection and orgasm) was normal in 18 (94.7%) patients surveyed 6 months postoperatively. In all of these patients nerve integrity was felt to be intact by the operating surgeon's visual assessment.[13]

Figure 12 Adequacy of a TME can be judged by appreciation of the intact mesorectal fascia. On gross inspection, the resected specimen should appear smooth and glistening.

Laparoscopic Rectal Resection with ANP

Laparoscopic techniques have been adopted for the operative treatment of rectal cancer. Studies comparing differences in rates of genitourinary dysfunction following laparoscopically assisted or open rectal cancer resections are limited. In 2002, one study reported no statistically significant difference in postoperative bladder dysfunction between patients undergoing laparoscopically assisted versus open mesorectal excision for rectal cancer. However, they did report significantly higher rates of impotence (33% versus 5%, $p=0.03$) and ejaculatory dysfunction (40% versus 5%, $p=0.01$) in men undergoing laparoscopic resection.[15] A larger, more recent study demonstrated similar rates of bladder dysfunction between patients undergoing either laparoscopic or open rectal resection, with trends toward worse overall sexual function and erectile function in men after laparoscopic resection.[16]

Conclusion

While ANP has gained acceptance in rectal cancer surgery, there are instances where its routine practice may not be justified. In patients with physical exam or radiologic findings suggestive of extensive pelvic disease, nerve preservation may not be technically feasible or oncologically sound. Direct tumor invasion of the autonomic nerves or presence of suspicious pelvic sidewall lymphadenopathy may require nerve sacrifice.

Genitourinary dysfunction results in significant morbidity when it occurs after rectal resection. Techniques for autonomic nerve identification and preservation, particularly during TME, can improve rates of postoperative sexual and urinary function.

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GI IMAGE

Primary Omental Mesothelioma: A Rare but Important Differential Diagnosis in Previous Asbestos Exposure

Ayad Harb · Elizabeth King · Helen Lloyd · Ziad Harb · John G. Payne

Abstract Primary omental mesothelioma is a malignant tumour of the mesothelial cells of the omentum, related to asbestos exposure. It is an extremely rare condition that presents both diagnostic and therapeutic challenges. We present a review of the related literature and report on a fatal case of primary omental mesothelioma in a 70 year old man, presenting with a painful abdominal mass. Radiological imaging was not diagnostic but useful in excluding other pathologies. Diagnosis relied on specific immunohistochemical analysis. The difficulty in diagnosis and management and the advanced stage of disease meant that prognosis was very poor. Our patient died within 3 weeks of diagnosis.

Introduction

Primary omental mesothelioma is a malignant tumor of the mesothelial cells of the omentum related to asbestos exposure. It is an extremely rare condition reported only twice before in medical literature. It was first described in 2000 as a separate entity and distinguished from generalized peritoneal mesothelioma. Omental mesothelioma presents both diagnostic and therapeutic challenges, with limited treatment options and a grave prognosis.

Case Report

The patient was a 70-year-old Hungarian gentleman, with previous occupational exposure to asbestos. He presented with a 3-month history of abdominal pain, increasing lethargy, loss of appetite, and weight loss of 20 kg. Examination revealed a distended and generally tender abdomen, with an exquisitely painful mass in the right upper quadrant. Blood tests showed microcytic anaemia, Hb 11, low albumin, and raised C-reactive protein. Tumor markers were tested—Ca125 of 360; CEA, Ca19.9, B-HCG, AFP were all within normal limits. Abdominal and chest radiographs on admission revealed no abnormalities. He had been previously investigated with ultrasound (USS) and three colonoscopies in Hungary, which had yielded no diagnosis.

A CT scan showed diffuse infiltration of the omentum and edema of the peritoneal fat throughout the abdomen and pelvis, the most prominent site of infiltration being adjacent to the hepatic flexure. No discrete bowel mass was seen, and the abdominal viscera appeared normal. The appearances were nonspecific and differentials of omental reaction, primary intraperitoneal neoplasm, or intraperitoneal tuberculosis were given (Fig. 1). The possibility of malignancy was considered; however, a barium enema and colonoscopy were both normal.

USS-guided percutaneous biopsies were taken of the omentum. Immunohistochemical staining suggested a mesothelial cell origin (Fig. 2). Tumor cells were positive for MNF116 and CK7, which identify epithelial cells in glandular and transitional tissues but are nonspecific for

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Figure 1 Nonspecific appearances and differentials of omental reaction, primary intraperitoneal neoplasm, or intraperitoneal tuberculosis.

mesothelioma. Staining was negative for CEA and PSA. To differentiate between mesothelioma and adenocarcinoma, more specific tissue makers were used: WT1, which is a tumor-suppressor gene found in kidney, spleen, and malignant mesothelial cells; calretinin, which is detected in most malignant mesothelial cells; and epithelial membrane antigen (EMA), which distinguishes between malignant mesothelioma and mesothelial hyperplasia. A diagnosis of malignant mesothelioma was made. The patient was referred to the mesothelioma-specialist clinic; however, he continued to deteriorate and died in the hospital 3 weeks after diagnosis.

Discussion

Primary omental mesothelioma is a rare malignant tumor of the mesothelial cells of the omentum, which has been reported twice before in medical literature, so little epidemiological data are known. Exposure to asbestos is likely to have a causative relation, as in the more common pleural and peritoneal mesotheliomas.

Mesothelioma of the abdominal cavity, in general, has an incidence of 2.2 cases per million and forms 10% of reported mesotheliomas. Most cases occur in patients over 50 years of age, but it has been also described in young adults, children, and neonates. The classic presentation of peritoneal mesothelioma features ascites, abdominal distension, or bowel obstruction.[1]

Pleural asbestosis often coexists, and coincidental pleural mesothelioma has been described. The proliferation of reported cases over the last two decades seems to indicate increased incidence and/or recognition. It is expected that the increasing incidence may not peak before 2016 in countries like the UK.

Our case of primary omental disease presented with a tender abdominal mass, which was associated with severe pain and change of bowel habit. Nonspecific signs of malignancy were also present, such as weight loss, anemia, and poor appetite. The vague nature of the symptoms led to a delay in diagnosis and treatment. Blood tests and tumor markers provided little diagnostic information.

Investigations with CT, USS, Barium enema, and colonoscopy were not diagnostic; however, they were helpful in excluding other pathologies. Angiography of the omental feeding vessels in omental mesothelioma was described by Marini and Walter.[2] The findings of left and right gastroepiploic artery hypertrophy were a nonspecific feature, which may aid in diagnosis. In our case, there was no ascites and so fluid aspiration cytology was not an option, as is often the case in peritoneal mesothelioma.[1] The most striking feature was the large abdominal mass, making direct biopsy either laparoscopically or percutaneously, the only reliable diagnostic modality in this particular case.

Omental mesothelioma appeared as fibroconnective tissue infiltrated by poorly differentiated carcinoma. Diagnosis was made by immunohistochemistry; the cells of malignant mesotheliomas being positive for calretinin, EMA, and WT1. A study by Ordonez in 2003 evaluated different tissue markers in malignant mesothelioma and concluded that calretinin, cytokeratin, and WT1 are the best positive markers for differentiating mesothelioma from adenocarcinoma.[3] The differential diagnoses of mesothelioma on histology are reactive mesothelial hyperplasia and
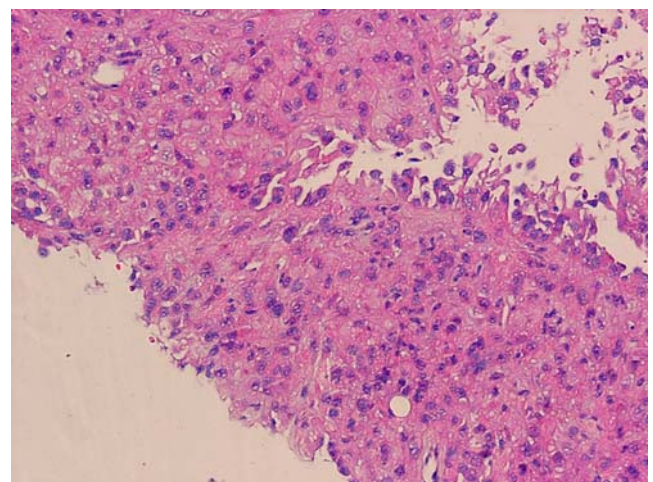
Figure 2 Immunohistochemical staining suggested a mesothelial cell origin.

the presence of metastatic carcinoma, particularly poorly differentiated adenocarcinoma. The main differential diagnoses from the CT appearance are abdominal TB and localized perforation with omental reaction.

Treatment options in abdominal mesothelioma are limited. There have been some encouraging results with a combination of surgical debulking and intraperitoneal or systemic chemotherapy. Intraperitoneal chemotherapy can cause less systemic toxicity while still decreasing the size of the tumor.[4] Survival time despite treatment is variable and seems to be related to the age and premorbid condition of the patient.[1,5]

The prognosis of the malignant mesothelioma in general remains extremely poor. Omental mesothelioma presents added difficulties in diagnosis and treatment is invariably delayed. Our patient died 3 weeks after diagnosis.

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ERRATUM

Erratum to: Obesity and Gastroesophageal Reflux: Quantifying the Association Between Body Mass Index, Esophageal Acid Exposure, and Lower Esophageal Sphincter Status in a Large Series of Patients with Reflux Symptoms

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During conversion of the proof of this article to the final copy, a mistake occurred in the last two rows of Table 3. The correct values of the composite pH score [mean (SD)] are 41.5 ± 48.0 for defective LES and 20.7 ± 25.4 for normal LES.

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