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Epidemiology and Pathogenesis of Diverticular Disease

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Abstract Diverticular disease is a common entity in the USA with an apparent increasing incidence worldwide. The pathogenesis of this disease process is likely multifactorial involving dietary habits, changes in colonic pressures and motility, and colon wall structural changes associated with aging. The following review addresses our current limited knowledge regarding the epidemiology and pathogenesis of diverticulosis and diverticulitis.

Keywords Diverticular disease · Diverticulosis · Diverticulitis · Epidemiology · Pathogenesis

Epidemiology

Diverticular disease has been considered a disease of Western society. Early reports noted its prominence mainly in the USA, Europe, and Australia and its rarity in Asia and Africa.[1] Though the exact assessment of the prevalence of diverticulosis is difficult, early estimates based on autopsy reports and barium studies range from 2–10%.[1] It is well known that disease frequency increases with age. During the 1960s, the prevalence of diverticulosis was estimated at 5% by the age of 40 years and up to 65% at 80 years of age.[2–4] Unfortunately, more recent evaluations are lacking, and it is unknown whether the incidence of diverticulosis remains stable. Interestingly, reports from Singapore and Japan note an increased prevalence of diverticulosis up to 19% and 23% respectively.[5,6] These changes are felt secondary to an increasing utilization of the Western diet.

Though most patients with diverticulosis remain asymptomatic, it is estimated that between 10% and 25% will develop diverticulitis with an average age of 62 years at presentation.[2,7] Early findings suggested that diverticular disease might be more common in men than women, though this finding seems to have reversed over time.[2,7] More recent studies based on admissions for acute diverticulitis now suggest a slight female predominance.[8,9]

Clinical evidence from Europe suggests that the overall incidence of diverticulitis is increasing.[10,11] However, a US comparison study covering a 15-year period revealed an increase of only 0.006% per year.[9] This study reported an absolute prevalence of 60/100,000 admissions for diverticulitis, much more common than the British findings (28/100,000).[11]

Diverticular Anatomy

Diverticula are most notable in the left colon, with up to 99% having some degree of sigmoid involvement.[3] They protrude most commonly in four rows between the antimesenteric and mesenteric taenia.[12] These pulsion diverticula are "false" diverticula where the mucosa and submucosa herniate through the muscularis propria without involving all layers of the bowel wall. These protrusions occur at points of blood vessel (vasa recta) penetration supplying this region. False diverticula also prevail in Asian populations, though a predominance for the right side of the colon is noted,[5,6] suggesting a possible genetic role as well.

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Pathogenesis of Diverticulosis and Diverticulitis

The true pathogenesis of diverticular disease may yet be unknown. However, evidence would suggest that dietary deficiency (fiber), colonic pressure and motility changes, and colonic structural alterations may be collectively responsible for diverticula formation.

After early observations that diverticular disease was nearly absent in developing countries, Painter and Burkitt[1] hypothesized that diverticulosis was due to radical diet changes in the late 1800s. They suggested that with industrialization, a decrease in fiber intake due to the refining of flour and cereals, along with an increased intake of refined sugar, was responsible. This came about with the introduction of roller milling of flour in 1880, removing much of the remaining fiber that led to the increased prevalence of diverticular disease 40 years later. Burkitt et al.[13] reported that the low-fiber diet consumed in the UK resulted in slower stool transit times and lower stool weights when compared to measurements from Africa where extremely high-fiber diets prevail, thus suggesting that fiber intake is beneficial against diverticula formation. Subsequently, comparison studies revealed that diverticular disease was more common in non-vegetarians than vegetarians, also concluding that lower fiber intake was responsible.[14] Finally, a 4-year summary of the Health Professionals Follow-up Study in American men noted that a low-fiber diet was inversely associated with symptomatic diverticular disease (relative risk, 0.58).[15] These and other similar findings would suggest a correlation between lack of fiber and diverticulosis.

It was therefore proposed that a low-residue diet allows for exaggerated contractions of the colonic circular muscle, dividing the lumen "more frequently or more efficiently" into a series of segments or "bladders." This results in raising the intracolonic pressure, generating increased outward force on the colon wall, leading to mucosal herniation.[16,17] Since these original descriptions linking intracolonic pressure to diverticula formation, other more recent demonstrations have confirmed that higher pressures and an exaggerated motility index (product of amplitude and duration of activity) occur in patients with symptomatic diverticular disease.[18,19] Still others seem more skeptic of a hallmark connection between diverticular disease and altered colonic motility.[20] A potential explanation for these conflicting opinions likely relates to the variation between motility measuring techniques. When measurements are obtained from within the true sigmoid colon, however,[18,19] an apparent link does exist between exaggerated colonic motility, increased pressures, and symptomatic diverticular disease. Most recently, 24-h manometric monitoring confirmed that symptomatic patients have abnormal motor and propulsive activities confined to the regions affected with diverticular disease.[21] What seems less clear, however, is whether these findings play a role in pathogenesis or are simply related to diverticular symptoms.

Aside from changes in colonic motility, Arfwidsson et al.[17] noted thickening of both the circular muscle layer and taenia of sigmoid diverticular specimens when compared with normal controls. He suggested that this "muscle hypertrophy" was not due to inflammation or diverticula formation but, rather, secondary to the increased motor activity. He proposed that these structural changes were present before diverticula formation, implying a causative role. Morson[22] also suggested that a primary muscle abnormality might exist and was the most consistent feature of diverticular disease. He noted that this thickening existed even without evidence of inflammation. Whiteway and Morson confirmed by light and electron microscopy the thickening of both the circular muscle and taenia coli in surgical specimens from patients with symptomatic diverticular disease. They noted that muscle cells retain a normal, non-hypertrophic appearance, and rather, the thickened taenias were due to increased elastin deposition, leading to contraction and secondary thickening of the circular layer.[23] They suggested that increased elastin deposition may result from increased colonic pressure, which in turn is due to the inability of low fiber, small caliber stools to distend the colon regularly. It is interesting to note that aging is associated with both increased collagen crosslinking[24] and progressive elastosis.[25] These structural changes may provide future insight into the etiology of diverticular disease and its elevated incidence associated with aging.

The most common complications of diverticular disease are bleeding and diverticulitis. Diverticular bleeding is due to rupture of the adjacent penetrating vasa recta into the diverticular lumen and occurs more commonly on the right side of the colon.[26] As diverticular bleeding is not the focus of this review, we will concentrate instead on diverticulitis. It is presumed that the numerous related factors already described contribute to diverticula formation. Similarly, the etiology of a subsequent inflammatory episode is also speculative. Diverticulitis has been described similarly to that of appendicitis in which a fecalith either lodges within the neck of the diverticulum or abrades the mucosal surface of the sac leading to secondary inflammation. This event then allows for a proliferation of bacteria, diverticulum distension, and localized ischemia. Eventually, perforation of variable extent may result, accounting for a range of symptoms.[27] It is probable that the same increased intracolonic pressure previously described is also responsible for pushing colonic contents into the diverticulum and inciting diverticular irritation.[17] Interestingly, a prior work evaluating the frequency of diverticular perforation noted that a significant percentage of these patients were taking nonsteroidal anti-inflammatory medications (NSAIDS) or opi-

ate analgesics.[28] Since it was previously shown that opiates increase intracolonic pressure,[16] a contributing role is possible. In addition, there is further evidence to suggest that NSAIDs may play a role in diverticular perforation, though the mechanism is unclear.[29]

It is apparent that diverticular disease is a common entity in our society, and world-wide, its incidence is increasing. Though the exact mechanism underlying this disease may yet be unknown, it appears likely that dietary changes, increased colonic pressure or alterations in motility, and structural changes within the colon wall contribute to its pathogenesis.

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The Natural History of Diverticulitis: Implications for Elective Surgery

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While there are some clear indications for emergent/urgent surgery in acute diverticulitis, the indications for elective colectomy following an acute episode of acute diverticulitis are less clear. At present, guidelines are available for the elective operative treatment of diverticulitis and are usually based on age of the patient and the number of recurrences. Many of these guidelines, however, are based on case series data, some of which have relatively small sample sizes. The aim of this paper is to summarize the recent literature regarding when to electively operate on patients who were treated nonoperatively during an acute episode of diverticulitis. More specifically, this paper will address the risk of recurrence of diverticulitis based on patient age and number of recurrences as well as discuss studies that have modeled the ideal time to operate on such patients. This review examines either large studies (population or hospital-system based) or studies using decision analysis techniques evaluating these issues.

According to the American Society of Colon and Rectal Surgery guidelines,[1] elective resection should be considered after one or two well-documented attacks of diverticulitis, depending on severity of the attack and age and medical fitness of the patient. In this regard, there have been two large diverticulitis studies published recently (2005) that have addressed these issues. One study by Broderick-Villa et al.[2] examined 3,165 patients who were initially hospitalized for acute diverticulitis and subsequently followed. Of the total cohort present with acute diverticulitis, 19% underwent emergent colectomy while 81% had nonoperative management. Of the 81% (n=2,551) who were initially observed, 185 underwent elective colectomy. Of the remaining 2,366 patients (i.e., those who did not have emergent surgery at the initial diverticulitis episode and also did not have subsequent elective surgery), 87% did not have a recurrence. Follow-up was for a mean of 9 years (range: 6–12 years). Of the 13% who had a recurrence, 9% had one recurrence, 3% had two recurrences, and 1.1% had three or more recurrences. In a multivariate analysis, age older than 50 years and a higher comorbidity score (as rated by the Charlson index) were associated with a risk of recurrence [Hazard ratios (HR) for <50 years 1.47, p=0.003; HR for Charlson Index 1.59, p<0.001]. Of note, gender and prior percutaneous abscess drainage were not associated with recurrence of diverticulitis (HR gender: 0.98, p=0.89; drainage: 1.11, p=0.84). The conclusions by these authors were that very few patients with acute diverticulitis treated nonoperatively have recurrences, and that this argues against routine elective colectomy following nonoperative management of acute diverticulitis.

In a separate study, Anaya et al.[3] performed a population-based retrospective cohort study using 25,058 patients hospitalized nonelectively for diverticulitis. Of the total cohort, 20% underwent nonelective surgery while 80% (20,136) were treated nonoperatively. Of the 20,136, 81% had no recurrence of diverticulitis and 19% (n=3828) had a recurrence. Of the 3,828 people who had a recurrence of diverticulitis, 692 underwent nonelective surgery, 1,510 underwent elective surgery, and 1,626 were treated nonoperatively. Emergent operations had more colostomies (56% vs. 15%) and higher 30-day mortality (3.1% vs. 1.1%).

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Interestingly, the authors examined clinical characteristics and outcomes based on age (<40 years, 40–50 years, >50 years). There were statistically significant trends for the youngest cohort to have higher recurrent admissions, more than one recurrent admission, and abscess formation. Moreover, the youngest cohort had higher rates of recurrence resulting in emergency colectomy and colostomy.

Although these results might persuade the reader to have a lower threshold for elective operation in the younger cohort, a closer examination of the data reveal that although the trends are statistically significant, the clinical difference may be less so. For example, the percent of <40 year olds who required emergent surgery and/or colostomy was 7.5% while the percent for the >50 year old cohort was 5.0%, for a difference of 2.5%. In point of fact, this would result in a number needed to treat of 13, i.e., 13 patients would need to undergo elective colectomy to prevent one emergent colectomy/colostomy. Anaya's conclusion from these data is that although younger patients are at risk for emergent colectomy and/or colostomy, it is evident that most young patients will not have a recurrence. Moreover, because only 7% will require emergent operation, the authors believe that elective colectomy after the initial hospitalization in a young patient does not appear to be warranted.

It is interesting that these results are consistent with cost-effectiveness/decision analyses that have modeled the optimal time to perform prophylactic surgery in diverticulitis. A study by Richards et al.[4] found that performing elective colectomy after the third attack of diverticulitis is cost saving compared to resection performed for an earlier attack. Similarly, Salem et al.[5] performed a decision analysis and found that colectomy after the fourth episode versus the second episode in patients older than 50 years resulted in 0.5% lower deaths, 0.7% fewer colostomies, and cost savings. In younger patients, performing colectomy after the fourth episode compared with the first episode also resulted in 0.1% fewer deaths, 2% fewer colostomies, and cost savings. While these advantages are admittedly small, the trends for all outcomes were consistent across the sensitivity analyses in both studies—specifically that nonoperative treatment is the dominant (i.e., advantageous) strategy in the modeled analyses.

Limitations

Given these recent studies that appear to support observation rather than operation for diverticulitis recurrence, it is important to note the potential shortcomings of these studies. Their results must be interpreted in the context of the study designs and their limitations. First, both of the patient database studies used inpatient files, and thus, outpatient treatment of diverticulitis was omitted. Second, the clinical knowledge obtained from discharge abstracts in inpatient databases is limited as such databases cannot give a complete clinical picture. Third, these analyses are based on treatment decisions in the respective cohorts, and thus, selection bias is likely to have been present and may be substantial. Fourth, the data and treatments (e.g., laparoscopic surgery, the issue of primary anastomosis without mechanical bowel preparation, etc.) may have differed or changed in the study periods, which collectively included patients from 1987–2003.

In summary, there are recent studies available that challenge the current guidelines for diverticulitis treatment. While such studies will probably not change the guidelines at present, they do provide added information for the provider to share when having a balanced discussion of treatment options, and thus a more informed decision by the patient.

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Imaging and Interventional Techniques in Acute Left-sided Diverticulitis

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Abstract Computed tomography is the most accurate and readily available imaging study in the diagnosis of acute, left-sided diverticulitis. It not only detects the presence of an abscess and guides percutaneous drainage of these abscesses, it may also guide subsequent surgical treatment based upon a modification of the Hinchey classification.

Keywords Acute left-sided diverticulitis · Contrast enema · Computed tomography

Plain Films

Even today, plain films are commonly requested at our institution in patients with acute abdominal pain. The findings are most commonly nonspecific. In my opinion, if imaging is contemplated in a patient presenting with true, acute abdominal pain, then a computed tomography (CT) should be performed.[1–3]

Water-soluble Contrast Studies

Today, contrast studies are almost never requested in patients with suspected acute, left-sided diverticulitis. A water-soluble contrast enema (CE) can be safely used in patients with acute diverticulitis,[4] and the findings are nonspecific. In the proper clinical setting, the findings of diverticula, spasm in the affected segment, fold thickening, asymmetric tethering of the colon wall, intramural sinus tract, mass effect, and both contained and free contrast extravasation can be reasonably diagnostic.[5] Additionally, most patients experience localized tenderness to palpation at the site of disease. CEs are sensitive in the diagnosis, but CT is more sensitive (CE=80% and CT=93% in one series and CE=92% and CT=98% in another).[6,7] Its use is limited, as a CE cannot identify extracolonic disease, especially pericolic and more distant abscesses.[6,7]

Sonography

Sonography can detect signs consistent with diverticulitis including bowel wall thickening and pericolic abscesses.[8,9] However, in the USA, this modality is rarely used to evaluate patients with suspected acute left-sided diverticulitis. For sonography to be accurate, a highly skilled/trained individual must be available 24 h a day for 7 days a week.

Computed Tomography

CT is the most accurate and readily available imaging study used to evaluate a patient with acute abdominal pain, including acute left-sided diverticulitis. CT diagnoses acute diverticulitis and its complications and can also be used for guiding percutaneous abscess drainage.

The CT findings were initially described by Hulnick et al. in 1984[10] and include diverticula, soft tissue stranding of the pericolic fat, colon wall thickening less than 5 mm, and abscess formation. Identifying diverticula is not absolutely

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Table 1 Hinchey Classification[11] and Modified Hinchey Classification (Adapted from Wasvary et al.[12]) of Acute Diverticulitis as It Applies to CT Findings (Modified from Kaiser et al.[13])

Hinchey classification[11]	Modified Hinchey classification (adapted from Wasvary et al.[12])	CT findings
–	0—Mild clinical diverticulitis	Diverticuli±colonic wall thickening
I—Pericolic abscess/phlegmon	Ia—Confined pericolic inflammation/ phlegmon	Colonic wall thickening with pericolic soft tissue changes
	Ib—Pericolic/mesocolic abscess	Ia changes + pericolic/mesocolic abscess
II—Pelvic, intraabdominal or retroperitoneal abscess	II—Pelvic, distant intraabdominal or retroperitoneal abscess	Ia changes+distant abscess (generally deep in the pelvis or in interloop regions)
III—Generalized purulent peritonitis	III—Generalized purulent peritonitis	Free gas associated with localized or generalized ascites and possible peritoneal wall thickening
IV—Generalized fecal peritonitis	IV—Generalized fecal peritonitis	Same findings as III

necessary for a CT diagnosis. Other findings detected by CT include small bowel obstruction, free intraperitoneal or extraperitoneal gas, colovesical fistula, thrombus or thrombosis of the mesenteric/portal veins caused by pyophlebitis, and hepatic abscess formation. In some instances, small, localized collections of gas are identified adjacent to the colonic wall and indicate localized, pericolic perforation.

CT findings relevant to clinical management can be classified into 0, Ia, Ib, and II using the modified Hinchey classification (Table 1).[7,11–13] In grade 0, the colonic wall on CT may be normal or thickened, but there are no pericolic fat soft tissue changes. Grades Ia, Ib, and II define a spectrum of findings starting with pericolic fat soft tissue changes and progressing to pericolic abscess and then peritoneal abscess (Figs. 1, 2, 3, and 4). CT cannot distinguish generalized purulent peritionitis (grade III) from fecal peritonitis (grade IV). In these unusual cases, there may be free intraperitoneal fluid and gas and/or extravasated oral or rectal contrast.

Published reports of the overall sensitivity and specificity of CT show a surprising range of 69–95% and 75–100%, respectively.[6,7,14–17] Only the recent, prospective, consecutive studies to determine the overall sensitivity and specificity in the diagnosis of acute left-sided diverticulitis have shown surprisingly high sensitivity and specificity of 90+%. It must be noted that even in these prospective series, not all patients with suspected diverticulitis were scanned. Thus, selection bias may artificially inflate the numbers.

The main problem with the sensitivity and specificity of CT is in distinguishing diverticulosis with muscular hypertrophy from mild acute diverticulitis (no acute inflammation vs. grade 0 diverticulitis).[15,18] With mild inflammation, pericolic fat changes can be absent. Further, asymptomatic patients who have had prior acute divertic-

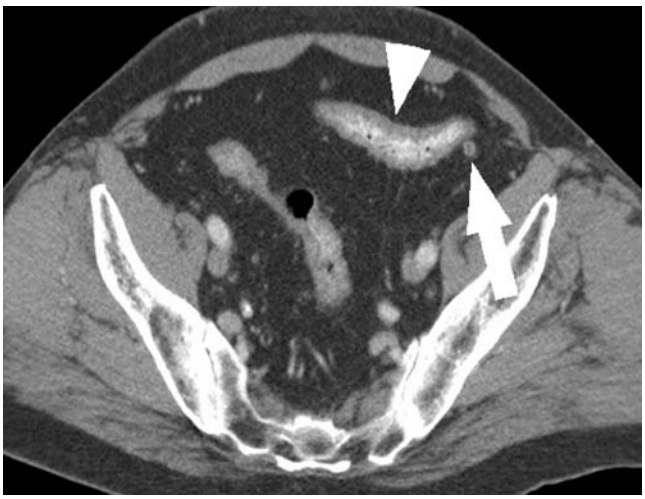

Figure 1 Grade 0, acute sigmoid diverticulitis—axial CT scan through the pelvis after oral, intravenous, and rectal contrast administration shows a thickened colonic wall (*arrowhead*) and a single diverticulum (*arrow*). There are no soft tissue changes in the pericolic fat. This could be normal.

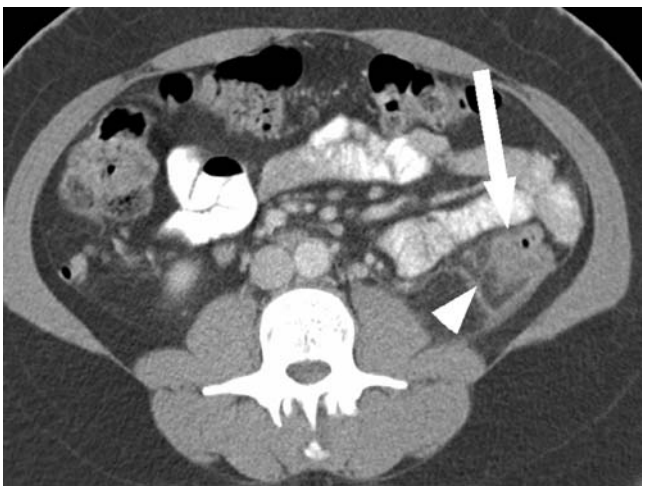

Figure 2 Grade Ia, acute descending colonic diverticulitis—axial CT scan through the lower abdomen after oral and intravenous contrast administration shows a thickened colonic wall (*arrow*) and soft tissue changes in the pericolic fat (*arrowhead*).

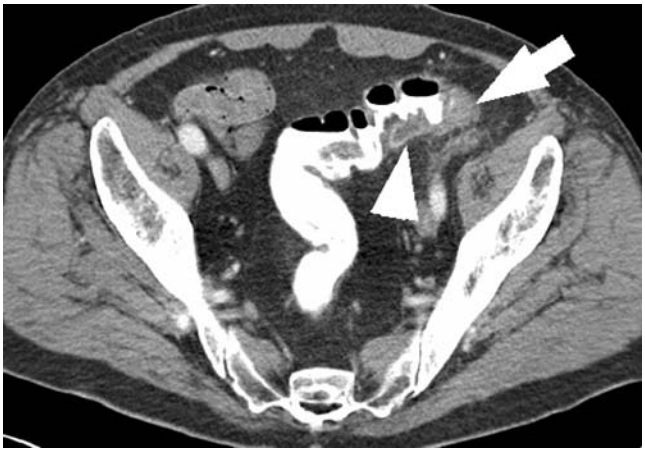

Figure 3 Grade Ib, acute sigmoid diverticulitis- axial CT scan through the pelvis after oral, intravenous and rectal contrast administration shows a thickened colonic wall (arrow) as well as a small pericolic/mesocolic abscess (arrowhead). There are also soft tissue changes in the pericolic fat, posterior to the sigmoid colon.

ulitis may have pericolic fat stranding and thickening of the root of the sigmoid mesentery, findings that mimic acute inflammation.

Differential Diagnosis in Left-sided Diverticulitis

The most important differential diagnosis is a perforated colon carcinoma. Perforated colon carcinomas generally produce larger, bulky, more asymmetrically or eccentrically placed masses when compared to acute diverticulitis.[19] The length of bowel involved is in general greater in acute diverticulitis. In my experience, in the proper clinical setting, a segment of thick-walled sigmoid colon, greater than 5 cm in length, is generally from acute diverticulitis rather than from carcinoma. Unfortunately, there is a considerable overlap between the two diseases.[20–22] Pericolic soft tissue stranding, pericolic vascular engorgement, and fluid at the root of the sigmoid mesentery favor acute diverticulitis.[20,22] Pericolic lymph nodes favor perforated colon carcinoma.[21,22] Regardless, every patient with suspected acute left-sided diverticulitis needs a mucosal study after the acute inflammation has been resolved.

Atypical radiographic findings in acute diverticulitis often are seen in immunocompromised patients.[23,24] In these cases, when an acute inflammatory response cannot occur, the presenting CT findings may only be intra- or extraperitoneal gas, without pericolic changes and abscess formation.

Another important differential consideration is acute epiploic appendicitis.[25] Patients with this condition present with signs and symptoms mimicking acute left-sided diverticulitis. The CT findings of the fat density epiploic appendix surrounded by soft tissue stranding are absolutely diagnostic and should not be confused with acute left-sided diverticulitis. As management of this condition is conservative, the specific diagnosis by CT is essential.

Other differential considerations include focal colitis from ischemia, infection (especially *C. difficile*) and inflammatory bowel disease, acute appendicitis with a deep pelvic abscess, pelvic inflammatory disease, and endometriosis. The findings under these conditions are more often distinct from diverticulitis,[26] but sometimes, it is impossible to make a specific diagnosis.

Intervention in Diverticular Abscess

When a diverticular abscess is detected, CT-guided percutaneous abscess drainage is often requested. In general, this can be performed safely and easily to remove the localized sepsis effectively.[27] Diverticular abscesses less than 3 cm in diameter cannot be successfully drained with a catheter, as the diameter of the pigtail of most drainage catheters is approximately 3 cm. Further, an abscess may be inaccessible to a catheter due the bony pelvis, urinary bladder, uterus, and surrounding bowel. In many instances, a transsciatic/transgluteal (Fig. 4) or other flexible and innovative approaches are used safely and effectively.[28–30] Unfortunately, patients are often uncomfortable with a transgluteal catheter.

It is important to note that if a catheter is placed, intravenous antibiotics remain essential in treatment. Before a drainage catheter is removed, a potential bowel connection must be excluded. Once catheter output has stopped, the catheter must be injected with iodinated contrast media, under fluoroscopic observation. No catheter should be removed if a communication to the colon exists.

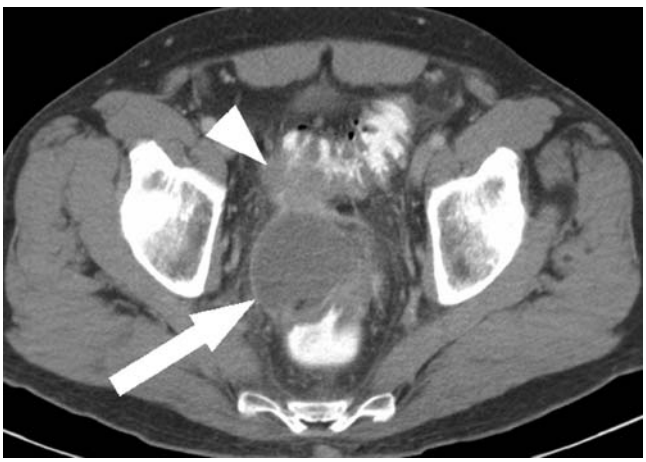

Figure 4 Grade II, acute sigmoid diverticulitis- axial CT scan through the pelvis after oral, intravenous and rectal contrast administration shows a thickened colonic wall (arrowhead) as well as a deep pelvic abscess (arrow). This abscess is best drained via a posterior, transsciatic/transgluteal approach.

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Acute Diverticulitis of the Left Colon: Value of the Initial CT and Timing of Elective Colectomy

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Abstract Computed Tomography is undeniably the most useful tool to confirm the suspected diagnosis of acute left-colonic diverticulitis and to objectively grade its severity into moderate diverticulitis (no signs of colonic perforation) and severe diverticulitis (signs of colonic perforation). Indeed, the severity of acute diverticulitis is statistically predictive of the risk both to need surgical treatment of the first episode of acute diverticulitis, and to follow a complicated evolution after successful conservative treatment of the acute phase. Consequently, CT brings a major contribution to define the place of surgery during the acute phase of diverticulitis, and, later on, inside the long-term evolution of the disease after initial successful conservative treatment.

Keywords Acute diverticulitis · CT · Elective colectomy

In 1990, in the early phase of our prospective experience with acute sigmoid diverticulitis, our first publication was entitled "Vol de nuit" (Night flight).[1] This reflected the chaotic state of knowledge in the management of acute diverticulitis.. We present in this paper the results of a prospective study examining treatment of acute diverticulitis, where many of the questions regarding the treatment of acute diverticulitis are answered. Between October1986 and 1997, we enrolled 542 patients in a prospective study at the University Hospital of Geneva. These patients (290 women and 252 men with a mean age of 64 years) were all admitted to our emergency center with a history and clinical findings suggestive of acute sigmoid diverticulitis. Apart from the patients requiring immediate surgery, all other patients underwent an abdominopelvic computed tomography (CT) and a water-soluble contrast enema (CE) within 72 h of admission. Radiological images were interpreted by radiologists blinded to clinical information. Diverticulitis was classified moderate or severe according to the following radiographic criteria: (1) CT scan—moderate diverticulitis was defined as a localized thickening of the colonic wall of 5 mm or more and signs of inflammation of the pericolic fat and severe diverticulitis was defined as colonic thickening with abscess and/or extraluminal air and/or extraluminal contrast; (2) CE—moderate diverticulitis was defined as a segmental lumen narrowing, a tethered mucosa with or without a mass effect and severe diverticulitis was defined when extravasation of contrast and/or the presence of extraluminal air were added to the former signs. Patients were included in the study when one or both radiological examinations were positive for diverticulitis or when it was histologically proven. After emergency evaluation, all patients were hospitalized in our surgical clinic. Conservative treatment consisted of 10 days of intravenous antibiotics, active against Gram-negative and anaerobic bacteria. The decision for conservative management or surgical intervention was determined by the surgical team.

This study is unique in four ways. First, it represents a selected group of patients with moderate to severe diverticulitis requiring hospitalization. Secondly, because Geneva has only one university hospital being in charge of about 600,000 people, these patients could not be hospitalized in another center, reducing the risk of selection bias. Thirdly, each patient admitted to our emergency unit with abdominal pain is always referred to one of our

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digestive surgeons. When acute diverticulitis is suspected, even if at first no surgical treatment is considered and the patient is sufficiently ill to warrant hospitalization, she or he will be directly admitted in our surgical unit. Finally, the diagnosis was confirmed in all patients with CT or CE, reducing the probability of false-positive rate, which occurs when the diagnosis is made on clinical grounds alone (50% of cases).[2,3]

We examined the role of CT to define the indications for elective colectomy after successful conservative management of a first episode of acute diverticulitis.

The Value of CT

In our prospective study based on 423 patients, of whom 355 had a CT, the value of initial CT in terms of diagnostic sensitivity, probability of failure of conservative treatment during the first hospitalization, and risk of complications after successful conservative treatment of the first acute episode was measured. Of the 132 patients who had a CT and in whom diverticulitis was proven at operation, the CT findings were truly positive in 123 cases, falsely negative in 4 cases, and falsely positive in 5, giving a sensitivity of 97%. Specificity could not be calculated because true negative cases were excluded by the criteria of inclusion. Of the 79 patients who were first treated with conservative treatment and later needed surgical treatment for clinical deterioration (failed medical treatment), 42 had a CT. Of these 42 patients, 32 (76%) had severe diverticulitis by CT scan, compared with 74 (24%) of 303 who had successful medical treatment ($p<0.0001$). Of the 106 patients with CT-severe diverticulitis, 32 (30%) required early operation compared with only 10 (4%) of 239 patients with CT-moderate diverticulitis ($p<0.0001$). Finally initial CT was found statistically predictive of high risk of secondary complications after initial successful medical treatment of acute diverticulitis [with a median follow-up of 46 months, CT-severe diverticulitis was present in 28 (47%) of 60 patients with secondary complications and in 44 (19%) of the 236 who had no complications ($p<0.0001$)].[4] This latter prognostic value was confirmed on long-term follow-up (9.5 years) of 118 patients where we found that the incidence of remote complications was the highest (54% at 5 years) for young patients with CT-severe diverticulitis and the lowest (19% at 5 years) for older patients with CT-moderate diverticulitis. In a univariate analysis, CT-severity and age were both significant, while when stratified for severity of CT, age was no longer significant.[5] We examined the CE to determine if it added to the CT scan in the management of these patients.. This is now dated because recent studies have clearly demonstrated the superiority of CT. It was reported in 2000 the clear superiority of CT compared to water-soluble CE in terms of sensitivity (98 vs 92%; $p<0.01$) and in diagnosing severe inflammation (26 vs 9%; $p<0.02$).[6]

Our experience on the crucial roles of CT in the evaluation of acute diverticulitis have been reported recently.[7]

The Place of Elective Colectomy

The timing of elective sigmoid resection and the risks of the medical management of the disease is still subject to debate.[8,9] The discussion is further complicated by the introduction of laparoscopic colectomy, with excellent immediate results in terms of morbidity, mortality, and cosmetic results.[10]

Many authorities agree that the indications for elective surgery include (1) patients with two or more previous acute attacks treated conservatively; (2) patients with one attack that is associated either with a contained perforation, or colonic obstruction, or with a fistula; (3) patients with a suspicion of colonic cancer that cannot be excluded by other means; and (4) immunocompromised patients, who should have surgery after the first episode. Surgery for younger patients after a first episode of acute diverticulitis is still a controversial topic.[11–13] These recommendations were based on the 40-year-old retrospective Parks'[14] study finding that chances of successful conservative treatment of acute diverticulitis were decreasing with recurrences. We used our prospective database to review these recommendations. Others have reported modern experiences with this disease.

Guzzo et al.,[15] retrospectively studied a group of 196 patients aged 50 years or younger successfully treated conservatively for a sigmoid diverticulitis and found after a median follow-up of 5.2 years that only 1 patient (0.5%) presented at a later date with perforation. Large recent multicentric retrospective studies studying the outcome of patients whose first episode of acute diverticulitis was conservatively treated confirmed that the risk of recurrent attacks was low.[16,17] Studies using statistical models proposed elective colectomy after the third[18] or the fourth attack of acute diverticulitis.[19]

We reported results from l18 patients with long-term follow-up after a first acute episode of sigmoid diverticulitis treated successfully conservatively and found after, a median follow-up of 9.5 years, that no patient died from complications of the disease and that no patient subsequently required emergency surgical treatment.[5] In contrast, in 73 patients who presented with severe diverticulitis and mesocolic (45) or pelvic (28) abscesses associated with acute diverticulitis, 23 (51%) patients with a mesocolic abscess and 20 (71%) of the 28 patients with a pelvic abscess needed surgical treatment either during their first hospitalization or later, after a median of 43 months follow-up. We concluded that sigmoid colectomy after percutaneous drainage was justi-

fied for pelvic abscess, while mesocolic abscess by itself was not an absolute indication for colectomy.[20]

Finally, I would like to stress the importance of accurately diagnosing acute diverticulitis with the use of CT during the acute phase. Most series reporting functional results of patients after elective colectomy are either retrospective and/or do not include CT evidence of diverticulitis. Consequently, the cohort of studied patients is largely altered by false-positive cases that weigh considerably on the interpretation of the results.

Only a few studies, mostly retrospective, centered their interest on functional postoperative results, and none of them had CT-proven diverticulitis.[21–24] These studies found that 7% to 27% of patients followed up between 11 and 48 months were still complaining of abdominal symptoms after colectomy. Authors attributed these persistent symptoms to irritable bowel syndrome,[21,22] insufficient length of resected colon[22] or inappropriate indications to surgery.[23]

Recently, we analyzed by questionnaire the functional results of 43 consecutive patients who had a laparoscopic sigmoidectomy from 3 to 76 months before (mean, 40 months) or after CT-proven diagnosis of acute diverticulitis (unpublished data). The surgical technique consisted of a total removal of the sigmoid colon, with take-down of the splenic flexure and an anastomosis in the rectum distally to the reunion of the taenias. The results of the questionnaire showed that four patients (9%) complained of new abdominal pain. Bowel function was reported as better for 24 patients (56%), unchanged for 16 patients (37%), and worse for 3 (7%). Forty-one patients (95%) said that they would recommend the surgery. Functional results after laparoscopic sigmoidectomy could probably be improved by rigorous surgical technique removing all the sigmoid colon and by better selection of patients based on CT-proven diverticulitis.

Elective sigmoidectomy is mandatory for patients with complicated diverticulitis (stenosis and fistula) and reasonable for patients with symptomatic recurrent diverticulitis not responding to conservative means. For patients with uncomplicated diverticulitis, responsive to medical management, recommendations for sigmoidectomy should be based on age and number of acute attacks.

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Operative Treatment of Recurrent or Complicated Diverticulitis

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Abstract Sigmoid diverticulosis remains a common disease in developed Western countries, and surgeons are frequently asked to manage diverticulitis and its complications. When to offer elective surgery to patients with uncomplicated, but recurrent, diverticulitis should be individualized, and practice recommendations by national societies continues to be debated. Complicated diverticulitis remains a surgically treated disease, and new technology such as colonic stents (for obstruction) and computed-tomography-guided percutaneous drainage (for abscess) have become bridging techniques to avoid two-stage operations in selected patients. Minimally invasive surgery for elective sigmoid resection has been shown to be safe and feasible and confers many patient-related short-term over traditional open surgery.

Keywords Recurrent and complicated diverticulitis · Open and minimally invasive approach

In developed Western countries, sigmoid diverticulosis is a common disease. Of the patients with sigmoid diverticulosis, 10% to 25% will experience diverticulitis and its complications.[1–4] Acute diverticulitis results when one or more quiescent diverticulum becomes obstructed, infected, and inflamed. If this inflammatory process progresses, complications such as abscess, phlegmon, fistula, obstruction, or free perforation can occur and is associated with significant morbidity and mortality.[5–7]

Surgical intervention is usually recommended for recurrent, uncomplicated diverticulitis and when the complications stated above occur. The ultimate goal of the surgeon is to remove the disease, prevent its recurrence, and restore bowel continuity when possible.

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Uncomplicated Diverticulitis

Whether or not to offer patients surgery in the setting of uncomplicated, but recurrent, diverticulitis depends on several factors. The age of the patient, the number of attacks, the frequency and severity of attacks, and patient-related risk factors (i.e., transplant patient) should be taken into consideration. Practice parameters and historic guidelines related to risk stratification continue to be debated.[8]

Complicated Diverticulitis

Abscess, Phlegmon, or Free Perforation

A phlegmon is a pericolonic mass secondary to a confined perforation and localized inflammatory process. If this inflammatory process progresses, formation of an intra-abdominal or pericolonic abscess may occur. Hinchey et al.[9] devised a grading system for the degree of perforation in this patient population: Stage I, diverticulitis associated with abscess; Stage II, diverticulitis associated with retroperitoneal or pelvic (distant) abscess; Stage III, diverticulitis associated with purulent peritonitis; Stage IV, diverticulitis associated with fecal peritonitis.

Computerized tomography (CT) and CT-guided percutaneous drainage has revolutionized the way we approach

perforated patients with an intra-abdominal abscess.[10,11] In the past, when patients developed an abscess, an emergent operation was often necessary to control sepsis. Currently, with antibiotics and CT-guided abscess drainage, sepsis can be successfully controlled without immediate surgery, and patients can be bridged for an elective one-stage operation. This has many advantages from a quality of life, cost, and morbidity/mortality standpoint. It is reported that up to 30% of patients with stomas created emergently, never have them reversed, and if they do, reversal may be associated with significant complications including death.[12–14]

Fistulizing Diverticulitis

A variety of diverticulitis associated fistulas can occur, including colovesical, colovaginal, coloenteric, colocutaneous, and colouterine, with the most common being colovesical.[15] One must always consider malignancy in these cases and diagnostic evaluation should be done. Most patients can be managed with a single-stage approach, which includes sigmoid resection, colorectostomy, and repair of the adjacent organ. Placement of the omentum between the effected organ and the new anastomosis may help prevent recurrent fistula.

Obstruction

Chronic inflammation from recurrent diverticulitis can lead to stricture formation and obstruction. Patients can present acutely, requiring emergent operation, or with chronic symptoms. The possibility of an occult malignancy should be considered in these cases. Resection and primary anastomosis may be difficult due to size discrepancy and inability to perform a bowel preparation. At our institution, we have had success using intraluminal colonic stents to temporarily relieve obstructed patients. This avoids an emergency, high-risk, two-stage operation and bridges them to an elective one-stage elective operation.

Choice of Operation and Technical Considerations

Many factors need to be considered when deciding on which operative approach to utilize. In the emergent setting of obstruction or perforation, the patients overall clinical status, degree of contamination, tissue quality, and possibility of malignancy guides the operative choice. For patients with a mass or phlegmon, ureteral stents may be useful for identification and protection of the ureters. Preservation of the superior hemorrhoidal artery improves blood supply to the anastomosis. If malignancy cannot be ruled out, en-bloc resection should be done. In patients with obstruction, some authors have advocated intraoperative colonic lavage as a useful adjunct to allow resection and primary anastomosis.[16]

In terms of how much bowel to resect, the entire sigmoid colon should be removed. Proximally, the descending colon can be taken at a level, not based on the presence or absence of diverticula but on the tissue quality.[17] The distal margin of resection should be at the level of the rectum identified by the coalescence of the tenia. This is a very important technical point because when distal sigmoid is left in place, recurrence rates double.[18] To assure a tension-free anastomosis, it may be necessary to mobilize the splenic flexure and, in rare cases, high ligation of the inferior mesenteric vein. A variety of anastomotic techniques have been described including hand-sewn or stapled, end-to-side, side-to-side, side-to-end, and end-to-end. Size discrepancy may play a role in this decision.

Typically, patients who present with uncomplicated diverticulitis, Hinchey Stage I, and selected patients with Hinchey Stage II, are offered a one-stage operation consisting of open or laparoscopic sigmoid resection and descending colorectostomy. For patients with Hinchey Stage III or IV, a two-stage open approach is often done, as tissue quality and overall poor condition of patients do not allow for safe primary anastomosis. A Hartmann procedure, with takedown and reestablishment of intestinal continuity 3–6 months later is done. Some groups have reported a one-stage approach for Hinchey III and IV patients, and in highly selected circumstances, this approach has been shown to be successful.[19] When adjacent pelvic structures are compromised by severe pelvic sepsis, acquiring the assistance of a gynecologic or urologic surgeon may be helpful.

Laparoscopic vs Open Approach

Minimally invasive approaches to diverticular disease have been reported and are now widely practiced.[20] Hand-assisted laparoscopic colectomy, laparoscopic-assisted colectomy, and pure laparoscopic colectomy have all been described. Most reports are on patients with recurrent uncomplicated diverticulitis, but the minimally invasive approach for patients with complicated diverticulitis, especially fistulizing disease, has been reported with good success.[21,22] Most studies demonstrate feasibility, safety, and advantages expected for minimally invasive surgery.[23–25] Most authors agree that, for the unstable patient with diffuse fecal peritonitis, an open approach is the most prudent.

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Evaluation of Surgical Outcomes and Gallbladder Characteristics in Patients with Biliary Dyskinesia

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Abstract
Introduction This study was designed to compare symptomatic outcomes following cholecystectomy in patients with biliary dyskinesia.
Materials and Methods From 1999 to 2006 at New York University Medical Center, 197 adults underwent hepatobiliary scintigraphy with cholecystokinin administration to evaluate gallbladder ejection fraction (GBEF). Biliary dyskinesia was demonstrated in 120 patients based on decreased GBEF of ≤35%. Forty-four patients underwent cholecystectomy, and data from chart review and telephone questionnaires were available for 42 patients. Patients reported symptomatic improvement whether gallstones were present (25/27, 92.6%) or absent (13/15, 86.7%) prior to cholecystectomy (p=0.90). The most common pathologic findings were chronic cholecystitis and cholesterolosis, regardless of the presence of gallstones. Additional data from 101 of the 120 patients with decreased GBEF demonstrated 74/101 (73.2%) patients were diagnosed with gastroesophageal reflux disease (GERD), and 59/101 (58.4%) patients were diagnosed with gastritis.
Results The results of this study suggest that biliary dyskinesia should be considered as part of the spectrum of symptomatic gallbladder disease that can be successfully treated with cholecystectomy and that biliary dyskinesia is associated with GERD and gastritis.

Keywords Gallbladder · Dyskinesia · Cholecystectomy · Gastroesophageal · GERD · GBEF · Chronic cholecystitis · Gastritis · Biliary

Introduction

Biliary dyskinesia is defined as abdominal symptoms attributable to the biliary tract in association with abnormal gallbladder function and the absence of gallstones. While surgery has been shown to benefit patients with this

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disorder, it is more difficult to predict which patients will improve symptomatically after surgery without the use of objective testing.[1,2] Hepatobiliary scintigraphy with cholecystokinin (CCK) provocation, more commonly referred to as the CCK-Tc-HIDA scan, has been developed to objectively test gallbladder function by quantifying gallbladder ejection fraction (GBEF). First described by Krishnamurthy in 1981,[3] the method of CCK administration has evolved along with the definition of abnormal GBEF. Regardless of this, the CCK-Tc-HIDA scan is currently used to objectively define patients with biliary dyskinesia who will benefit from cholecystectomy.

Using GBEF measured by CCK-Tc-HIDA, several groups report that patients with biliary dyskinesia improve after cholecystectomy.[2,4–12] However, other investigators do not support this conclusion.[13–15] Furthermore, despite the fact that bile stasis can lead to the formation of gallstones in a dysfunctional gallbladder,[16–19] biliary dyskinesia is often not considered as one of several processes that may contribute to gallstone formation. In patients with impaired gallbladder function and gallstones, it is not apparent which process is responsible for symptoms.

The purpose of this study was to evaluate the role of cholecystectomy in symptomatic patients with impaired gallbladder function whether or not gallstones were present and to evaluate biliary dyskinesia as part of a spectrum of gallbladder disease that includes gallstone formation. We retrospectively reviewed medical records of symptomatic patients with decreased GBEF, defined as ≤35% using CCK-Tc-HIDA scan, and compared the effect of cholecystectomy on patients with biliary dyskinesia and on patients with decreased GBEF and gallstones. We hypothesized that surgical outcomes would be similar between these two groups.

Materials and Methods

From 1999 to 2006 at the New York University Medical Center (NYUMC), 197 adult patients underwent hepatobiliary scintigraphy with cholecystokinin provocation to evaluate gallbladder ejection fraction. Of these 197 patients, 120 displayed decreased GBEF in association with abdominal discomfort. The cutoff for decreased GBEF was defined as 35%, consistent with what was initially defined by Krishnamurthy et al.[20] using a 3-min CCK infusion technique. Of the 120 patients with decreased GBEF, 44 subsequently underwent cholecystectomy, and these included 16 patients with gallstones identified prior to evaluation of GBEF. Chart review and unblinded, unscripted telephone interviews were conducted by the first author on these 44 patients to determine the nature and outcome of symptoms before and after cholecystectomy. This study was approved by the NYU Institutional Review Board (IRB), and data acquisition and storage was compliant with the IRB's guidelines.

We sought to determine the effect of cholecystectomy on patient symptoms. Improvement was defined as absence or lessening, as assessed by the patient, of one or more symptoms involved within 6 months of the operation. Surgical outcomes of these patients were first evaluated based on the presence of preoperatively diagnosed gallstones. Preoperative studies used to evaluate abdominal pain included transabdominal ultrasound (U/S), endoscopic ultrasound, computed tomography (CT) scan, magnetic resonance cholangiopancreatography (MRCP), and endoscopic retrograde cholangiopancreatography. Patients were distributed to either Group 1, patients with no gallstones noted before surgery, or Group 2, patients with gallstones detected before surgery. Outcomes of these two groups were compared.

Surgical outcomes were further evaluated in the same 44 patients based on the presence of gallstones noted at any point in their course, including postoperatively once the gallbladder could be opened and grossly evaluated. Patients who did not demonstrate gallstones before and/or after cholecystectomy were defined as Group A, while patients with gallstones found at some point during treatment were defined as Group B. Outcomes of these two groups were compared.

Pathologic findings of these 44 patients treated with cholecystectomy were studied. The most common findings were evaluated and compared between Groups A and B.

Finally, we sought to determine if there were any associated factors common among patients with decreased GBEF. Chart review of all 120 patients with decreased GBEF was conducted to determine any patient similarities, regardless of treatment eventually pursued. Shared characteristics were further evaluated as appropriate.

Statistical analysis was conducted using the student t-test or using the Fisher exact test with a two-tail p-value.

Results

Patients, Group Distributions, And Symptoms

Of the 120 patients with decreased GBEF, 43 were men, and 77 were women (ratio=1:1.8). Group 1, or patients with no gallstones detected before surgery, included 28 of 44 patients; eight were men, and 20 were women (ratio=1:2.5). These patients did not exhibit gallstones, and they were given a preoperative diagnosis of biliary dyskinesia. Group 2, or patients with gallstones detected before surgery, included 16 of the 44 patients treated with cholecystectomy; six were men, and ten were women (ratio=1:1.7; Fig. 1). These patients did not fit the current definition of biliary

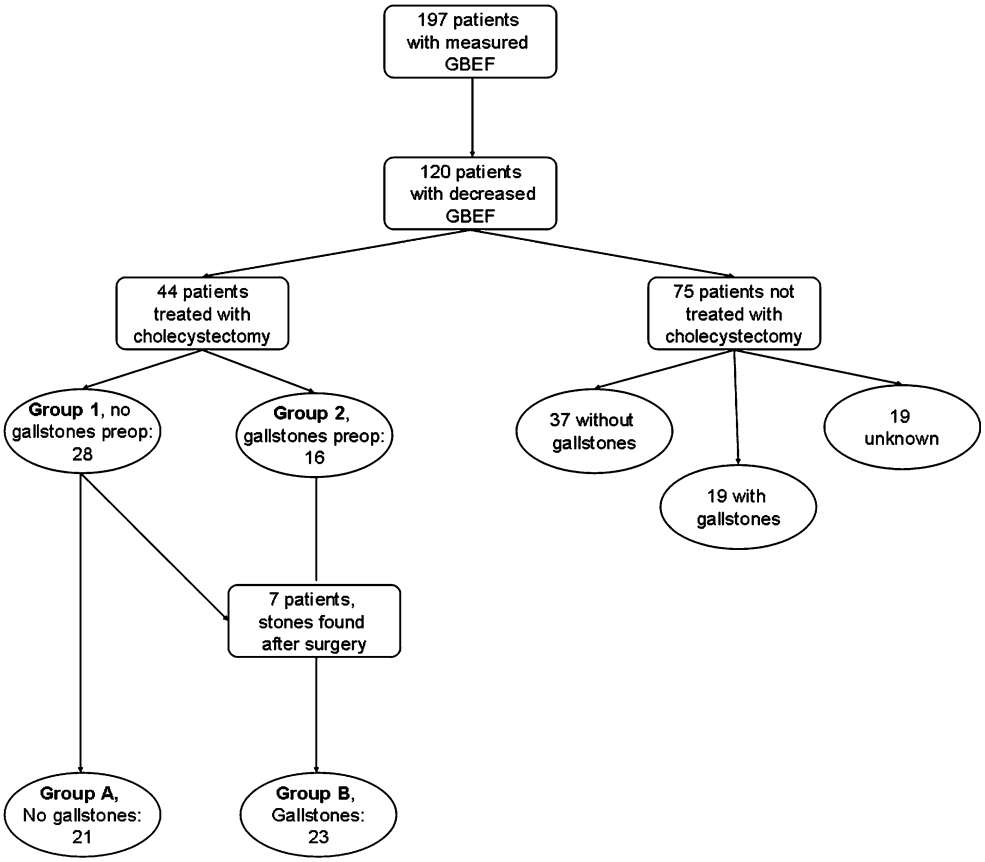

Figure 1 Flow chart demonstrating classification of patients undergoing hepatobiliary scintigraphy with cholecystokinin administration. Of 197 patients who underwent CCK-hepatobiliary scintigraphy at NYUMC between 1999 and 2006, 120 displayed decreased GBEF. Forty-four of those patients underwent cholecystectomy. The cholecystectomy group was subdivided into two groups depending on whether gallstones were diagnosed preoperatively (*Group 1* = patients with no stones detected before surgery, *Group 2* = patients with stones detected before surgery). The cholecystectomy group was further subdivided into two groups to include those patients who had gallstones detected after gallbladder specimen evaluation (*Group A* = patients with no gallstones found at any point of treatment, *Group B* = patients with gallstones found at some point in treatment).

dyskinesia because they demonstrated gallstones. There was no difference between the two groups when comparing the average GBEF (Group 1 mean=16.5%, range 0–33%; Group 2 mean=20.2%, range 8.4–32%; p=0.58).

After the gallbladder specimen was examined, seven patients in Group 1 were found to have gallstones and were assigned to Group B (patients with gallstones detected at some point in treatment). These seven patients no longer fit the current definition of biliary dyskinesia. Group A, or patients with no gallstones detected at any point in treatment including after gallbladder specimen evaluation, included 21 of 44 patients; five were men, and 16 were women (ratio= 1:3.2). Group B, or patients with gallstones detected at some point in treatment including upon gallbladder specimen evaluation, included 23 of 44 patients; nine were men, and 14 were women (1:1.6; Fig. 1). There was no difference between the two groups when comparing the average GBEF (Group A mean=18.7%, range=4.4–33%; Group B mean=16, range=0–32%; p=0.67).

The most common symptoms experienced by these patients included right upper quadrant pain or discomfort, epigastric pain or discomfort, back pain or discomfort, bloating, nausea, and vomiting. These symptoms may or may not have occurred postprandially. Food intolerance was also common. There were no differences between any groups based on presenting symptoms.

Outcomes of Cholecystectomy in Groups 1 and 2

In Group 1 (Table 1), 27 of 28 patients' information were available for postoperative follow-up. Of these 27 patients, 25 experienced improvement after surgery (92.6%). In Group 2, 15 of 16 patients were available for follow-up. Of these 15 patients, 13 experienced improvement after surgery (86.7%). When comparing surgical outcomes of Group 1 and Group 2, there was no difference between those patients with impaired gallbladder function whether or not gallstones were demonstrated preoperatively (p=0.90).

Table 1 Outcomes After Cholecystectomy in Symptomatic Patients with Decreased GBEF

	Number of patients improved	Number of patients not improved
Group 1, no preop gallstones (n=27)	25/27 (92.6%)	2/27 (7.4%)
Group 2, preop gallstones (n=15)	13/15 (86.7%)	2/15 (13.3%)
Group A, no gallstones at any point in treatment (n=21)	20/21 (95.2%)	1/21 (4.8%)
Group B, gallstones found at some point in treatment (n=21)	18/21 (85.7%)	3/21 (14.3%)
Overall (n=42)	38/42 (90.5%)	4/42 (9.5%)

n Number of patients with complete follow-up

Table 2 Pathologic Findings of Gallbladders After Removal from Symptomatic Patients with Decreased Gallbladder Ejection Fraction

Finding	Number of patients with no gallstones at any point in treatment (Group A)	Percent*	Number of patients with gallstones at some point in treatment (Group B)	Percent*	Total number of patients	Percent of Total*
Chronic cholecystitis	12	60	18	81.8	30	71.4
Cholesterolosis	3	15	3	13.6	6	14.2
Normal	4	20	0	0	4	9.5
Cystic lesion	2	10	1	4.5	3	7.1
Acute cholecystitis	0	0	2	9.0	2	4.7
Pseudopyloric metaplasia	1	5	1	4.5	2	4.7
Trabeculation	1	5	1	4.5	2	4.7
Chronic cholelithiasis	1	5	1	4.5	2	4.7
Mucosal hyperplasia	2	10	0	0	2	4.7
Mucosal elevation	0	0	1	4.5	1	2.3
Adenomyomatosis	1	5	0	0	1	2.3
Rokitansky-Aschoff Sinus	1	5	0	0	1	2.3

* Percent of patients with complete reports

Outcomes of Cholecystectomy in Groups A and B

Seven of 28 patients in Group 1 demonstrated gallstones on specimen evaluation after cholecystectomy. Tests that were falsely negative in an attempt to detect gallstones in these seven patients included U/S in 6/7, MRCP in 3/7, and CT scan in 4/7, and more than one test may have been performed per patient. These seven patients were subtracted from Group 1, and the remaining 21 patients with no gallstones detected at any point in treatment and, thus, truly fitting the definition of biliary dyskinesia were defined as Group A. All 21 of these patients were available for follow-up. Of 21 patients, 20 improved with surgery (95.2%), and 1/21 patients did not (4.8%).

The seven patients with gallstones found on gallbladder specimen evaluation were added to the 16 patients within Group 2, and these 23 patients were collectively called Group B. Of these 23 patients, 21 were available for follow-up. 18/21 patients improved after cholecystectomy (85.7%) while 3/21 patients did not (14.3%). There was no difference in surgical outcome between Groups A and B ($p=0.60$).

Overall Outcome in Patients with Decreased GBEF, Regardless of Gallstones

Overall, 38 of the 42 symptomatic patients with decreased GBEF who were available for follow-up (90.5%) improved after surgery while four of 42 patients (9.5%) did not, regardless of the presence of gallstones (Table 1).

Pathology

Pathology reports were available for review in 42 of the 44 patients with decreased GBEF who underwent cholecystectomy. The most common abnormalities in these 42 patients included chronic cholecystitis ($n=30$, 71.4%) and cholesterolosis ($n=6$, 14.2%; Table 2). Four of 42 patients had gallbladders with no pathologic abnormalities (9.5%), and all four of these patients experienced symptomatic improvement (100%). None of these four patients demonstrated gallstones at any point in treatment (Group A).

When comparing Groups A and B (Table 2), pathology reports were available for 20 of 21 patients in Group A who did not have gallstones diagnosed at any point in treatment and for 22 of 23 patients in Group B who had gallstones diagnosed at some point in treatment. There was no statistically significant difference between Groups A and B when evaluating the presence of chronic cholecystitis ($p=0.22$) or cholesterolosis ($p=1.00$). When comparing the two groups for the finding of a normal gallbladder, Group A had four patients with a normal gallbladder whereas Group B had none, although this did not meet statistical significant ($p=0.08$).

Table 3 Methods for Diagnosis of GERD in 101 Patients with Low Gall Bladder Ejection Fraction Based on EGD Gross Findings, Esophageal Biopsy, X-Ray (Including Esophagram, Upper Gastrointestinal Series, and CT Scan), and Clinical History (Including History of Esophageal Fundoplication and Charted Diagnosis)

Findings consistent with GERD	Number of patients
EGD gross observation	42
Biopsy	19
X-ray observation	7
Clinical history	16

GERD Gastroesophageal reflux disease, *EGD* esophagogastroduodenoscopy

Table 4 Methods for Diagnosis of Gastritis in 101 Patients with Low Gall Bladder Ejection Fraction Based on EGD Gross Findings, Gastric Biopsy, X-ray (Including Upper Gastrointestinal Series In One Patient Only), and Clinical History (Including Charted Diagnosis)

Findings consistent with gastritis	Number of patients
EGD gross observation	44
Biopsy	34
X-ray observation	1
Clinical history	16

EGD Esophagogastroduodenoscopy

Clinical Factors Associated with Decreased GBEF

Of the 120 patients with decreased GBEF, sufficient information was available from 101 charts to determine potential common associations among these patients. Upon further evaluation, 74 of these 101 (73.2%) patients were diagnosed with gastroesophageal reflux disease (GERD) based on one or more of the following: esophagogastroduodenoscopy gross observation of esophageal inflammation, erosions, and hiatal hernia; esophageal biopsy; X-Ray observations, including esophagram demonstrating reflux or hiatal hernia, upper gastrointestinal series demonstrating reflux or hiatal hernia, and CT scan demonstrating hiatal hernia; and clinical history, including history of esophageal fundoplication and otherwise charted diagnosis in medical history (Table 3). GERD was present in these patients at a higher frequency (73.2%) than what is expected in the general population (approximately 20–40% of the population based on symptoms, between 2% and 7% based on objective findings).[21–23] Gastritis was found in 59 of 101 (58.4%) patients based on criteria similar to those used for GERD (Table 4). *Helicobacter pylori* infection was diagnosed in 24 of 101 (23.7%) patients based on endoscopic biopsy, chart history of eradication, and/or urease breath test, and this approximates the 13–32% seen in the general population.[24]

Discussion

Biliary dyskinesia is defined as the presence of abdominal symptoms that can be attributed to the biliary tract in the absence of gallstones and associated with abnormal gallbladder emptying. The method most frequently used to objectively demonstrate gallbladder emptying is hepatobiliary scintigraphy with cholecystokinin provocation, more commonly referred to as the CCK-Tc-HIDA scan. Recent studies evaluating the role of cholecystectomy for the treatment of biliary dyskinesia after diagnosis using the CCK-Tc-HIDA scan suggest that there is a favorable symptomatic outcome after cholecystectomy in patients with low GBEF.[2–12] These studies found that a majority of patients (between 79% and 100%) with biliary dyskinesia improved after cholecystectomy. Despite this, some debate exists whether low GBEF helps distinguish patients who would benefit from cholecsytectomy.[13–15]

Our study further supports that biliary dyskinesia, when diagnosed using the CCK-T_C-HIDA scan, can be surgically treated with favorable outcome. In this study, the cutoff value for normal GBEF was defined at 35%, consistent with Krishnamurthy et al.[20] who used a 3-min CCK infusion time. While it has been demonstrated that the duration of continuous CCK administration is important for an accurate test result, and while some have demonstrated that a cutoff of 40%[12] or 50%[6] is more appropriate after administering CCK over 45 min, we used the more stringent value of 35% in this analysis with a CCK administration between 3 and 45 min. Using this criterion, we retrospectively discovered that 92.6% of patients with abdominal symptoms and decreased GBEF who did not demonstrate gallstones on preoperative imaging studies (Group 1) improved symptomatically after cholecystectomy, and this was not different from the 86.7% of patients with decreased GBEF and gallstones diagnosed preoperatively (Group 2) who improved after surgery. Furthermore, when excluding the seven patients within Group 1 whose gallstones were only grossly visualized in the gallbladder following cholecystectomy, 95.2% of patients with no gallstones (Group A) improved symptomatically after cholecystectomy. This was not different from the 85.7% of patients with gallstones in Group B who improved after cholecystectomy. These similarities in outcomes suggest that biliary dyskinesia should be considered as part of a spectrum of gallbladder disease that improves after cholecystectomy.

When evaluating gallbladder pathology, chronic cholecystitis and cholesterolosis were the two most common findings in patients with decreased GBEF. Chronic cholecystitis was found in the gallbladders of 60% of patients

Table 5 Association of GERD, Gastritis, and *Helicobacter pylori* in Patients with Gallbladder Dysfunction

	GERD		Gastritis		*H. pylon*	
	n	%	*n*	%	*n*	%
Patients with decreased GBEF	74	73.2	59	58.4	24	23.7

GERD Gastroesophageal reflux disease, GBEF gallbladder ejection fraction

with decreased GBEF and no gallstones visualized at any point in treatment, and this is consistent with what has already been published.[6–9,11,12] Comparing the gallbladder pathology of patients in Groups A and B, we found that there was no difference in the existence of chronic cholecystitis ($p=0.22$) or of cholesterolosis ($p=1.00$), which were the most common findings in both groups. Furthermore, although four patients in Group A demonstrated normal gallbladder pathology while none of the patients in Group B did, this observation did not meet statistical significance. This similarity in gallbladder pathology further supports that biliary dyskinesia should be considered as part of the same spectrum of gallbladder disease as gallstones, and perhaps patients with biliary dyskinesia and normal gallbladders represent the earliest time point in the pathophysiology of calculous gallbladder disease.

Careful investigation into characteristics shared by patients with low GBEF demonstrate that a majority of these patients had gastroesophageal reflux disease (73.2% of the 101 patients reviewed). The incidence of GERD within this population is two times what is expected in the general population.[21–23] Three recent reports have suggested that a high incidence of GERD is associated with gallbladder dysfunction;[25–27] however, this relationship has not been definitively explained. An association between the two diseases is apparent when considering the following. First, as it has been shown that physiologic levels of plasma cholecystokinin can cause relaxation of the lower esophageal sphincter (LES),[28] it is possible that the patterns of secretion of cholecystokinin in patients with gallbladder dysfunction differs from that of normal subjects. Second, it is possible that secondary hypergastrinemia from chronic proton pump inhibitor use affects gallbladder function (Table 5). This hypothesis has been supported by Morton and colleagues;[27] Chowdhury et al., who suggested that gastrin and CCK may compete for a common receptor in the cat gallbladder;[29] and Ryan et al., who found that gastrin causes a decrease in CCK's ability to increase intraluminal pressure in the opossum gallbladder.[30] Furthermore, increased levels of gastrin may exacerbate symptoms from a diseased gallbladder because excess gastrin hyperstimulates an already poorly responding organ. Lastly, because acetylcholine causes LES contraction as well as gallbladder contraction, a defect in acetylcholine signaling in patients with biliary dyskinesia and GERD may explain the association of these two conditions. Overall, an association between GERD and gallbladder dysfunction is an interesting finding that, with further research, may both provide insight into the physiology of these disorders as well as eventually lead to better overall management of patients with these disorders.

A high proportion of patients with low GBEF also had gastritis (58.4% of the 101 patients reviewed). It is more difficult to compare this result with the general population because there is such a vast range of asymptomatic people who demonstrate evidence of gastritis in populations studied (between approximately 18% and 70%).[24,31] A possible relationship between the two disorders should still be investigated. As 71.4% of patients in this study whose pathology records were available for review after cholecystectomy had evidence of chronic cholecystitis, one possible explanation linking gastritis and a dysfunctional gallbladder may be the association of a general upper gastrointestinal inflammatory state which may or may not be related to infection. This is particularly interesting as Stathopoulos and colleagues found that 74% (14 of 19) of patients with symptomatic gallstones and moderate to marked gastritis had evidence of *H. pylori*[31] and as *H. pylori* has been identified in gallbladders with chronic cholecystitis.[32,33] With this possibility in mind, we looked for documentation of *H. pylori* infection in the 101 patients with decreased GBEF available for study, finding that 23.7% displayed evidence of this bacterium. This approximates the 13–32% anticipated in the general population.[24] Further studies to evaluate gallbladder specimens of patients with low GBEF for the presence of *H. pylori* are warranted.

Conclusion

From this study, we conclude that patients with biliary dyskinesia improve symptomatically after cholecystectomy, that both biliary dyskinesia and gallstones should be considered as part of a similar spectrum of gallbladder disease that responds to cholecystectomy, and that there is an association of gallbladder dysfunction with GERD and possibly gastritis.

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Impact of Gastro-esophageal Reflux on Mucin mRNA Expression in the Esophageal Mucosa

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Abstract
Introduction Changes in the expression of mucin genes in the esophageal mucosa associated with uncomplicated gastro-esophageal reflux disease have not been evaluated even though such changes could be associated with reflux-induced mucosal damage. We therefore sought to identify reflux-induced changes in mucin gene expression using a cell line and biopsies from the esophageal mucosa in patients with and without reflux.
Methods MUC-1, MUC-3, MUC-4, and MUC-5AC gene expressions were investigated in the HET-1A cell line following exposure to acid (pH 4) and/or bile (120 μM of a bile salt milieu), and in esophageal mucosal biopsies from controls, subjects with non-erosive gastro-esophageal reflux, and subjects with reflux associated with ulcerative esophagitis (erosive). The mucosal biopsies were also evaluated for IL-6 mRNA expression (inflammatory marker) and CK-14 mRNA expression (mucosal basal cell layer marker). Gene expression was determined using real-time reverse transcriptase-polymerase chain reaction analysis.
Results In the cell line studies, there were differences in mRNA levels for all of the evaluated mucins following treatment with either acid or the acid and bile combination. In the studies which evaluated tissue specimens, IL-6 and CK-14 mRNA levels increased according to degree of reflux pathology. The expression of MUC-1 and MUC-4 in mucosa from patients with erosive reflux was lower than in subjects without reflux and in patients with non-erosive reflux, whereas the expression of MUC-3 and MUC-5AC was increased (although these differences did not reach significance at $p<0.05$). When mRNA expression data for tissue samples from all groups were combined, significant correlations were identified between IL-6 vs. CK-14 and IL-6 vs. MUC-3, MUC-3 vs. CK-14 and MUC-3 vs. MUC-5AC, and for MUC-1 vs. MUC-5AC. The correlation between IL-6 and CK-14 was also significant within the control and non-erosive reflux groups. The correlation between IL-6 and MUC-3 was significant within the control and erosive reflux groups, and the correlation between MUC-1 and MUC-5AC was significant within the erosive reflux group.
Conclusions The results of this study suggest that the profile of mucin expression in the esophageal mucosa is influenced by the pH and composition of the gastro-esophageal reflux. Further work should explore the response of these genes to acid and bile reflux, and their role in the etiology of mucosal damage in gastro-esophageal reflux.

Keywords Gastro-esophageal reflux · Mucin · mRNA expression · Esophagus

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Introduction

Gastro-esophageal reflux disease is a common problem that affects up to 50% of Western populations. It occurs when excessive quantities of gastric contents, with or without duodenal contents, reflux into the esophageal lumen. This is characterized by an inflammatory response in the esophageal mucosa, and in some patients who experience prolonged pathological reflux, other pathology such as columnar metaplasia (Barrett's esophagus) or adenocarcinoma can arise. Some patients with gastro-esophageal reflux have a microscopically visible esophageal mucosal injury, but no endoscopically visible mucosal ulceration, whereas other patients with reflux have endoscopic evidence of ulcerative esophagitis. Notably, duodeno-gastro-esophageal reflux is more common in patients who have ulcerative esophagitis, i.e., erosive reflux disease, compared to patients who have non-erosive reflux disease.[1] It is believed that the combination of acid and bile in the refluxate could be responsible for a more severe mucosal injury, and this probably contributes to the development of mucosal ulceration.[2] Some patients with reflux will progress to Barrett's esophagus, which is the only known precursor lesion to esophageal adenocarcinoma.[3]

The normal esophagus is not exposed to excessive amounts of gastric acid or bile, and it lacks a viscous adherent mucus gel barrier.[4] The esophageal submucosal glands do, however, secrete soluble mucus to aid in lubrication,[5] and in response to excessive exposure to acid, the esophageal mucosa secretes increased quantities of viscous mucus. This response is impaired in patients with ulcerative esophagitis.[6,7] The main components of the mucus layer are large glycoproteins: the so-called mucins (MUC),[8] which are produced by specialized epithelial cells. Mucin genes are expressed throughout the human gastrointestinal tract (including the esophageal epithelium) in a site-specific manner.[9] Advances in the detection of the expression of mucin genes and the proteins they produce have provided new insights into the role of the mucus layer and its potential relevance to gastrointestinal disease.[8] For instance, MUC-3 mRNA expression is much stronger in biopsies collected from Barrett's esophagus mucosa, yet virtually absent in normal mucosal biopsies.[10] It is certainly possible that changes in the expression of mucin genes could be associated with the development of the mucosal damage caused by gastro-esophageal reflux, and even its progression to Barrett's esophagus.

The expression of mucin genes has not been evaluated in the context of gastro-esophageal reflux disease in patients who do not have Barrett's esophagus, even though this uncomplicated reflux almost certainly precedes the development of metaplasia in the reflux–Barrett's esophagus–adenocarcinoma sequence. Furthermore, changes in mucin mRNA expression, if present, might provide clues to biological processes associated with reflux-induced esophageal mucosal damage, and it is also conceivable that alterations in mRNA expression in the esophageal squamous epithelium might precede the development of intestinal metaplasia. Furthermore, alterations in mucin mRNA expression might also provide specific biomarkers for testing the efficacy of treatments for gastro-esophageal reflux.

For these reasons, we sought to identify changes in mucin gene expression which are associated with gastro-esophageal reflux, using an esophageal mucosal cell line and esophageal mucosal biopsies from patients with and without reflux.

Methods

Mucin gene expression was initially investigated in an esophageal cell line which was exposed to acid and bile, and then in a panel of biopsies taken from control subjects, subjects with non-erosive gastro-esophageal reflux disease, and subjects with gastro-esophageal reflux disease associated with ulcerative esophagitis. The specific genes of interest were MUC-1, MUC-3, MUC-4, and MUC-5AC. When examining the mucosal biopsy specimens, we also evaluated IL-6 mRNA expression, as this has previously been shown to correlate with reflux-induced mucosal inflammation,[11–13] and we evaluated CK-14 mRNA expression, an increase of which has been shown to correlate with hyperplasia of the basal cell layer of the esophageal mucosa, i.e., one of the earliest reflux-induced histopathological changes.[14–16]

Cell Culture and Acid/Bile Treatment

The HET-1A cell line was used to model the response of normal human esophageal epithelium to acid and/or bile exposure. This is a keratinocyte cell line that was derived from the esophagus of a human male and then immortalized with SV-40 large T antigen.[17] HET-1A cells were seeded at a density of 10^5 cells/well (in 2 mL LHC-9 medium) in six-well plates. The cells were cultured for 2 days until they reached approximately 40–50% confluence. At this stage, the cells were subjected to 3 days of pulsatile acid and/or bile treatment, with three 5-min periods of exposure per day, each pulse separated by approximately 4 h. The acid pulse medium consisted of LHC-9 medium acidified to pH 4 using hydrochloric acid (HCl). The bile pulse medium consisted of LHC-9 medium adjusted to pH 7 using HCl and containing 120 μM of a bile salt milieu. The bile salt milieu contained glycocholate, taurocholate, glycochenodeoxycholate, taurochenodeoxycholate, glycodeoxycholate,

and taurodeoxycholate (Sigma-Aldrich®, USA) in a molar ratio of 20:3:15:3:6:1. This concentration and molar ratio mimics the median seen in reflux esophagitis.[18] The acidified bile medium consisted of LHC-9 medium adjusted to pH 4 containing the previously mentioned molar concentrations of bile milieu. After each 5-min period of exposure, the cells were rinsed with untreated LHC-9 medium, this was discarded, and then 2 mL of untreated LHC-9 medium was added to the cells. After the last 5-min period of exposure on day 3, the cells were left for 3 h to recover and then lysed in 1 mL of TRIzol® (Invitrogen, USA) for RNA extraction. The cells were approximately 90% confluent at this time. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was performed for all mucin genes (as described below). Experiments were repeated a minimum of three times.

Subjects and Tissue Collection

Since 2004, as part of an ongoing tissue collection protocol, patients who are undergoing upper gastrointestinal endoscopy for a range of clinical conditions have been invited to provide additional endoscopic biopsy samples for laboratory research. For the current study, we selected individuals from whom we had collected fresh tissue samples who also met the following criteria:

1. Normal subjects (n=12)—Individuals who at endoscopy had a visibly normal esophageal mucosa, no other endoscopic indicators of gastro-esophageal reflux disease, and no symptoms (or previous history) of gastro-esophageal reflux disease.
2. Non-erosive reflux disease (n=15)—Individuals who at endoscopy had no evidence of ulcerative esophagitis, but had typical symptoms of gastro-esophageal reflux disease (heartburn and regurgitation), and at endoscopy they had evidence of mechanical incompetence of the gastro-esophageal junction—Hill grade III or IV.[19]
3. Erosive reflux disease (n=12)—Individuals who had typical symptoms of gastro-esophageal reflux disease (heartburn and regurgitation), and mucosal ulceration (ulcerative esophagitis—Savary Miller grade I to IV) was visible at endoscopy.

The biopsies used for this study were taken from the distal esophagus, 5 cm proximal to the squamo-columnar junction. Biopsies were placed immediately into RNA-later® (Ambion, USA) for storage using the manufacturer's protocol. The biopsies were stored at −20°C until required for this study. Biopsies from metaplastic columnar mucosa from five additional patients with non-dysplastic Barrett's esophagus were also analyzed for comparative purposes. In samples from these patients, Barrett's esophagus with intestinal metaplasia was verified by ABPAS/D histochemical staining and histopathological evaluation. Biopsies from the duodenal mucosa and proximal gastric mucosa were obtained from three further subjects, and these samples served as positive control tissues for the expression of intestinal and gastric mucins, respectively.

All biopsies were removed from storage and thawed. A small piece (20% to 30%) of the biopsy tissue was removed from each biopsy sample, placed in formalin, and then assessed using routine histochemical and histopathological methods to ensure that the tissue of origin was correctly identified and that it consisted only of esophageal squamous epithelium. The remainder of the biopsy was used for gene expression analysis. Tissue collection was approved by the Flinders Clinical Research Ethics Committee and the Repatriation General Hospital Clinical Research Ethics Committee.

RNA Extraction and Reverse Transcription

Following removal of 20% to 30% of each tissue biopsy for histopathology, the remainder of the biopsy was transferred to a 1.5-mL snap-top tube containing 500 μL of TRIzol® (Invitrogen Life Technologies, NY, USA). Tissue was homogenized using a plastic pestle attached to a Dremmel® MultiPro™ drill, and total RNA was extracted according to the manufacturer's protocol. The concentration of RNA was determined using a Biophotometer (Eppendorf®, North America Inc, Westbury, USA). RNA quality was determined by electrophoresis through a 1% agarose gel. All RNA samples were confirmed to be undegraded by visualization of distinct 28S and 18S rRNA species. The final RNA solution was stored at −80°C until required for cDNA synthesis.

DNAse treatment of total RNA was performed prior to reverse transcription in order to minimize PCR signal arising from carry over genomic DNA. The Ambion DNAfree™ kit was used. To 1 μg of each RNA sample (i.e., 5 μL of 200 ng/μL RNA), 2 μL of sterile water, 1 μL 10× DNase I buffer, 1 μL tRNA (2.5 μg/μL), and 1 μL r-DNAse I were added. After a quick spin, the samples were incubated for 30 min at 37°C in an Eppendorf® Mastercycler. Two microliters of DNAse inactivation reagent was added to a total volume of 12 μL in each tube, and the samples were centrifuged at 10,000 rpm for 5 min.

A new set of tubes was prepared, which contained 1 μL dNTPs (10 mM each in stock), 1 μL pd(N)6 (250 ng/μL stock), and 10 μL of the corresponding centrifuged RNA (equal to 1 μg) per sample. These samples were incubated at 65°C for 5 min then incubated on ice for at least 1 min. A second mastermix was added, which contained 4 μL 5× first strand buffer, 1 μL 0.1 M DTT, 2 μL sterile water, and 1 μL Superscript III™ RT (200 U/μL; Invitrogen™, USA)

per sample. Reactions included a reverse transcriptase negative control sample, i.e., containing 1 μL sterile water instead of 1 μL Superscript III RT enzyme. Reverse transcription was performed in an Eppendorf® Mastercycler, with an initial incubation at 25°C for 5 min, followed by incubation at 50°C for 60 min. The reaction was terminated by heating at 70°C for 15 min.

Real-time Polymerase Chain Reaction

Real-time PCR was performed using 1/130th of the cDNA reaction (all amplicons except MUC3 and MUC5AC), or 1/13th of the cDNA reaction (MUC3 and MUC5AC). PCR amplification was performed in 20-μL final volumes containing 3 μL of cDNA template, 2 μL of each forward and reverse primer, 3 μL of sterile water, and 10 μL of 2× Quantitect SYBRGreen Master Mix (Qiagen, Germany). Thermocycling utilized a Rotorgene 6000 cycler (Corbett, Australia) with an initial denaturation at 95°C for 15 min, followed by 45 cycles with different annealing temperatures for every amplicon (Table 1), and with a final extension at 72°C for 4 min. Triplicate reactions were performed on all samples. PCR products were verified by nucleotide sequencing.

Cycle thresholds were determined using the relative quantitation analysis module in the Rotorgene 6000 Series software (Corbett Research, Australia). The amplification efficiency of each primer pair was estimated from a real-time PCR dilution curve generated using serial twofold dilutions of genomic DNA, and 95% confidence intervals for the amplification efficiencies were estimated from the mean least squares slope of the standard curves and their associated standard errors. Quantitative real-time RT-PCR analysis was then performed using Q-Gene software,[20,21] with the amplification efficiency confidence intervals applied to the relative concentration analyses of both the genes of interest and the housekeeping gene. Gene of interest expression data was normalized by dividing by the corresponding levels of beta-actin for each sample. Expression values for the tissue samples were set relative to expression in the HT-29 colon adenocarcinoma cell line, and the HET-1A cell line samples were set relative to gDNA. In cases where more than one biopsy per patient was analyzed, the results were averaged.

Table 1 PCR Conditions and Primer Sequences

Amplicon	Primer sequence	Annealing temperature (°C)
MUC-1	F GGTGGCAGCAGCCTCTCTTA	55
	R CCCTGAAGAACCTGAGTGGA	
MUC-4	F GGCCAGGTTCTCCTATTTCC	54
	R TCCAGTCTCCCAAAAGCAAT	
MUC-3	F CGCTCCCACGGGCTATGAAG	60
	R AGAACGCACTGGCCCTGGTGA	
MUC-5AC	F CTGAGGGTCTCAGGAATGACGC	60
	R TGTACTGAGGAGGGGAGCGC	
IL-6	F GCAATAACCACCCCTGACC	55
	R TAAAGCTGCGCAGAATGAGA	
CK-14	F ACGATGGCAAGGTGGTGT	54
	R GGGATCTTCCAGTGGGATCT	
β-actin	F TTGCCGACAGGATGCAGAAG	59
	R GCCGATCCACACGGAGTACT	

Statistical Analysis

Dot plots of gene expression from the normal, non-erosive reflux disease, and erosive reflux disease groups were generated, and statistical analyses were performed using KaleidaGraph® (Synergy Software). Apparent reflux-related changes in gene expression between these three groups were assessed for statistical significance using the Kruskal–Wallis rank sum test with a post hoc Bonferroni adjustment. Spearman rank sum correlations between the expression levels of two markers were calculated using the online program Spearman Rank Sum Correlation Tester (http://www.wessa.net/rankcorr.wasp).

Results

Mucin Expression in Cell Lines: Acid and/or Bile Treatments

Figure 1 shows the results of a typical HET-1A acid and bile treatment experiment performed in triplicate. Compared with the pH 7 control, mean MUC-1 and MUC-4 mRNA levels were raised (but this response was variable across triplicates for MUC-1) following treatment with either bile alone or acid (pH 4) alone (Fig. 1a and c). However, the combined treatment with both acid and bile was followed by a decrease in MUC-1 and MUC-4 mRNA. MUC-3 expression was markedly higher in cells treated with either acid or the acid and bile combination (Fig. 1b). Responses varied within triplicates for the pH 4 treatment group of MUC-3. MUC-5AC mRNA levels were also increased following treatment with either acid or the acid and bile combination (Fig. 1d), although MUC-5AC mRNA levels were lower following treatment with the acid and bile combination compared to treatment with the acid only. Overall, there were differences in mRNA levels for all of the evaluated mucins following treatment of the cells with either acid or the acid and bile combination.

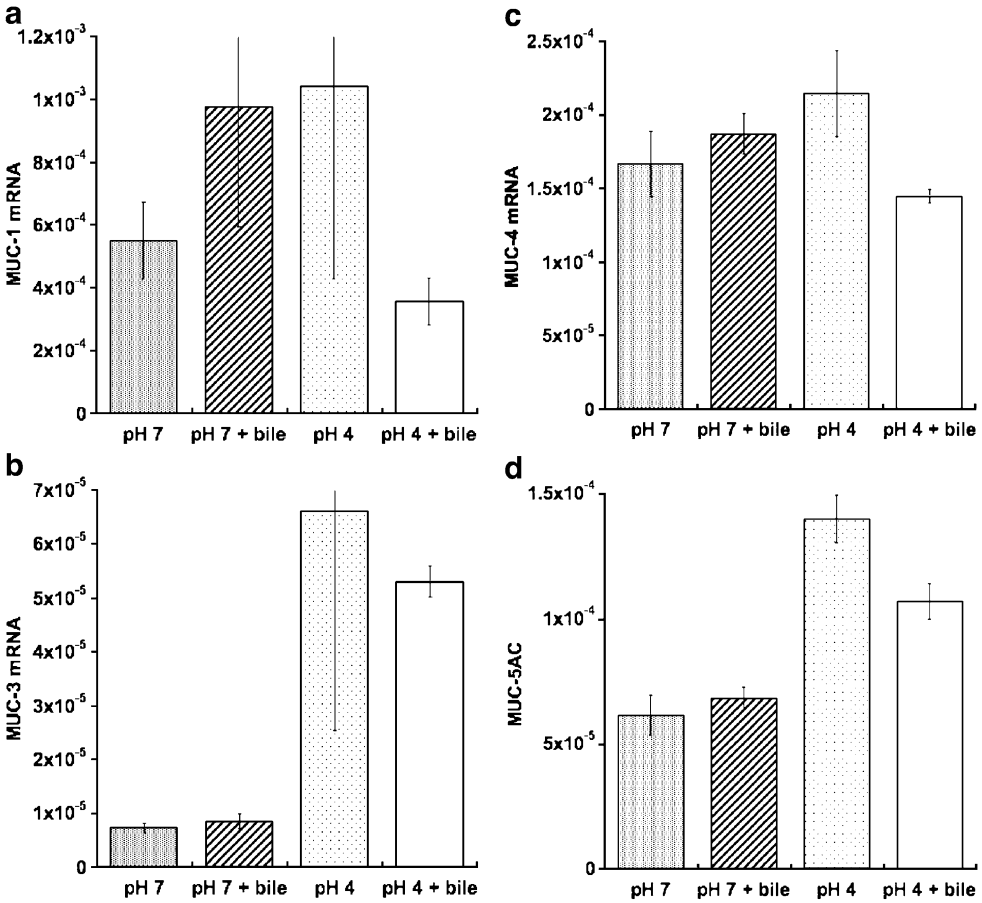

Figure 1 MUC-1 (**a**), MUC-3 (**b**), MUC-4 (**c**), and MUC-5AC (**d**) expression in HET-1A cells following exposure to acid and bile. Data is expressed as mean ± SEM.

IL-6 Expression in Esophageal Mucosal Biopsies

IL-6 mRNA levels in biopsies from the esophageal mucosa in patients without reflux, patients with non-erosive reflux disease, and patients with erosive reflux disease were measured, and the results are shown in Fig. 2a. Generally, levels increased according to the degree of reflux pathology, i.e., consistent with increasing inflammation. The median values were 1, 3, and 7 for the normal, non-erosive reflux, and erosive reflux groups, respectively. Comparison of the three groups showed significant differences in IL-6 mRNA levels (p=0.010, Kruskal–Wallis test). Post hoc testing demonstrated significant differences between the normal and non-erosive reflux groups (p=0.006) and the normal and erosive reflux groups (p=0.013). There was no significant difference between the non-erosive and erosive reflux groups (p=0.526).

CK-14 Expression in Esophageal Mucosal Biopsies

Figure 2b depicts the sample values and medians for CK-14 mRNA levels in the three groups. The levels of CK-14 were similar in the samples from patients without reflux vs. patients with non-erosive reflux (median=0.008 and 0.009, respectively). The levels in the samples from patients with erosive reflux were increased threefold (median=0.024, p=0.02 Kruskal–Wallis test). Post-testing showed no significant difference between the normal and non-erosive reflux groups (p=0.591), but significant differences between the normal and erosive reflux groups (p=0.014) and between the non-erosive and the erosive reflux groups (p=0.019).

Mucin Expression in Squamous Epithelium from Control and Reflux Subjects

The expressions of MUC-1, MUC-3, MUC-4, and MUC-5AC measured in the mucosal biopsy specimens from patients without reflux, patients with non-erosive reflux disease, and patients with erosive reflux disease groups are summarized in Fig. 3. While the mean and median values for MUC-1 (Fig. 3a) and MUC-4 (Fig. 3c) expression in mucosa from patients with erosive reflux were lower than in patients without reflux and in patients with non-erosive reflux, the differences in MUC-1 and MUC-4 expression for the three groups were not statistically significant (p=0.055 and p=0.133, respectively, Kruskal–Wallis test). Similarly, the increases in mean MUC-3 (Fig. 3b) and MUC-5AC (Fig. 3d) expression for the non-erosive reflux vs. no reflux groups and for the erosive reflux vs. no reflux

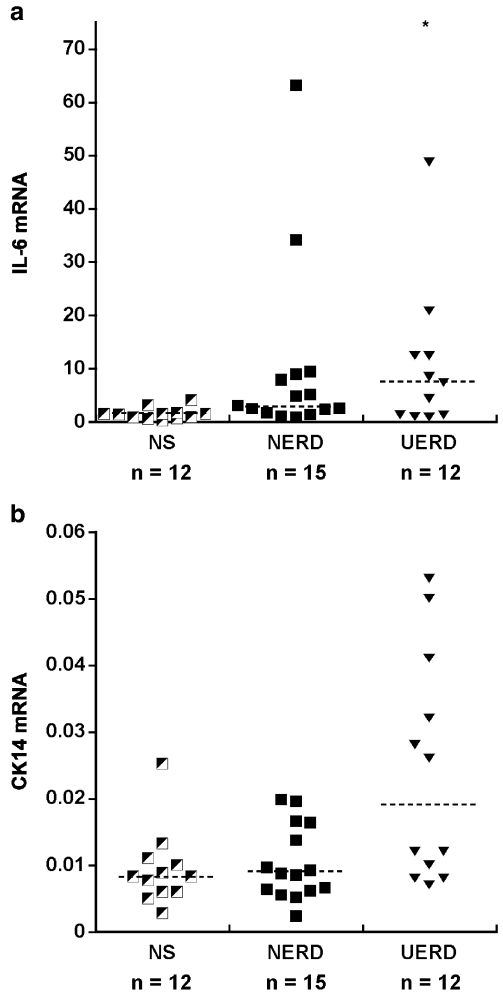

Figure 2 IL-6 (**a**) and CK-14 (**b**) mRNA mRNA levels in esophageal mucosal biopsies from controls (*NS*), patients with non-erosive reflux (*NERD*), and erosive reflux (*UERD*). All data points shown and median for each group (*asterisk*, outlying data point).

groups were also not statistically significant ($p=0.077$ and $p=0.390$, respectively, Kruskal–Wallis test).

Correlations Between Gene Expression Levels

Correlation between the expression levels for different genes was tested for all possible combinations using the Spearman rank order correlation test. When mRNA expression data for all tissue samples from all three groups were combined, significant correlations were identified between IL-6 vs. CK-14 and IL-6 vs. MUC-3, MUC-3 vs. CK-14 and MUC-3 vs. MUC-5AC, and for MUC-1 vs. MUC-5AC. The Spearman rho values for these comparisons are shown in Table 2. Correlations which did not reach statistical significance for any combination are not shown. Correlations for all three tissue groups combined have 37 degrees of freedom. The significant correlations from the larger combined group of samples were further investigated to determine if the correlation was confined to one or more tissue subgroups (also shown in Table 2). Once separated into the separate groups, there were 13 degrees of freedom for results from the non-erosive reflux group and 10 degrees of freedom for results from each of the non-reflux and erosive reflux groups.

The IL-6 vs. CK-14 correlation was only significant for the non-reflux and the non-erosive reflux groups. A significant correlation between IL-6 and MUC-3 was present for the non-reflux and the erosive reflux groups. For MUC-1 vs. MUC-5AC, the correlation was only significant for the erosive reflux group. The MUC-3 vs. CK-14 and the MUC-3 vs. MUC-5AC correlations were not significant when analyzed for specific groups.

Mucin Expression in Gastric, Duodenal, and Barrett's Esophagus Epithelium

Esophageal biopsies from patients without reflux, as well as esophageal biopsies from patients with Barrett's esophagus, mucosal biopsies from the proximal stomach, and duodenal mucosal biopsies were tested for mRNA levels of MUC-1, MUC-3, MUC-4, and MUC-5AC to validate the assays for the expression of these genes. Mean mucin mRNA levels, according to tissue type, are depicted in Fig. 4. MUC-1 expression was increased threefold in biopsies from Barrett's esophagus mucosa and approximately 15-fold in proximal gastric mucosa, compared with esophageal biopsies from patients without reflux (Fig. 4a). MUC-4 mRNA levels are shown in Fig. 4c. All tissue types demonstrated a decrease in expression compared to esophageal biopsies from patients without reflux. Figure 4b shows that MUC-3 expression levels are higher in proximal gastric mucosa, much higher in Barrett's esophagus mucosa and greatest in duodenal mucosa, compared with esophageal biopsies from patients without reflux. Figure 4d indicates that MUC-5AC expression was increased in Barrett's esophagus mucosa and even more so in proximal gastric mucosa and that MUC-5AC was not expressed in duodenal epithelium.

Discussion

Mucin gene function is important for the integrity of mucosal cell function. These genes can be classified into two main families: (1) mucins which are secreted and may participate in mucus gel formation (including MUC-2, MUC-5AC, MUC-5B, and MUC-6), and (2) membrane-bound or signaling mucins (including MUC-1, MUC-3, and MUC-4). Membrane-bound mucins are involved in initiating or modulating intracellular signals and are important for cell function.[22,23] Mucin genes MUC-1 to MUC-6 have all been shown to be expressed in some or all of the following tissues: normal esophageal mucosa, metaplastic columnar

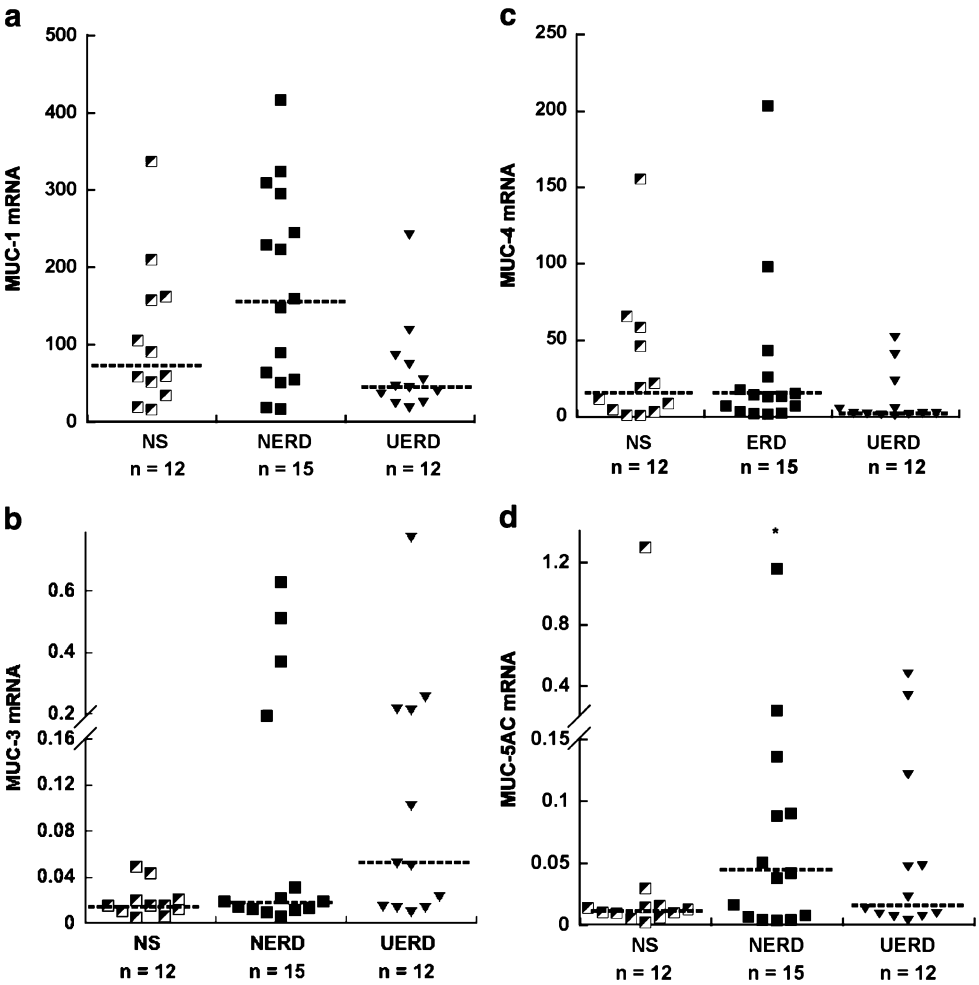

Figure 3 MUC-1 (**a**), MUC-3 (**b**), MUC-4 (**c**), and MUC-5AC (**d**) mRNA levels in esophageal mucosal biopsies from controls (*NS*), patients with non-erosive reflux (*NERD*), and erosive reflux (*UERD*). All data points shown and median for each group (*asterisk*, outlying data point).

esophageal mucosa (with or without intestinal metaplasia), and in esophageal adenocarcinoma.[10,24–26] However, to date, the function of these genes in the context of uncomplicated gastro-esophageal reflux disease, either erosive or non-erosive, has not been investigated. This is perhaps surprising because gastro-esophageal reflux is the precursor of intestinal metaplasia (Barrett's esophagus), and this is the major risk factor for the development of esophageal adenocarcinoma. Hence, early changes in the way in which genes function in the esophageal mucosa in the presence of pathological gastro-esophageal reflux might help us to understand how metaplasia occurs and perhaps allow us to eventually identify individuals who are at risk of progression to Barrett's esophagus and eventually cancer.

For these reasons, we studied mucin gene expression in the esophageal mucosa in control subjects without gastro-esophageal reflux and compared this to expression in the mucosa of patients with non-erosive and erosive gastro-esophageal reflux disease. We specifically chose to investigate the expression of four mucin genes. The secreted mucin gene MUC-5AC was selected because MUC-5AC mRNA expression has not been previously detected in the superficial epithelium or deep glands of the normal esophageal mucosa, whereas it is strongly expressed in the superficial layers of metaplastic columnar epithelium and to a lesser extent in the deep glands in metaplastic columnar epithelium with or without specialized intestinal metaplasia.[24] Furthermore, MUC-5AC expression is markedly higher in mucosa of the proximal stomach compared to Barrett's esophagus with intestinal metaplasia.[25,26]

We also selected three signaling mucins: MUC-1, MUC-3, and MUC-4 for investigation. MUC-1 mRNA is strongly expressed in the superficial epithelium and the deep glands of the gastric cardia,[26] and it is also expressed in the mid layer of the stratified squamous epithelium of the normal human esophagus, but its expression is absent in the deep submucosal glands of the esophagus.[24] Furthermore, MUC-1 mRNA levels are elevated in the superficial epithelium and deep glands of metaplastic columnar epithelium in patients with Barrett's esophagus.[24] MUC-3 is not expressed in the normal esophagus. It appears to only be expressed in the superficial columnar epithelium in Barrett's esophagus in the presence of specialized intestinal metaplasia.[10,24,25] MUC-4 mRNA is expressed in the mid layer of the stratified squamous epithelium of the human esophagus, but is absent from the deep submucosal

Table 2 Rho and p Values for Expression Correlations for All Genes Across All Tissue Groups and Separate Tissue Groups (*$p<0.05$)

mRNA	All tissue groups combined	No reflux	Non-erosive reflux	Erosive reflux
IL-6 vs. CK-14	0.531 ($p=0.001$)*	0.712 ($p=0.018$)*	0.604 ($p=0.024$)*	0.340 ($p=0.254$)
IL-6 vs. MUC-3	0.654 ($p<1.000\times10^{-6}$)*	0.630 ($p=0.037$)*	0.493 ($p=0.064$)	0.734 ($p=0.015$)*
MUC-3 vs. CK-14	0.336 ($p=0.038$)*	0.337 ($p=0.258$)	−0.014 ($p=0.952$)	0.554 ($p=0.064$)
MUC-3 vs. MUC-5AC	0.412 ($p=0.011$)*	0.154 ($p=0.603$)	0.457 ($p=0.085$)	0.336 ($p=0.263$)
MUC-1 vs. MUC-5AC	0.356 ($p=0.028$)*	0.056 ($p=0.849$)	0.407 ($p=0.126$)	0.678 ($p=0.024$)*

glands.[24] MUC-4 expression is lower in the superficial epithelium and deep glands of metaplastic columnar epithelium.[10]

Other groups have studied the relative pattern of mucin mRNA expression in the normal esophagus and stomach, as well as in Barrett's esophagus.[10,24–26] Buisine et al.[27] showed that MUC-3 is highly expressed in the adult duodenum, but by comparison MUC-1, MUC-4, and MUC-5AC are virtually absent. These studies used in situ hybridization, Northern analysis, and endpoint RT-PCR. These techniques are semi-quantitative and are unable to detect subtle changes in mRNA levels. Our study used the more sensitive and quantitative real-time RT-PCR technique, designed specifically to detect small changes in mucin expression that may occur in reflux subjects. Because quantitative RT-PCR has not been used in this setting elsewhere, we validated the methodology used in our current study by testing mucin mRNA expression in tissue samples from stomach, duodenum, and Barrett's esophagus, and the patterns of expression were found to be comparable to the outcomes of other studies.

Overall, our results support the hypothesis that the expression of mucin genes in the esophageal mucosa is influenced by the composition (pH and bile) of the refluxate in gastro-esophageal reflux. In the cell culture study, we observed increases in the expression of all tested mucin genes in cells exposed to pH 4. These differences were similar to the trends observed in the studies undertaken using biopsy samples, i.e., the median MUC-1, MUC-3, and MUC-5AC mRNA levels were higher in the esopha-

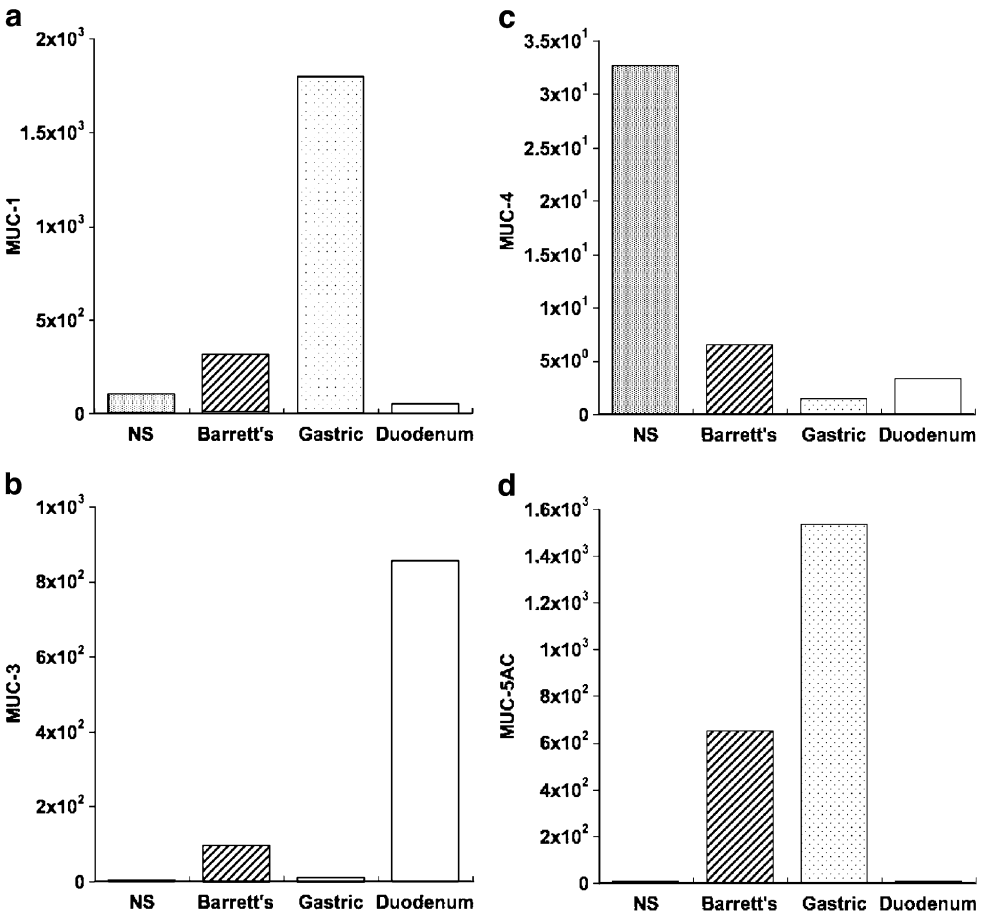

Figure 4 Mean MUC-1 (**a**), MUC-3 (**b**), MUC-4 (**c**), and MUC-5AC (**d**) mRNA levels in normal esophageal mucosal biopsies (*NS*), Barrett's esophagus, proximal stomach, and duodenum.

geal mucosa from patients with non-erosive reflux although the difference between this group and the control group did not reach statistical significance. However, it is possible that increases in the expression of these mucin genes are occurring in the esophageal epithelium due to exposure to intra-luminal acid. With the exception of MUC-3, we also observed decreases in mucin gene expression in the Het-1A cells which were treated with a combination of acid and bile. Similar differences were also seen in the esophageal mucosa from patients with erosive reflux, with the expression levels for MUC-1, MUC-4, and MUC-5AC less than those seen in samples from control subjects and patients with non-erosive reflux. While again the differences between groups did not reach statistical significance, it is conceivable that protective roles of MUC-1, MUC-4, and MUC-5AC in the esophageal epithelium are abolished or reduced when gastro-esophageal reflux is associated with ulcerative esophagitis, and this could be associated with the combination of acid and bile in the refluxate.

The significant correlations between MUC-3 expression and IL-6 (a marker of inflammation) and MUC-3 expression and CK-14 (a basal epithelial layer marker) suggest that MUC-3 could have a role in esophageal mucosal protection during inflammation, and this may therefore have implications for our understanding of the repair of reflux-induced mucosal damage. Furthermore, co-expression of MUC-1 and MUC5-AC in mucosa from patients with erosive reflux disease may indicate the metaplastic potential of esophageal epithelium in this state. This is because MUC-1 and MUC-5AC are known to be co-expressed in proximal gastric mucosa and Barrett's esophagus, but not in normal esophageal mucosa. Our results, which show for the first time that these two markers are co-expressed in esophageal epithelium from patients with erosive reflux (but not controls or patients who did not have ulcerative esophagitis), indicate possible progression or an early step at the molecular level towards columnar metaplasia.

Unlike previous reports, our study has investigated the effect of gastro-esophageal reflux on the expression of mucin genes. This is probably the earliest step in the progression to esophageal adenocarcinoma via the Barrett's esophagus to cancer sequence. We took active steps to ensure that the tissues used in this study were correctly classified, and the potential for biopsy sampling error has been largely eliminated by obtaining histopathology on part of each biopsy sample. However, we did not use 24 h ambulatory pH monitoring to categorize patients, but rather relied on endoscopic criteria and clinical symptoms. While it has been argued that pH monitoring is the "gold standard" for the identification of patients with gastro-esophageal reflux, it should be recognized that this test can be associated with false positive and false negative outcomes. Some studies have shown that up to 50% of patients with reflux can have a normal 24-h pH test.[28] Furthermore, we have collected tissue samples from many patients undergoing endoscopy in our institution, and for the current study we were conservative in our classification of patients, and when there was doubt about the clinical diagnosis we excluded the specimens from the individuals concerned. In addition, the endoscopic criteria for identifying ulcerative esophagitis are well accepted in clinical practice, and there is little doubt that this category of patients had gastro-esophageal reflux disease. An additional potential confounder is the possible use of proton pump inhibitor medication. However, as most of the patients in the two reflux categories were taking this medication at the time of endoscopy, the gradation of severity was probably not influenced by medication use.

Previous studies using animal models or clinical tissue samples have shown that IL-6 levels are elevated in the esophageal mucosa in the presence of reflux esophagitis.[11–13] For this reason, we evaluated IL-6 as a possible surrogate tissue marker of reflux. Our results demonstrated an increase in IL-6 levels across the three study groups, in a manner which was consistent with increasing severity of reflux. Hyperplasia of the basal cell of the esophageal mucosa is also evident in patients with gastro-esophageal reflux, and it is probably caused by epithelial repair mechanisms in response to reflux-induced damage.[14] The presence of CK-14 has been shown to be restricted to the basal cell layer within the esophageal mucosa, and hyperplasia of this layer is associated with increased expression.[15,16] Hence, we evaluated CK-14 as another potential surrogate tissue marker of reflux. Its expression also correlated with increasing severity of reflux. These results provide additional support to the hierarchy of our study groups—controls vs. non-erosive vs. erosive reflux. Also supporting this is the significant correlation between IL-6 and CK-14 mRNA levels.

The differences between gene expression levels for the patient groups failed to reach statistical significance at the level of $p<0.05$. However, the data trends suggest that if the sample size was larger, many of these trends might have reached statistical significance. When we commenced this study, we were not sure what magnitude of difference (if any) we were likely to find, and for this reason a sample size calculation was not feasible. Hence, a larger sample size may yield significant differences, and further work is warranted to explore this possibility.

Overall, our results suggest that the profile of mucin expression in the esophageal mucosa is influenced by the pH and composition of gastro-esophageal reflux. Further work should explore the response of these genes to acid and bile reflux and explore their role in the etiology of mucosal damage in gastro-esophageal reflux, as well as its progression to Barrett's esophagus and beyond.

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Esophageal pH Profile Following Laparoscopic Total Fundoplication Compared to Anterior Fundoplication

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Abstract In Barrett's esophagus, total abolition of reflux may give maximum protection against the development of malignancy. To determine whether laparoscopic anterior fundoplication gives the same degree of antireflux control as a total fundoplication, we analyzed a prospectively followed cohort of patients from randomized controlled trials of laparoscopic antireflux surgery. There were 167 patients who returned for routine esophageal pH studies within 6 months of surgery (123 laparoscopic total fundoplications and 44 anterior fundoplications). There was no difference in percentage time pH <4 between fundoplication groups, but the total number of reflux episodes was significantly different (total fundoplication, four reflux events vs. partial fundoplication, six reflux events; $p=0.03$). It is difficult to believe that this difference is either biologically or clinically important. In patients with a second esophageal pH study more than 5 years later, both the percentage time pH <4 (0.1% total fundoplication vs. 2.7% partial fundoplication; $p=0.004$) and total number of reflux episodes (three total fundoplication vs. 24 partial fundoplication; $p=0.002$) were significantly different. However, the postoperative esophageal acid exposure was within the normal range for both total and partial fundoplication, so whether the statistical difference is clinically important, remains a moot point.

Keywords Laparoscopic fundoplication · Gastro-esophageal reflux · Esophageal pH monitoring · Randomized controlled trial

Introduction

Laparoscopic total fundoplication is regarded as the gold standard in the surgical treatment of gastro-esophageal reflux disease. However, it may result in troublesome postoperative dysphagia and wind-related problems. For this reason, modifications to the procedure have evolved. Randomized controlled trials show that partial fundoplications result in less dysphagia and less wind-related problems than total fundoplications, although these benefits are offset to a degree by an increased likelihood of recurrent reflux symptoms.[1–5]

Subjective reflux symptoms are not the same thing as objective evidence of control of reflux.[6–9] It is not known whether a laparoscopic partial fundoplication gives the same objective degree of antireflux control as a laparoscopic total fundoplication. This may have particular application in Barrett's esophagus where maximum protection against reflux is sought on the grounds that refluxed material may contribute to the development of malignancy.

Objective evidence of reflux is measured using ambulatory pH monitoring. Distal esophageal acid exposure beyond the normal range (95th percentile: 4.45%) for normal healthy control subjects is used to stratify patients with "abnormal' gastro-esophageal reflux.[10,11] However, evidence is lacking regarding different types of fundopli-

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cation and their effect on objectively assessed reflux control.

This study was designed specifically to determine the objective antireflux control of laparoscopic total and anterior partial fundoplication by examining their postoperative esophageal acid reflux profiles. Clinical subjective outcomes have been extensively reported previously in randomized trials.[1–5]

Materials and Methods

The study group was a prospectively followed cohort of patients who underwent laparoscopic fundoplication as a part of one of six randomized controlled trials involving various fundoplication wrap types that examined different outcomes.[1,2,5,12,13] Twenty-four-hour esophageal pH studies were routinely performed 3 to 6 months after surgery for these trial patients as part of their postoperative objective assessment. All patients gave written informed consent prior to randomization, surgery and postoperative testing. All the trials received ethics committee approval.

All patients who returned for the routine postoperative esophageal pH test were included as this provided objective unbiased data for the study. The investigators were blinded as to whether the patients had symptoms or not. Patients who presented at other time points for postoperative esophageal pH testing because of postoperative problems were excluded because these would introduce bias.

Patients were grouped into those who underwent a total fundoplication and those who underwent a partial fundoplication (anterior 180° or anterior 90°). Details of the various types of laparoscopic fundoplication have been described previously.[5,14,15]

Ambulatory pH monitoring was undertaken with patients off acid suppression for at least 5 days. A Digitrapper Mk III (Medtronic Functional Diagnostics, Denmark) was used with an antimony pH probe (Medtronic Functional Diagnostics, Zinetics Inc, Utah, USA) passed transnasally and placed 5 cm above the proximal border of the lower esophageal sphincter. Data were uploaded to a personal computer and analyzed using EsopHogram ver2.01 (Polygram for Windows ver2.04, Synectics Medical ©1996). An acid reflux event was defined as a drop in pH below pH 4.0 for longer than 5 s.

Outcome measures include distal esophageal acid exposure (percentage of time pH below 4), total number of reflux episodes, and indicators of no reflux at all (zero time percentage for pH below 4 and an absence of any reflux episodes). Comparisons were made between the laparoscopic total and partial fundoplication groups.

A small group of patients underwent two routine postoperative 24-h esophageal pH studies at least 5 years apart. Subgroup analysis was performed using the same outcome measures, comparing the time period between the two studies and between the total and partial fundoplication groups.

Data were stored and retrieved from a computerized database, Filemaker Pro version 8.5, (Filemaker Corporation, Santa Clara, CA, USA) and analyzed with SPSS version 11 for Windows (SPSS Inc, Chicago, IL, USA). Outcome measures are expressed as median [interquartile range] or proportions (percentages) as appropriate, whereas demographic data are expressed as mean (95% confidence interval) or proportions (percentages). Categorical variables were compared using Fisher's exact test, continuous variables with a parametric distribution were compared using the independent samples t test, and nonparametric data was compared using the Mann–Whitney U test. Statistical significance was accepted as $p<0.05$.

Results

From 1994 to 2006, 435 patients participated in various laparoscopic fundoplication trials[1,2,5,12,13] of which 173 (40%) patients returned for routine postoperative 24-h esophageal pH studies. Six patients were excluded due to technical failure during monitoring, resulting in 167 studies for analysis. The pH study group did not differ from the remaining trials patients in the proportion of wrap types. There was also no difference in their 3-month postoperative symptomatic outcomes. However, the pH study group had a higher mean age (50 years vs. 45 years; $p=0.00$) and fewer males (53% vs. 64%; $p=0.03$) compared with the remaining trials patients who declined to undergo postoperative pH testing.

In the postoperative pH study group, there were 123 (74%) total fundoplications, 25 (15%) anterior 180° fundoplications, and 19 (11%) anterior 90° fundoplications. The demographic characteristics between the total and partial fundoplication groups were well matched. The mean age was 50 years for both the total fundoplication group (95% CI 48–52) and partial fundoplication group (95% CI 46–54). The number of males was 67 (55%) in the total fundoplication group and 23 (52%) in the partial fundoplication group.

Table 1 lists the comparison of the main outcome measures. The distal esophageal acid exposure time (% time pH <4), proportion of patients with zero percent time pH <4, and proportion of patients with no reflux episodes did not differ significantly between the total and partial fundoplication groups. In contrast, the partial fundoplication group had a significantly higher number of total reflux episodes, occurring during upright posture (Table 2).

Table 1 Acid Reflux Profile Following Total and Partial Fundoplication

	Total Fundoplication $n=123$	Partial Fundoplication $n=44$	p
Duration of pH study, hours[a]	22.7 (22.3–23.1)	23.1 (22.8–23.3)	0.31[c]
% time pH <4[b]	0.2 [0–1]	0.5 [0.1–1.7]	0.08[d]
Total no. of reflux episodes[b]	4 [1–9]	6 [3–15]	0.03[d]
No. of long reflux (>5 min) episodes[b]	0 [0–1]	0 [0–2]	0.08[d]
No. of patients with 0% time pH <4	39 (32%)	10 (23%)	0.34[e]
No. of patients with zero reflux episodes	17 (14%)	4 (9%)	0.60[e]

Values are: [a] mean (95% confidence interval), [b] median [interquartile range] or proportion (percentages).
[c] Independent samples t test
[d] Mann–Whitney U test
[e] Fisher's exact test

The anterior 180° fundoplication and the anterior 90° fundoplication were similar in regard to the postoperative acid reflux profiles (Table 3).

There were 34 patients with Barrett's esophagus: 20 (16.3%) underwent total fundoplication and 14 (31.8%) underwent partial fundoplication. This difference was significant ($p=0.05$). There was no difference in the distal esophageal acid exposure and total number of reflux episodes between the total and partial fundoplication groups in the patients with or without Barrett's esophagus.

Seventeen out of 167 patients said that their heartburn was not controlled postoperatively. Twelve (9.8%) had total fundoplication while five (11.4%) had partial fundoplication. Only two of these patients (both partial fundoplication) had recurrent reflux on pH testing.

Table 2 Acid Reflux Profile in Upright and Supine Position Following Total and Partial Fundoplication

	Total Fundoplication	Partial Fundoplication
Upright	$n=120$	$n=44$
% time pH <4	0.3 [0.0–1.3]	0.5 [0.1–2.4]
No. of reflux episodes	3 [1–8]	6 [3–15]
No. of long reflux episodes (>5 min)	0 [0–0]	0 [0–2]
Supine	$n=119$	$n=44$
% time pH <4	0.0 [0.0–0.0]	0.0 [0.0–0.1]
No. of reflux episodes	0 [0–0]	0 [0–2]
No. of long reflux episodes (>5 min)	0 [0–0]	0 [0–2]

Values are median [interquartile range].

Table 3 Acid Reflux Profile Following Partial Fundoplication

	Anterior 180° Fundoplication $n=25$	Anterior 90° Fundoplication $n=19$	p
% time pH <4	0.5 [0.1–1.6]	0.4 [0.2–1.8]	0.77[a]
Total no. of reflux episodes	6 [1–12]	6 [3–26]	0.20[a]
No. of patients with 0% time pH <4	6 (24%)	4 (21%)	1.00[b]
No. of patients with zero reflux episodes	3 (12%)	1 (5%)	0.62[b]

Values are median [interquartile range] or proportion (percentages).
[a] Mann–Whitney U test
[b] Fisher's exact test

A small subgroup of patients underwent a second routine 24-h esophageal pH study at least 5 years after surgery. The first (6-month) postoperative acid reflux profile of each subgroup was similar to that of the main group from which they were taken. The mean time elapsed between studies was not significantly different between fundoplication groups (Table 4). For the pH study performed more than 5 years later, the partial fundoplication group had significantly higher distal esophageal acid exposures (% time pH <4) and significantly greater total number of reflux episodes compared to the total fundoplication group (Table 4).

Discussion

It is difficult to persuade patients to have routine postoperative 24-h pH monitoring. We have studied the patients who agreed to return for a postoperative pH study. The only differences we could find between this group and the patients who declined a further pH study was a higher mean age and fewer males in our study group. We think it is

Table 4 Acid Reflux Profile of Fundoplications After More Than 5 Years

	Total Fundoplication $n=16$	Partial Fundoplication $n=9$	p
Time elapsed between studies, years[a]	8.7 (8.3–9)	7.8 (6.5–9.1)	0.24[c]
% time pH <4[b]	0.1 [0–0.4]	2.7 [0.7–7]	0.004[d]
Total no. of reflux episodes[b]	3 [0–13]	24 [21–39]	0.002[d]

Values are [a] mean (95% confidence interval), [b] median [interquartile range] or proportion (percentages).
[c] Independent samples t test
[d] Mann–Whitney U test

unlikely that these differences will have had much effect on the results of the study.

Symptomatic outcome is the prevailing "yard-stick" for randomized studies comparing fundoplications with varying degrees of fundal wrap. Randomized studies suggest a total fundoplication has superior or equivalent reflux symptom control to posterior (Toupet) fundoplication,[16,17] and anterior fundoplication,[1–5,18] but is associated with greater unwanted side effects. Complete control of reflux symptoms has been reported for partial and total fundoplications in the early postoperative period.[18] However, over time a trend toward greater reflux symptom recurrence is reported for anterior fundoplications.[1–5,18]

Very few randomized studies report postoperative objective assessments at short- or long-term follow-up. From the limited data available, it seems that both laparoscopic total and anterior 180° fundoplication result in normalization of esophageal pH assessed at 3–5 months after surgery (median percentage time pH <4 were 2.5% and 2.8%, respectively).[18] This is similar to the 3–6 month postoperative acid reflux profile of our two study groups, which did not differ for distal esophageal acid exposure and indicators of zero reflux (proportion of patients with 0% time pH <4 and those with no reflux episodes).

If the distal esophageal acid exposure and total number of reflux episodes had approached previously established norms in the anterior fundoplication patients then such a difference might have been accepted as having clinical significance. However, the number of reflux events recorded was very small in both groups compared to the accepted upper limit of normal, which is 46.9 reflux episodes in a standard 24-h esophageal pH study.[11] It is hard to believe that the small but statistically significant difference, in reflux events we found, has any clinical or biological significance.

Evidence is also lacking for long-term objective reflux assessment after fundoplication. In our study of 24-h esophageal reflux testing 5 years after surgery, the partial fundoplication group showed significantly higher distal esophageal acid exposure and number of reflux episodes. However, the median percentage of reflux and number of reflux events were still well within the limits of normality (2.7% and 24 refluxes, respectively) at a median follow-up of 8.7 years. Furthermore, the small number of subjects (n= 25) with objective reflux assessed at 5 years after surgery, raises the possibility that the difference found was a type 1 statistical error. Nevertheless, the fact that the upper quartile limit of the number of reflux episodes in the total fundoplication group (13) was less than the lower quartile limit of the number of reflux episodes in the partial fundoplication group (21) suggests the finding was real.

If there is some quantitative relationship between the number of reflux episodes and the development of cancer, then these differences might be of importance. However, with such low acid exposure this seems inherently unlikely to us. This study therefore does not provide any very compelling evidence that a total fundoplication is superior to an anterior partial fundoplication in its ability to prevent gastro-esophageal reflux.

Because of small subject numbers, we have combined the two types of anterior fundoplication used for the comparison against total fundoplication. Analysis of the three wrap types in separate groups (total vs. anterior 180° vs. anterior 90° fundoplication) did not alter the results.

By chance there were significantly more patients with Barrett's esophagus in the partial fundoplication group. However, the presence of Barrett's esophagus did not significantly alter the outcome in either the total or the partial groups, although once again the numbers are small enough to raise the possibility of a type 1 error. We have previously found, with relatively short-term follow-up, no significant difference in outcome between patients with Barrett's esophagus and those with no Barrett's esophagus.[19]

As the notion that a fundoplication prevents cancer remains unproven[20] and as the long-term results of anterior partial fundoplications in symptomatic terms remain as good as total fundoplication,[4,21] the premise that a total fundoplication should be undertaken in patients with Barrett's esophagus has not been established.

Conclusion

The results of this study show that both total and anterior partial fundoplication lead to normalization in distal esophageal acid exposure. In the short term, anterior fundoplication offers a similar degree of antireflux control to a total fundoplication. Long-term postoperative objective assessment of reflux in a small number of subjects indicates reflux control may weaken slightly over time for the anterior fundoplication, relative to a total fundoplication. However, for the majority of patients, both total and anterior fundoplication result in sustained normalization of esophageal pH.

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Mortality Rate Associated with 56 Consecutive Esophagectomies Performed at a "Low-Volume" Hospital: is Procedure Volume as Important as We are Trying to Make it?

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Abstract
Background Esophagectomy procedures have been associated with high morbidity and mortality rates. Recent articles in the literature have focused on the relationship between operative volume and the rates of mortality and morbidity in association with esophagectomy. The common theme among these publications is the statistically significant correlation between high-volume centers (typically defined as at least 10 esophagectomies per year) and lower mortality rates. The authors hypothesized that an individual surgeon's expertise with the various esophagectomy procedures would better correlate to mortality rates than the absolute number performed in an institution per year.
Study Design The study involved a retrospective cohort of a single surgeon over a 7-year period (August 17, 1999–December 23, 2006). Selection criteria included all patients who had undergone a transhiatal esophagectomy, transabdominal with diaphragmatic split esophagectomy, or Ivor-Lewis esophagectomy procedure by a single surgeon (PP) during the specified time period. The main outcome measures were 30-day mortality and postoperative complications.
Results Over the 7-year study period, 56 esophagectomies were performed (average, eight per year). The 30-day morbidity and mortality rates were 48% (27/56) and 3.57% (2/56), respectively.
Conclusions If low-volume esophagectomy centers are to be defined in the literature as completing <10 procedures per year, then these data represent such an institution. While several authors have demonstrated a correlation between lower mortality rates and high-volume esophagectomy hospitals, our results support surgeon experience as potentially being more significant than absolute number of procedures performed in an institution per year.

Keywords Esophagectomy · Morbidity · Mortality

Introduction

High morbidity and mortality rates have been associated with esophagectomy procedures.[1–13] Recent articles in the literature have focused on the relationship between operative volume and the rate of mortality and morbidity in association with esophagectomy.[3,14–16] Begg et al.[3] examined a retrospective cohort of Medicare patients over the age of 65 and demonstrated a direct link between mortality rate from esophagectomy and the number of esophagectomies a hospital performed per year. Thirty-day mortality rate varied from 20% in hospitals performing one esophagectomy per year to 4% in those performing greater than 20 esophagectomies per year. Similarly, Dimick et al.,[16] in 2005, also demonstrated a trend toward reduction in esophagectomy-related mortality with increased hospital volume. They examined in-house mortality rates between low- (≤7 esophagectomies/year) and high-volume (>7 esophagectomies/year) institutions. Their results showed mortality rates as high as 15.3% for low-volume centers and 7.5% for high-volume hospitals. Thus, they concluded that the greater the number of esophagectomies a hospital performed each year, the lower their mortality rate. Similarly, a meta-analysis in 2004 focused on hospital volume and mortality rates from esophagectomy.[15] In this study, low-volume centers were defined as having per-

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formed two to 10 esophagectomies per year, while medium- and high-volume centers performed 11–20 and >20 per year, respectively. It concluded that "only with the experience of more than 20 esophagectomies per year can a significant reduction of the mortality, down to 4.9%, be achieved." This was determined after finding significantly higher rates of mortality in low-volume hospitals (median 18% mortality rate) when compared to high-volume centers (median 4.9% mortality rate). The present study is a retrospective cohort study conducted to examine the morbidity and mortality rates from a low-volume hospital, single-surgeon's experience compared to rates documented in the literature.

Materials and Methods

We queried the surgeon's operative case log to identify patients who had undergone an esophagectomy. Data were queried using ChartMaxx (MedPlus Inc., Mason, OH, USA) and then analyzed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Data were collected for a 7-year period beginning August 17, 1999 and ending December 23, 2006. We chose the time frame of 7 years to obtain an inclusive operative experience of a single surgeon in a community-based, tertiary care teaching hospital. The beginning date was selected as this was when the surgeon first began performing this operation. The surgeon is a graduate of a general surgery residency in 1994 and has been a fellow of the American College of Surgeons since 1996 and currently performs esophagogastrectomies solely at the studied institution. The surgeon has not completed any advanced training for this operation. The surgeon did not perform any other esophagectomies at any other institution during this time period.

Patients were selected for inclusion if they had undergone a transhiatal esophagectomy, transabdominal with diaphragmatic split esophagectomy, or Ivor-Lewis esophagectomy during the specified time period. The surgeon did not perform an esophagectomy utilizing any other technique than those included in this study during this time period; however, the surgeon currently performs a minimally invasive esophagectomy. The transabdominal approach with diaphragmatic split esophagectomy was first described by Denk in 1913 but has since been described by others.[17–19] Lesions which were just proximal to the gastroesophageal junction, were gastric stomal tumors of the distal esophagus, and those with Barret's and high-grade dysplasia were selected for the transabdominal approach. While there were other esophagectomies performed by two other surgeons (two in 1999 and one in 2001) at the studied institution during this time period, these were not included in this study.

The esophagogastrotomy anastomosis was performed by a single-layer, hand-sewn technique using absorbable suture. The proximal gastrectomy was performed to achieve a sufficient oncologic resection and allow utilization of the remaining stomach as the conduit. There was no effort made to tubelarize the stomach. Variables queried for included patient age at the time of operation, race, comorbid conditions including positive social histories, operative time (skin incision to skin closure), intra- and postoperative complications, estimated blood loss, and length of hospital stay. Preoperative evaluation including esophagogastrodoudenotomy (EGD), endoscopic ultrasound (EUS), positron emission tomography (PET) scan, or computed tomography (CT) were also queried. Perioperative adjuvant treatments with chemotherapy or radiation were noted.

Results

Over the 7-year study period, 56 esophagectomies were performed (Fig. 1). The male to female ratio was 4.6:1. The patients ranged in age from 34–84 years (mean age, 60.3 years). Patient comorbidities are listed in Table 1; the most common comorbidity was hypertension (found in 41% of the patients). Operative time averaged 165 min (range, 107–436; median, 146), the average estimated blood loss was 560 ml (range, 50–2,000; median, 400), and the length of hospital stay averaged 16.7 days (range, 8–121; median, 12). All cases were performed on an elective basis. The surgeon billed for 7,487 procedures ranging from central line insertions to complex abdominal operations during the studied time period. The preoperative workup of patients including EUS, EGD, CT, or PET scanning was not uniformly completed. Of the esophagec-

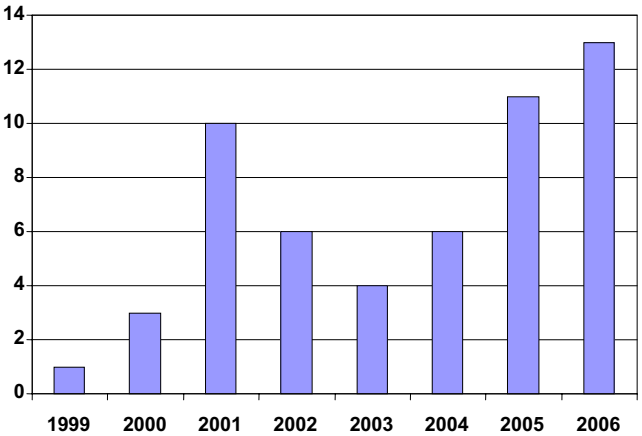

Figure 1 Esophagectomies per year by a single surgeon.

Table 1 Patient Demographics

	Number of cases	Percentage (%)
Caucasian	53	94.6
African American	3	5.4
Hypertension	23	41.1
GERD	13	23.2
Hypercholesterolemia	7	12.5
Diabetes mellitus	6	10.7
Coronary artery disease	13	23.2
Atrial fibrillation	4	7.1
COPD	7	12.5
Hypothyroidism	3	5.4
Cancer		
Breast	1	1.8
Lung	1	1.8
Tobacco use	29	51.8
Alcohol use	7	12.5

tomies performed, 45 (80.4%) were via a transhiatal approach, seven via transabdominal with diaphragmatic split approach, and four via an Ivor-Lewis procedure. Intraoperative complications included three (5.3%) splenectomies, two (3.6%) chyle leaks, and one (1.8%) inferior vena cava injury. All of the patients were cared for in the postoperative setting by the surgeon who performed the operation; a pulmonary critical care specialist was involved in the management of some of the patient's pulmonary issues. The surgical team managed all other critical care issues in the postoperative setting.

Nutritional parameters were assessed uniformly and each patient was required to have a prealbumin of greater than 15 prior to undergoing operation. This was achieved via Dobb-hoff tube feeding placement in the preoperative setting. If patients were selected for preoperative neo-adjuvant chemotherapy and radiation therapy, the surgeon closely followed these patients to ensure that adequate nutritional status was maintained.

The 30-day mortality was 3.57% (2/56) during the study period. The two deaths occurred in 2003 and 2005. Estimated 30-day-risk-adjusted mortality was calculated at 7.67% with a 95% confidence interval of 0%, 10.51%.[20] The postoperative 30-day morbidity included 33 (59%) complications (Table 2). Postoperatively, there was one wound infection that healed successfully by secondary intention after being opened at the patient's bedside. There were four (7%) chyle leaks that developed in the acute postoperative period. Pulmonary complications were the most frequent cause of postoperative morbidity, with an 18% (10/56) incidence (seven related to the development of pneumonia). Newly diagnosed cardiac issues were evident in seven patients; six of these patients developed new-onset atrial fibrillation. Anastomotic leaks were found in six

Table 2 Postoperative Complications

	Number of cases	Percentage (%)
Cardiac	7	12.5
Pulmonary	10	17.8
Anastomotic leak	6	10.7
Vocal cord paralysis	1	1.8
Chylous leak	4	7.1
Wound infection	1	1.8
Stricture	4	7.1

(10.7%) patients and one (1.8%) patient was diagnosed with a transient vocal cord paralysis.

Pathological reports confirmed 35 of the surgical specimens as adenocarcinoma (Table 3). Seventeen patients received preoperative chemo/radiation therapy. Ten (59%) of these patients had ulcerations at the tumor site but no tumor was identified at the time of resection, indicating a complete clinical/pathologic response to preoperative neo-adjuvant therapy. Tumor-free margins were reported after pathological evaluation in 55 (98%). Lymphatic or vascular invasion was identified in 12 (21.4%) of the cases. Pathology reported evidence of Barrett's esophagus in 23 of the 56 patients and 23 of the surgical specimens were reported to have positive lymph nodes.

Discussion

While esophagectomies have historically been associated with significant morbidity and mortality rates, current literature suggests that these rates are higher in institutions that do not perform the operation frequently. The classification between low- and high-volume centers remains to be debated, as several definitions exist in the literature.[3,14–16] After careful review of several articles, the distinction between low- and medium-volume centers remains elusive; we have chosen to define high-volume centers as those

Table 3 Pathological Findings

Finding	Number of cases
Adenocardinoma	35
Mucinous adenocarcinoma	1
Squamous cell carcinoma	1
Enteric cyst/leiomyoma	1
Ulceration	10
Dysplasia	3
Benign stricture	2
GIST	3

performing 10 or more esophagectomies per year and those doing less than that as low-volume centers. Utilizing this classification, our institution is defined as a low-volume center. Even though the number of esophagectomies per year has been increasing, the single surgeon reviewed in this report has performed an average of eight per year over each of the past 7 years (range, 1–13; median, 6.5). Therefore, this study was conducted at a low-volume center. It is imperative to note that this surgeon currently is the primary surgeon performing esophagectomy procedures at this institution; however, during the study period, three other esophagectomies were performed at the institution.

Current data cite mortality rates as high as 20% associated with esophagectomies.[3,15] When these statistics are categorized between low- and high-volume hospitals, there have been a few reports of statistically significant decreases in 30-day mortality. Some authors have gone so far as to conclude the procedure is safer and should only be performed in high-volume centers.[3,14–16] Contrary to this belief, this study demonstrates that the overall number performed each year may not be as significant to overall outcome as other factors may be. Reports of 30-day mortality rates at high-volume centers are recorded in the literature as being as low as 4.9%.[15] In comparison, our results represent a comparable patient population in terms of comorbid conditions and preoperative nutritional status as those already presented in the literature. The results of a single surgeon performing 56 consecutive esophagectomies at a low-volume center include a low incidence of death: two out of 56 patients (3.57%). After taking into account the associated risk factors of the study population, the estimated 30-day-risk-adjusted mortality (7.67%) was nearly twice the observed. It is interesting to note that, while it may have been intuitive that the two deaths occurred earlier in the surgeon's experience, this was not the case with the deaths occurring in 2003 and 2005. This suggests that other factors likely played a more significant role in the mortalities than surgeon experience did. Although this 30-day mortality rate is lower than in previous reports, this surgeon has a similar rate of morbidity at approximately 59% when compared to previously published reports.

In an attempt to identify factors which may explain our favorable outcomes, we would like to draw attention to a few potential correlated factors. As explained previously, the preoperative optimization of each patient's nutritional status is of utmost importance. The surgeon is stringent on this issue, making a point to closely follow each patient even as they undergo preoperative adjuvant therapies. All patients were evaluated in the preoperative setting by a cardiologist and pulmonologist. This routine ensured that these specialists were immediately available and familiar with the patient in the postoperative period if any issues arose. The operative times have been minimized partly due to the use of an ultrasonic scalpel which rapidly speeds the dissection and may in part contribute to the results achieved. While it was not recorded in this study, the surgeon works closely with the anesthesiologist to have the patient expeditiously extubated immediately following completion of the case and makes every effort to prevent prolonged intubation in the postoperative period, if appropriate. Ambulation is begun on postoperative day 1 to promote increased activity and decrease potential complications associated with prolonged immobility. These factors may show correlation to help explain our results; however, we have not performed a logistic regression analysis nor made any attempt to suggest that a causality relationship may exist.

The retrospective nature and rather small number of patients are obvious limitations of this paper. Unfortunately, there was a lack of uniformity in preoperative workup in the patient population studied. This inherently has led to an inability to quantify any meaningful relationship of outcomes to preoperative staging. It must be mentioned that there were three other esophagectomies performed at the studied institution, two in 1999 and one in 2001, by three other surgeons. These were not included in this analysis. Any speculation regarding these patients is beyond the scope of this paper. Regarding the absolute number of esophagectomies performed at the single institution during the study period, the increase in volume, if the three other esophagectomies were included, would still not be significant to classify as high volume (average cases per year, 8.4 vs. eight).

The importance of surgeon volume in mortality related to a variety of operations is not a new one and in fact has been studied rather extensively.[21] Much of the current literature supports lower mortality significantly being associated with increasing surgeon and hospital volumes. We argue that while this may play a component of lower mortality, it is but a single piece of the puzzle. The issue of surgeon experience has also remained a key issue. Some have argued for patient preference to select a more weathered set of hands to operate on them than a fresher, younger surgeon's. We report on a single surgeon whose hands are continuing to become worn with experience, yet whose mortality rates compare favorably to the most calloused of those presented in the literature. With increasing talk of procedure volume being used as a quality indicator and a benchmark for "centers of excellence," our results indicate that good surgical outcomes are a far more complex issue with surgeon experience remaining a key component.

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Improved Reflux Monitoring in the Acute Gastroesophageal Reflux Porcine Model Using Esophageal Multichannel Intraluminal Impedance Measurement

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Abstract
Background Acute animal models are needed to obtain further insights into the mechanism of gastroesophageal reflux disease. Existing acute models use imprecise methods to detect reflux. The aim of the present study was to evaluate the potential of esophageal multichannel intraluminal impedance measurement (MII) to improve the quality of results of acute reflux porcine models.
Materials and Methods MII was used in ten pigs to monitor gastroesophageal reflux. Measurements were obtained (A) before and (B) after mobilization of the esophagus and (C) after myotomy. The results were compared to those obtained when reflux was monitored by esophageal drainage of intragastrically infused blue solution (DBS).
Results The times to first appearance of reflux were 67% (A), 86% (B), and 57% (C) of those by DBS when detected by MII; $p<0.05$. The respective values for intragastric pressures needed to provoke reflux were 46%, 76%, and 66%; $p<0.05$. Although the lower esophageal sphincter pressure decreased by 69% after (B) the intragastric pressure needed to provoke reflux increased by 140%; $p<0.05$.
Conclusions MII improves the detection of reflux in the acute reflux porcine model. The finding that after sole mobilization of the esophagus the intragastric pressure needed to provoke reflux was increased although the finding that the pressure of the lower esophageal sphincter was decreased needs further investigation.

Keywords Gastroesophageal reflux disease · Lower esophageal sphincter · Pathophysiology · Acute porcine model · Myotomy · Esophageal intraluminal impedance measurement · Methylene blue

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Introduction

The pathophysiology of gastroesophageal reflux disease is still not clearly understood. There are several approaches to explain the pathomechanism of gastroesophageal reflux disease (GERD) of which the concepts of a lower esophageal sphincter (LES) deficiency[1–4] and transient LES relaxations[5] are the most important. Although both approaches explain GERD in parts, neither is perfect. To obtain further insights into the nature of GERD and its treatment modalities, appropriate experimental models also for investigations on a more macrophysiological level are needed.

There are already several animal gastroesophageal reflux (GER) models existing. The ones which are the closest to clinical conditions induce GER by cardiomyotomy or

partial myectomy and use 24-h pH measurements for reflux monitoring.[6–9] Although those models are very close to reality, they contain the disadvantage of being very laborious and not practicable for acute (nonsurvival) experiments. The animals have to be put under general anesthesia not only for the intervention tested but also before every placement of the pH catheter. The 24-h pH measurement itself has then to be done in an awake state. However, such a complex and, for both the investigator and the animals, stressful procedure may not be needed for every hypothesis to be tested. Therefore, other investigators used acute GER animal or viscera models. In those models, GER was induced the same way by cardiomyotomy or partial myectomy, but it was visualized by esophageal drainage of blue solution (DBS) which was infused into the stomach.[10,11] Alternatively, intraoperative endoscopy was performed to detect GER.[12] The drawbacks of those models are that they work with dead organisms and/or they accept a quite imprecise form of visualization of GER. A living organism, however, is a prerequisite when the functioning of the LES is to be analyzed and the precise detection of GER is essential to guarantee the validity and reproducibility of reflux experiments. For this reasons, we were looking for an alternative form of GER monitoring, which would be applicable in the acute GER living animal model.

Esophageal multichannel intraluminal impedance measurement (MII) is a new method to record GER directly without being reliant on the acidity of the refluxate.[13,14] The technique is based on changes in resistance to alternating current between metal electrodes produced by liquid or gas moving inside the esophageal lumen. Since the method has already led to an improvement in the clinical diagnostics of GERD[15], it is supposable that it will also help to improve the monitoring of GER in acute animal reflux models. The aim of the present study was to evaluate the potential of MII to improve GER monitoring in a conventional acute living porcine model. The questions to be answered were if, in fact, the detection and definition of GER could be done reliably and, also, if it could be done faster and more precisely by MII compared to DBS.

Methods

Study Design

Ten Landrace pigs with a mean weight of 29±3 (range 24–34) kg were studied in a standardized GER animal model similar to that by Watson et al.[11] who used cardiomyotomy and DBS to induce and visualize GER in living pigs. The differences in our model were that we used three-channel manometry instead of sleeve manometry and intragastric pressure instead of intragastric volume to determine the LES resting pressure and to assess the LES competence, respectively. Besides intragastric pressure leading to GER, we compared time to first appearance of GER for both methods (MII and DBS) applied for the detection of GER. Three stages of esophagogastic dissection were tested: (1) before mobilization of the esophagogastric junction, (2) after mobilization of the esophagogastic junction but before esophagocardial myotomy, and (3) after esophagocardial myotomy. The primary hypothesis to be tested was that MII detects GER reliably in every experiment. The secondary hypothesis was that GER is detected earlier and, as a consequence, at a lower intragastric pressure by MII compared to DBS.

Animal Preparation

General anesthesia was induced with Isofluran and N_2O and no muscle relaxation was used. After an upper midline laparotomy, the left hepatic lobe was mobilized and the lesser omentum incised for an appropriate exposition of the hiatal region. The greater omentum was dissected along the gastroepiploic vessels and the short gastric vessels were divided. For continuous intragastric infusion and simultaneous intragastric manometry, an 8-French double luminal manometry tube (type MMS5702, MMS Deutschland GmbH, Bottrop, Germany) was introduced through a small ventral gastrotomy into the corpus of the stomach and fixed with a purse-string suture. A second ventral gastrotomy was performed close to the pylorus, which served for cleansing and emptying of the stomach and for a correct positioning of the measuring unit (Fig. 1). After completion of the first set of measurements, the esophagogastric junction was circularly mobilized starting at the left diaphragmatic pillar up to the ventral hiatal border and back along the right diaphragmatic pillar. Thereafter, the second set of measurement was obtained. Finally, a ventral longitudinal myotomy was performed over a distance of 5 cm of the distal esophagus and 1 cm of the proximal gastric cardia and the last set of measurements was obtained. At the end of the experiments, all animals were sacrificed by intracardiac injection of high-dose potassium chloride while still being under general anesthesia.

Measuring Unit

For the detection of GER and measurement of the LES resting pressure, a particularly composed measuring unit was used. It consisted of a 16-French gastric tube (MEDICOPLAST, Jllingen, Germany), a MII probe containing 12 measuring segments of 2 cm located between 2 and 26 cm (type MMS5702, MMS Deutschland GmbH, Bottrop, Germany), and a 14-French perfused six-channel manometry tube (type E6-0-0-5-5-5, MMS Deutschland

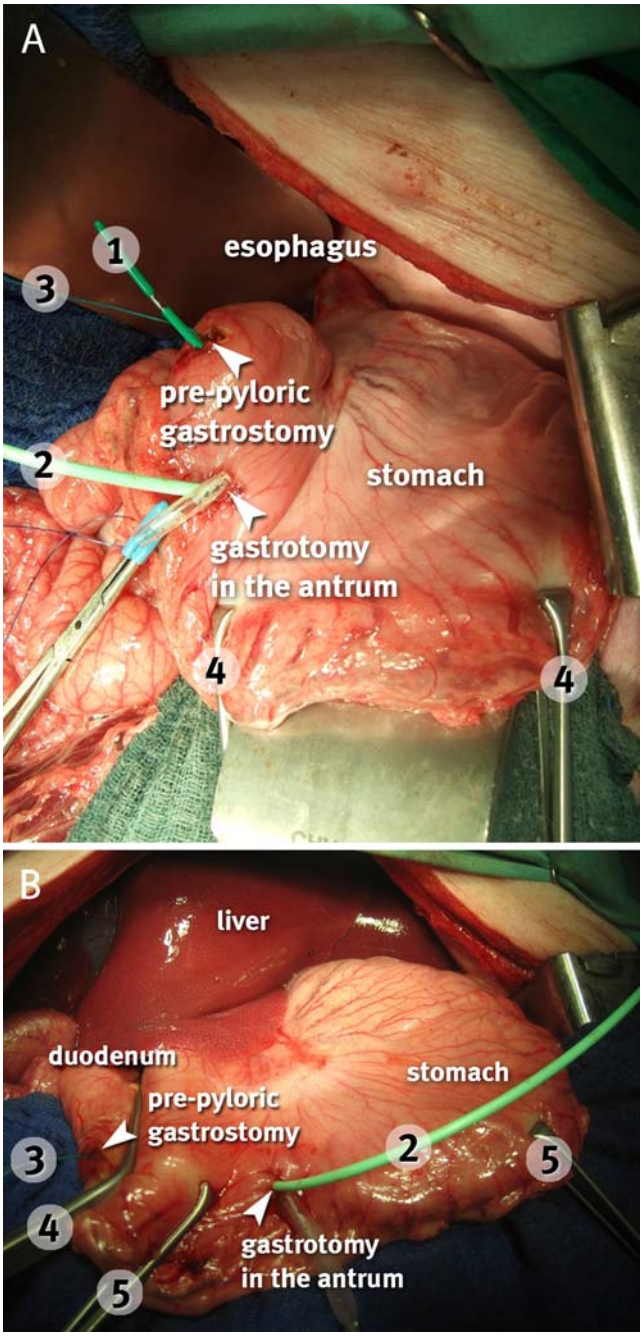

Figure 1 Instrumentation of the porcine stomach for an acute gastroesophageal reflux model in which esophageal multichannel intraluminal impedance measurement and esophageal drainage of transgastrically infused blue solution as methods of gastroesophageal reflux detection are compared. **a** Placement of the measurement unit by thread guidance. **b** Completed instrumentation. *1* measurement unit, *2* gastric manometry tube, *3* guide thread, *4* vascular clamp, *5* Alice clamp.

GmbH, Bottrop, Germany) of which only the three channels at the tip—each orientated 120° from the other—were used. The three tools were fixed to each other with hydrophobic scotch tape as follows: The sensors at the tip of the manometry tube were placed next to the measuring electrode between the third and fourth measuring segment—8 cm from the tip—of the MII probe. The tip of the gastric tube was positioned another 5 cm higher—corresponding to 5 cm above the LES—in the center of the sixth measuring segment of the MII probe (Fig. 2).

Instrumentation

For the placement of the measuring unit, first a 16-French gastric tube with obturator (MEDICOPLAST, Jllingen, Germany) was transorally introduced into the esophagus under laryngoscopic control. Next, the measuring unit was fixed to the end of the gastric tube with a thread. By pulling the gastric tube out of the gastrotomy next to the pylorus, the measuring unit was pulled through into the stomach (Fig. 1). To identify the high-pressure zone of the LES, the catheter was withdrawn until the pressure measured at the tip of the manometry tube (type E6-0-0-5-5-5, MMS Deutschland GmbH, Bottrop, Germany) markedly rose. The maneuver was repeated until the correct position was confirmed three times. Finally, it was checked by digital palpation whether the tip of the manometry tube was positioned in the distal esophagus compatible with a practical anatomic location of the LES. The distance of the tip from the incisor teeth was recorded to control a stable position during the whole experiment.

Measurement Protocol

Measurement of LES and Intragastric Pressure

After the appropriate placement of the measuring unit, the LES resting pressure was recorded as the mean of the values measured by the three sensors at the tip of the esophageal six-channel manometry tube (type E6-0-0-5-5-5, MMS Deutschland GmbH, Bottrop, Germany). Thereafter, the esophagus and the stomach were completely emptied by suction through the esophageal tube and the prepyloric gastrotomy, respectively. Next, the prepyloric region was separated from the rest of the stomach by a vascular clamp to prevent leakage. Further on, the stomach was filled continuously with blue solution (10 ml methylene blue in 1,000 ml 0.9% sodium chloride) through the perfused gastric double luminal manometry tube (type MMS5702, MMS Deutschland GmbH, Bottrop, Germany) at a rate of 100 ml/min using a rotation pump (MMS Solar Urodynamic system, MMS Deutschland GmbH, Bottrop, Germany). Simultaneously, the intragastric pressure was recorded.

Detection and Definition of GER

GER detected by MII was defined as a decrease in impedance of at least 50% from the baseline registered in

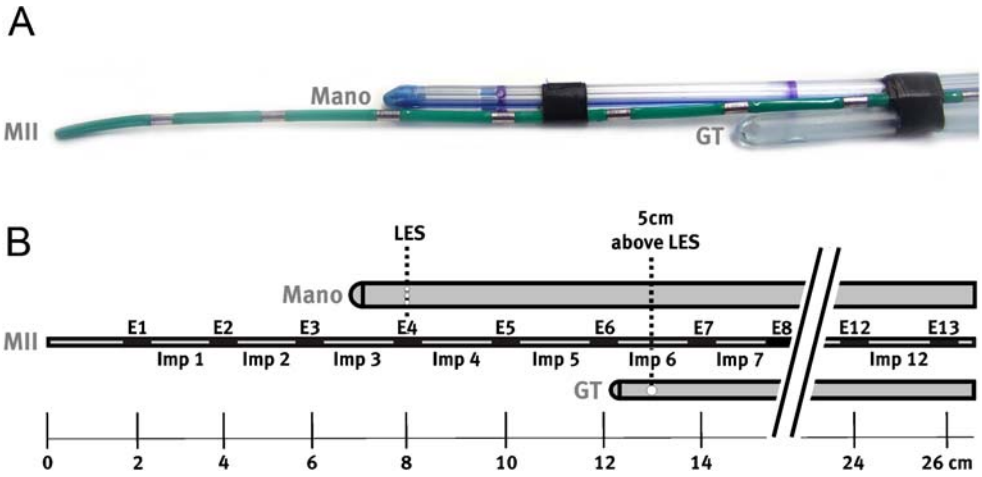

Figure 2 a Image and **b** scheme of the particularly composed measuring unit consisting of a multichannel impedance catheter, a six-channel manometry tube, and a gastric tube which are fixed to each other with hydrophobic scotch tape. Impedance electrode (*E*), gastric tube (*GT*), impedance measuring segment (*Imp*), lower esophageal sphincter (*LES*), manometry tube (*Mano*), multichannel intraluminal impedance catheter (*MII*).

the sixth measuring segment from the tip or the third measuring segment above the LES, respectively (corresponding to the tip of the gastric tube used for DBS). The decrease of impedance had to be sequential, beginning in the most distal measuring segment and reaching at least the seventh measuring segment from the tip of the MII probe. The point of intragastric pressure registration was set at the beginning of the decrease of impedance (Fig. 3). GER detected by DBS was defined as the visibility of blue solution in the gastric tube confirmed by all three investigators involved in the experiment. During the measuring process, an intermittent suction was connected to the gastric tube.

Data Acquisition

The respective time to first appearance of GER detected by MII and DBS and the respective intragastric volumes and pressures at that point of time were registered.

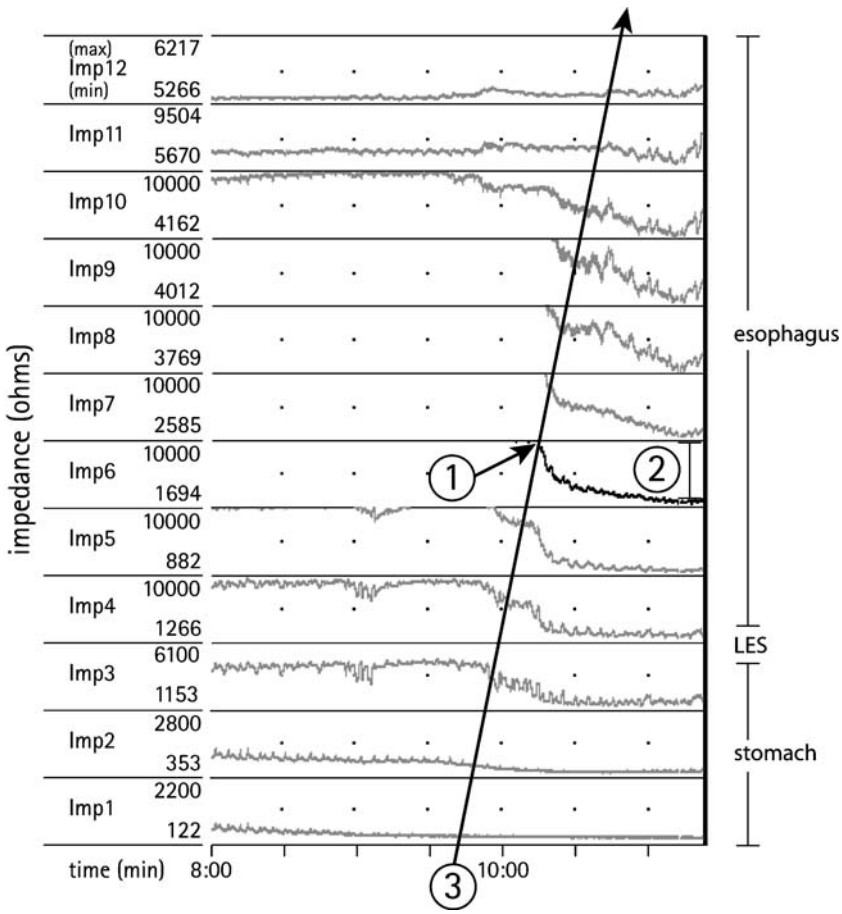

Figure 3 Example of a gastroesophageal reflux event detected by esophageal multichannel intraluminal impedance measurement. Lower esophageal sphincter (*LES*); *1* start of the reflux event defined as a decrease of impedance in the sixth measuring segment (corresponding to 5 cm above the lower esophageal sphincter), *2* a more than 50% decrease of impedance, and *3* a sequential decrease of impedance beginning in the more distal measuring segments and reaching at least the seventh measuring segment.

Measurements were repeated three times: once before mobilization of the esophagogastric junction, once after mobilization of the esophagogastric junction but before esophagocardial myotomy, and once after esophagocardial myotomy.

Animal Care

All experiments were conducted in accordance with the German Law for Animal Protection and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication 86-23, revised 1985). They were approved by the local Committee on Animal Care of the Government of Karlsruhe, Germany.

Statistical Analysis

Statistical analysis was performed using the SAS software (Release 9.1, SAS Institute, Inc., Cary, NC, USA). The results are expressed as mean and standard deviation. Comparisons of time to first appearance of GER and intragastric pressure leading to GER between both methods and between the different stages of preparation were tested using paired Student's t test. The relationship between intragastric pressure and volume was examined using the Pearson's correlation coefficient and the corresponding p value. Two-sided p values were always computed and an effect was considered statistically significant at a p value of ≤ 0.05.

Results

Operative Procedure

All animals remained alive during the whole experiment until they were sacrificed and none had to be excluded from the final analysis due to any reasons. The mean operating time was 112±21 min (range, 75–135). No intraoperative complications occurred. The instrumentation could always be done without any relevant difficulties.

Comparison of MII Versus DBS

Both MII and DBS detected GER reliably. MII, however, detected GER always earlier than DBS and the respective time to first appearance of GER was significantly shorter for MII than for DBS in every set of measurements (Table 1). For both instruments, there was a significant correlation between intragastric volume and intragastric pressure (Fig. 4). Since infusion rate of blue solution was constant over time and intragastric volume did correlate with intragastric pressure, intragastric pressure at the moment of detection of GER was also lower when GER was identified by MII compared to DBS (Fig. 5).

Comparison of the Different Stages of Mobilization

The LES resting pressure decreased significantly from 10.4± 7.4 mmHg before mobilization to 3.2±2.5 mmHg after mobilization and additionally, although not significantly, to 1.6±4.6 mmHg after myotomy (Fig. 6). Although the LES resting pressure decreased, the intragastric pressure needed to provoke GER detected by MII increased from 2.7± 3.1 mmHg before mobilization to 6.5±5.3 mmHg after mobilization of the esophagogastric junction and decreased again to 4.2±3.6 mmHg following myotomy. The respective values were 5.9±3.3, 8.5±6.9, and 6.4±5.9 mmHg when GER was detected by DBS. The increase after mobilization was significant when MII was used for the detection of GER but it was not when DBS was used. The decrease after myotomy was not significant in either group (Fig. 5).

Discussion

An ideal GER model for acute experiments should be easy to handle, reliable, efficient, and precise. To improve existing acute GER models for living animals in that regard, we evaluated MII as a GER detection instrument and compared it to DBS, the established way to identify GER in acute animal models.[10,11] Animal preparation and instrumentation could always be done in a reasonable operating time of near to 2 h. GER was identified reliably by both MII and DBS. However, GER was always detected earlier, within shorter time and, as a consequence, at a lower intragastric pressure by MII. These findings suggest that, in fact, the use of MII allows for an easy, reliable, and, compared to DBS, more efficient and more precise detection of GER in acute animal models. This again may imply a generally more precise investigation of the esophagogastric closing mechanism. For example, we were able to significantly demonstrate a relation between the intragastric pressure and GER after mobilization of the esophagogastric junction when GER was identified by MII, which was not possible when DBS was used for the investigation. Incidentally, this could mean that fewer animals might be needed to reveal any other existing differences when MII is used for the detection of GER. Fewer animals needed imply lower research costs and probably less difficulties in getting the approval of ethical committees.

The superiority of MII may mainly be related to its complete computer-based online data recording. This offers the opportunity to revise all the data and graphics collected.

Table 1 Time to First Appearance of GER Detected by MII and DBS in the Respective Set of Measurements

	Time to GER detected by MII (s)	Time to GER detected by DBS (s)	*p* value[a]
Before mobilization	99±103	148±115	<0.001
After mobilization	205±210	238±230	0.009
After myotomy	124±145	223±203	0.029

GER Gastroesophageal reflux, *MII* esophageal multichannel intraluminal impedance, *DBS* drainage of blue solution
[a] Student's *t* test

The point of impedance decline and the related pressures at the LES and in the stomach can be displayed and revised, which allowed for a reliable analysis of the recorded data. The exactness of a point determined once can be verified interindividually and corrected if needed. While DBS represents quite a crude detection technique of GER depending on the attention of the observer and his naked eyes, MII contains the potential to record every smallest GER episode, which can be identified by the observer even when it has been overlooked at the first view.

There may be some limitations for the direct comparison of MII and DBS. Thus, the moment of GER detected by DBS was defined somehow arbitrarily by naked eye, whereas a very sensitive instrument was used for MII. To reduce the risk of a systematic error in this regard, the solution drained from the esophagus was observed by three different investigators. The moment when the first investigator noted a blue colorization was taken for the point in time of GER. Of course, the accuracy of DBS might be improved by use of colorimetry. However, as colorimetry is not the common way of identifying methylene blue in DBS at present[7,8,12], we did not use it in the present experiment. This might be the issue of further investigations. Another limitation might be the potential attenuation of color by absorption and drainage of methylene blue through lymphatic vessels. However, we considered this risk as minimal as the perfusion of the stomach was always done with freshly mixed solution of 10 ml methylene blue in 1,000 ml sodium chloride 0.9% and the longest measurement period was less than 12 min.

The idea may exist that pH measurement contains the same benefits as MII but that it might be even more exact and better available than MII, so that it should be used instead of MII even for acute animal experiments. However, according to our experience, pH measurement is very difficult to handle in the acute animal experiment. Since pH measurement is relying on changes in acidity, the stomach has to be infused with acidic solution while the esophageal milieu should at least be neutral at the starting of the experiment. When subsequently, experiments have to be repeated, e.g., after different steps of dissection, it is almost impossible to do this in a standardized manner. Additionally, due to a slower decrease,[14] the starting point of pH decline is more difficult to identify than the starting point of

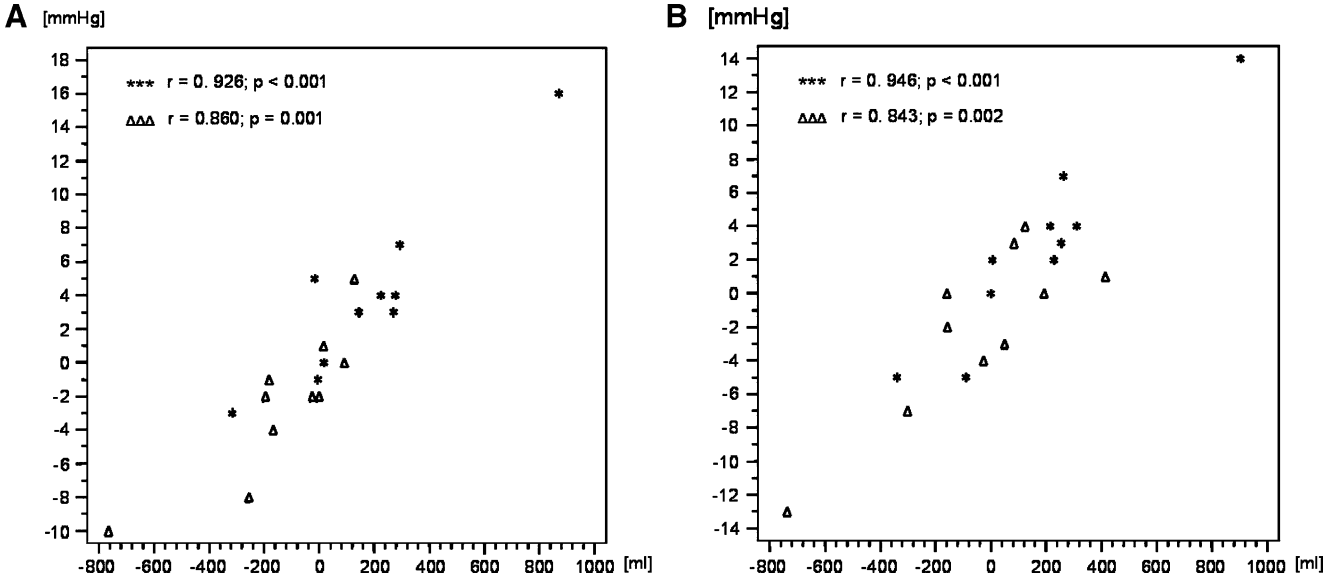

Figure 4 Pearson correlation of differences in intragastric pressure and volume measured at the moment of gastroesophageal reflux detected **a** by esophageal multichannel intraluminal impedance measurement and **b** by esophageal drainage of transgastrically infused blue solution. [(*asterisks*) differences between the states before and after mobilization, (*triangles*) differences between the states before and after myotomy].

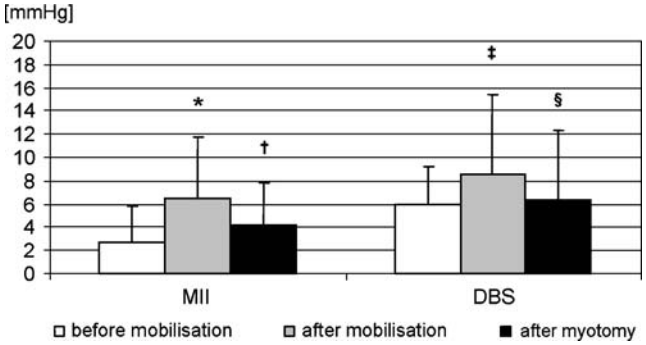

Figure 5 Respective intragastric pressure at which GER was detected by MII and DBS in the evaluated GER porcine model. Esophageal multichannel intraluminal impedance (*MII*); drainage of blue solution (*DBS*). *p=0.047 (versus "before mobilization"); †p=0.125 (versus "after mobilization"), ‡p=0.172 (versus "before mobilization"); §p=0.220 (versus "after mobilization").

a drop in impedance. These drawbacks may be some of the reason why pH measurement has only been used in ambulant survival experiments so far.[6–9]

The restrictions of our modified GER model still are that GER cannot be quantified, no information on transient sphincter relaxations can be obtained, and the induction of GER is quite unphysiological. This may not be relevant for the investigation of certain aspects of GERD such as the relation of LES *resting* pressure and efficiency of GER control or the evaluation of operative antireflux procedures. However, these limitations may make the investigation of neurophysiological aspects of GERD and the evaluation of antireflux medication impossible. In these settings, the more physiological but also more laborious ambulatory GER models using 24-h pH measurements for reflux monitoring are needed.[6,7,9,16]

While evaluating the presented animal GER model, a surprising observation was made. According to previous studies, which demonstrated the correlation of LES pressure and LES competence[1,3,4], we expected that with each stage of preparation the LES resting pressure and the intragastric pressure needed to provoke GER would decrease simultaneously. However, what we found was that, despite a decline of the mean LES resting pressure to a third of the baseline value, the mean intragastric pressure needed to provoke GER was more than doubled after simple mobilization of the esophagogastric junction (Figs. 5 and 6). These findings seem to be contradictory to the results of other animal experiments, which focused on the effect of antireflux procedures demonstrating an increased LES pressure and a better GER control after different types of fundoplication.[10,11,17,18] However, in contrast to those experiments, we were looking at the LES *resting* pressure and GER control after sole mobilization of the esophagogastric junction without performing a fundoplication. Therefore, according to our data, it seems also to be true that even after sole mobilization of the esophagogastric junction GER control improves although the LES resting pressure may decrease. This indicates that LES competence must not necessarily rely on a rise of LES *resting* pressure.

This phenomenon might be due to a lengthening of the esophagus by mobilization of the esophagogastric junction. This would be in accordance with other authors who concluded that an adequate intra-abdominal length of the esophagus is an important prerequisite of a sufficient LES. The first of them was Allison[19] who stipulated the restoration of the cardia below the diaphragm to improve the effect of the hiatal muscle sling on the distal esophagus. DeMeester and coworkers[1–4] provided the in vitro as well as the manometric and pH metric in vivo data, which demonstrated the importance of the length of the LES for a sufficient antireflux mechanism. Finally, Stelzner postulated the LES to function like a stretching closure. Based on several experimental and clinical observations, he hypothesized that the spiral muscle fibers of the esophagus achieve the best closing effect when they are stretched, similar to the mechanism of finger traps.[20] Our finding might be an additional element supporting this hypothesis.

A lengthening of esophagus by mobilization would also best explain the decrease of the LES resting pressure in our experiment. As we did not change the position of the manometry during the experiment, it is conceivable that the measurements at different stages of dissection were performed at different sites. Furthermore, it might be that as a result of mobilization the three-dimensional pressure profile of the LES was changed. Korn et al.[21], for example, showed in a recent work that a change of the perimeter of the cardia, which could be the consequence of mobilization, has a significant influence on the three-dimensional pressure profile of the LES. However, it might also be that mobilization led to a real decrease in LES resting pressure in our experiment and that in spite of that a better LES competence was achieved due to a more effective function of the stretched LES. This again would support the hypothesis of Stelzner et al.[20]

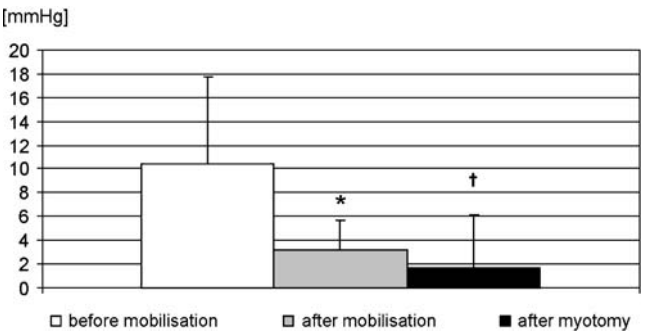

Figure 6 Respective LES pressures before mobilization, after mobilization but before myotomy and after myotomy in the evaluated gastroesophageal reflux porcine model. *p=0.005 (versus "before mobilization"); †p=0.191 (versus "after mobilization").

It remains speculative how and why the LES resting pressure effectively changed between different stages of dissection in our experiment. However, this does not change the essential fact that LES competence improved after mobilization of the esophagogastric junction. This finding raises the question of the relevant step for GER control in surgical antireflux procedures. It seems that this step might be the sole mobilization of the esophagogastric junction which is the common path of all antireflux procedures—fundoplications as well as procedures which strive for GER control solely by hiatoplasty and cardiopexy. In that case, the main function of a fundoplication would be the durable anchoring of the cardia below the diaphragm. However, much more studies are required to strengthen this idea, and the presented porcine GER model may be a help in this regard.

Conclusion

Our acute GER porcine model modified by MII as GER detection instrument proved to be user-friendly, reliable, efficient, and more precise than it was when DBS was used for GER detection. This improvement of acute GER animal models may be helpful to conduct more experimental studies for the better understanding of both the pathophysiology of GERD and the mechanism of antireflux surgery.

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Laparoscopic Local Resection Based on Sentinel Node Evaluation for Early Gastric Cancer: A Preliminary Report

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Abstract We previously reported that lymphatic mapping using isosulfan blue can be used to identify sentinel nodes (SNs). This study was undertaken to evaluate the feasibility of using the SN technique in treating early gastric cancer and to explore its usefulness for minimal invasive surgery. Twenty-three patients with early gastric cancer who underwent SN biopsy were retrospectively evaluated. Based on SN evaluation, individualized surgery was performed in five patients with T1N0M0 gastric cancer. When pathological examination of frozen sections revealed metastasis in SNs, we performed a standard D2 gastrectomy. Laparoscopic local resection was applied when the SN biopsy was negative. Our results showed that the success rate with SN biopsy in early gastric cancer was 100%, as were the accuracy, sensitivity, and specificity. All five patients with early gastric cancer had SNs negative for metastases both by frozen section and by postoperative pathology. Thus, all these patients underwent laparoscopic local resection without extended lymphadenectomy. We conclude that SN biopsy is a useful tool to individualize the operative procedure, and laparoscopic local resection can be safely performed using SN guidance in selected patients with early gastric cancer.

Keywords Early gastric cancer · Laparoscopic local resection · Sentinel node navigation

Introduction

Early gastric cancer (EGC) is defined as adenocarcinoma of the stomach in which the depth of invasion is limited to the mucosa and/or submucosa, regardless of lymph node metastatic status. A treatment widely used for EGC in Japan and in some European countries has been total or subtotal gastrectomy with D2 lymphadenectomy.[1] However, the incidence of lymph node metastasis determined by histology in mucosal and submucosal gastric cancer is 2–4% and 13–20%, respectively.[2] Thus, standard lymph node dissection is unnecessary for patients without lymph node metastasis. Recently, several new surgical procedures for the treatment of EGC with minimal invasiveness, such as laparoscopic, endoscopic, and surgical gastric resections without lymphadenectomy, have been evaluated and described in the literature, especially in studies from Japan.[3] Between 1992 and 2001, 1,428 EGC patients were treated by laparoscopic wedge resection and 260 by intragastric mucosal resection in Japan. Endoscopic mucosal resection has also been widely accepted as a curative therapeutic strategy in selected EGC patients. However, the above-mentioned methods have several restrictions. Firstly, these methods must be applied under strict criteria, which need accurate preoperative evaluation. However, endoscopic ultrasound (EUS) for tumor depth evaluation correctly distinguishes between mucosal and submucosal in approximately 77–84% of tumors; thus, additional treatment may be given to understaged patients.[4] Secondly, lymphadenectomy may not be performed with these methods, and the lymph node status cannot be evaluated even in patients with lymph

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node metastases. Minimal invasive surgery should be performed only when the complete postoperative absence of tumor can be assured. This requirement includes not only the local situation but also lymph node involvement.

The sentinel node (SN) is the first possible site of metastasis along the route of lymphatic drainage from the primary lesion. SN mapping has been a focus of attention in the management of various solid tumors by individualized minimally invasive surgery. Several studies supporting the validity of the SN concept for gastric cancers have been reported in the past 5 years.[5] In particular, T1N0 gastric cancer seems to be a good entity for which to modify the therapeutic approach. SN-based minimally invasive surgery as an individualized tailored surgery has been reported in studies from Japan.

We reported previously that lymphatic mapping using isosulfan blue dye can be used to identify SNs and that the SN concept can be applied to solid tumors including breast and gastric cancer.[6,7] In this study, the feasibility of SN detection in EGC was retrospectively evaluated, and our preliminary experience of laparoscopic local resection under SN-guided surgery is reported.

Table 1 Clinicopathological Characteristics of 23 Patients with EGC

	Patients	
	Number	Percent
Age[a] (years)	51.4±13.1 (26–29)	
Sex		
Male	14	60.9%
Female	9	39.1%
Tumor location		
Upper 1/3	1	4.3%
Middle 1/3	4	17.4%
Lower 1/3	18	78.3%
Tumor size[a] (cm)	3.2±1.6 (0.5–7.0)	
Histology		
Differentiated	6	26.1%
Undifferentiated	16	69.6%
Unknown	1	4.3%
T1 invasion		
Mucosa	13	56.5%
Submucosa	10	43.5%

[a] Mean±SD with range in parentheses

Materials and Methods

Feasibility of SN Mapping in EGC

A total of 23 EGC patients with signed informed consent, who had SN biopsy performed during the operation at the Department of Surgical Oncology, Sir Run Run Shaw Hospital between June 2000 and September 2005, were retrospectively analyzed. The clinical characteristics of the patients are shown in Table 1. The SN technique was started immediately after laparotomy; intraoperative endoscopy was performed to localize the tumor, and then isosulfan blue dye (lymphazurin 1%; USSC, Ben Venue Labs, Bedford, OH) was injected into the peritumoral submucosal layer. After SN biopsy,[6] subtotal or total gastrectomy with D2 lymphadenectomy was performed. All specimens were sent for hematoxylin–eosin (H&E) staining examination. Immunohistochemistry was not routinely performed unless the SNs were negative for metastases by H&E staining. The lymph node groups and stations were classified according to the guidelines of the Japanese Research Society for Gastric Cancer. The diagnostic accuracy, sensitivity, specificity, and false-negative rate of SN biopsies were calculated using the definitions given by Veronesi et al.[8]

Laparoscopic Local Resection Based on SN Evaluation in EGC

Based on the retrospective analysis of SN biopsy in 23 patients with EGC, we then performed laparoscopic local resection based on SN evaluation in patients under the following conditions: preoperative diagnosis of a single mucosal gastric cancer, less than 4.0 cm in diameter, with no involved nodes based on endoscopic examination, EUS, and computed tomography. The lesion was located anteriorly or on the great curvature with sufficient distance from the cardia. The indications were widened if the patients' performance status may not allow for radical surgery. Signed informed consent was obtained from all patients before the surgery.

The surgical procedures were all performed laparoscopically under pneumoperitoneum with carbon dioxide. After the lesion was localized by intraoperative endoscopy, 1 ml of isosulfan blue dye was injected into the submucosal layer at the four leading edges of the carcinoma (Fig. 1a). A local resection line at the serosal surface, approximately 2 cm outside the margin of the lesion, was marked using an electrocoagulation hook with the help of endoscopy from inside the stomach. Within 5 min of dye injection, lymphatic flow from the lesion was visible (Fig. 1b). The perigastric area dyed by isosulfan blue was dissected using an ultrasonic scalpel and extracted. Several nodes, including the dyed ones, were isolated (Fig. 1c). After perigastric area dissection, local resection was performed at the resection line. The resected specimen with the marked resection margin (Fig. 1d) and the perigastric tissue including the dyed nodes were assessed by frozen section analysis during surgery. When pathological examination of the frozen sections revealed that the lesion was invasive to T1 with a clear resection margin and the SNs were negative for metastasis, we then completed the operation after the gastric defect was closed. Extended D2 gastrectomy was

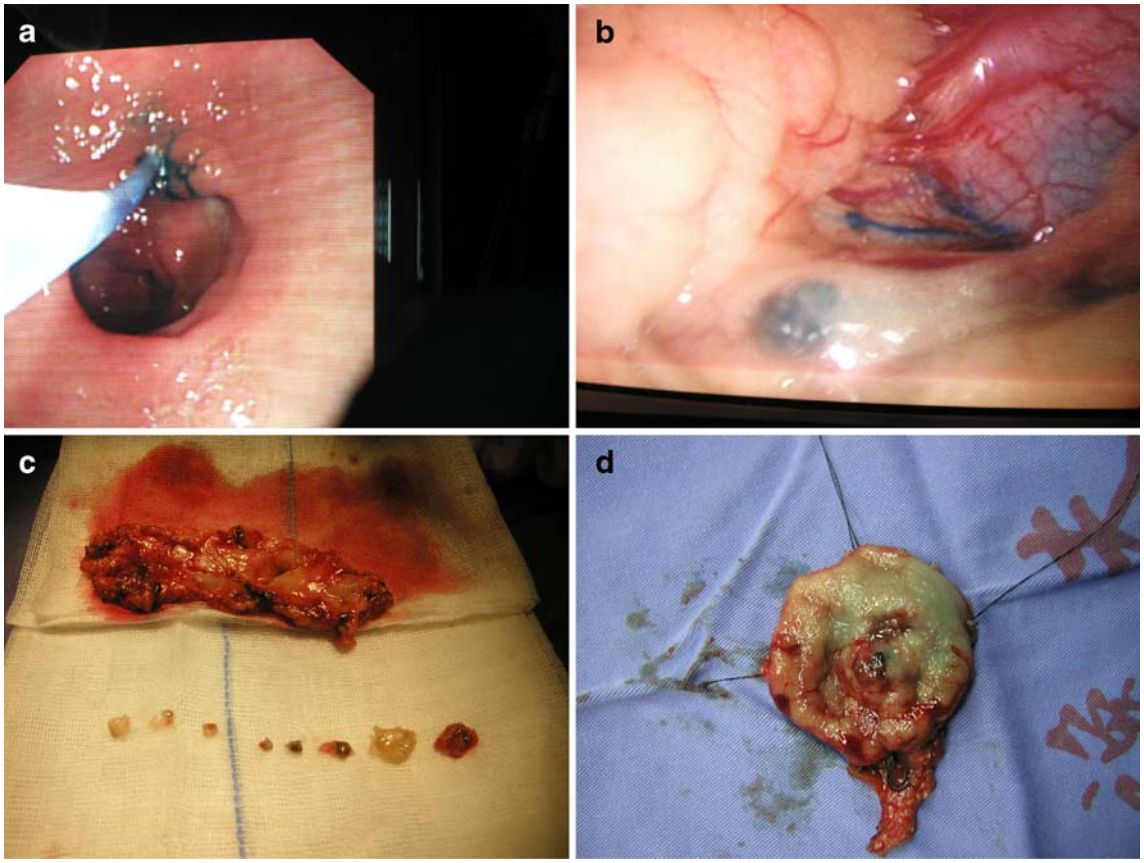

Figure 1 Procedure of laparoscopic local resection with sentinel node biopsy. **a** Isosulfan blue was injected into the peritumoral submucosal layer after the lesion was localized by intraoperative endoscopy. **b** Within 5 min of dye injection, lymphatic flow from the lesion was visible. **c** Sentinel nodes were isolated while perigastric tissue was extracted. **d** Local resection line was marked at the four edges of the resection margin.

applied if the result of the SN biopsy was positive, and delayed radical surgery was carried out if SNs were negative in frozen sections but positive by permanent examination.

The patients were followed with physical examination, ultrasonography, and/or radiographic and endoscopic upper gastrointestinal examination every 3 months during the first 2 years and every 6 months during the next 3 years. The patients were followed up until death or until the date of last follow-up of June 30, 2007. The median follow up interval was 17 months (range, 9–21 months).

Results

Feasibility of SN Mapping in EGC

From the 23 patients with EGC, a total of 555 nodes were harvested. The median number of retrieved nodes per patient was 20. Seven (1.3%) of these were positive for carcinoma by permanent examination. Four (17.4%) patients had histological evidence of metastasis to nodes, which were all located at the N1 station, and all these patients had tumor invasion to the submucosal layer.

SNs were detected in all patients. The total number of SNs was 67, with a median of 2.9 (range, 1–7) per patient. Twenty-two patients had SNs only at the N1 station, and one (4.3%) had SNs at both N1 and N2 stations. Positive SNs were found by routine H&E staining in three patients, and two of them also had positive nonsentinel lymph nodes. SN micrometastases were identified by immunohistochemical examination in one patient who had no histological evidence of nodal involvement, and these micrometastases were found only within SNs. All the positive SNs were located at the N1 station. The accuracy, sensitivity, and specificity of SN biopsies in EGC patients are shown in Table 2.

Laparoscopic Local Resection Based on SLN Evaluation in EGC

From September 2005, we performed laparoscopic local resection under SN guidance for five EGC patients. All were confirmed to have EGC both by frozen sections and permanent examination. SN biopsy was successfully achieved in all patients, and all SNs were negative for metastases by intraoperative frozen section and routine H&E staining as well as immunohistochemistry. Thus, under the guidance of SN

Table 2 Sentinel Node Biopsy in 23 Patients with EGC

	No. of patients	Percent
Regional lymph node positive	4	4/23 (17.4%)
Sentinel node positive	4	4/23 (17.4%)
Sentinel node only positive	2	2/4 (50%)
Sentinel node negative and non-sentinel node positive	0	0/4 (0%)
Sensitivity	4	4/4 (100%)
Specificity	19	19/19 (100%)
Accuracy	23	23/23 (100%)
False negative rate	0	0/4 (0%)

biopsy, the five patients received laparoscopic local resection without extended lymphadenectomy (Table 3). All recovered well after surgery, and no recurrence or distal metastasis was detected during follow–up.

Discussion

In this study, we retrospectively analyzed the validity of SN mapping in gastric cancer and found that SN biopsy was a useful tool to evaluate the lymph node status in patients with EGC. On this basis, we found that laparoscopic local resection with SN-based evaluation is a feasible procedure for selected patients.

Previous studies reported that over 80% of EGC patients with no lymph node metastasis had received unnecessary lymphadenectomy although excellent 5-year survival rates were achieved.[9] To improve the treatment of EGC, a variety of minimally invasive strategies include local resection without reducing the curability have been introduced to avoid unnecessary tissue resection. The critical criteria for determining whether local resection is appropriate remain the local absence of tumor and the absence of lymph node metastases.[10]

For local resection of the tumor, it is debatable which margin is necessary in early lesions. Yokota et al.[11] considered that the resection line should be at least 1 cm from the tumor margin or there would be a high risk of local recurrence. Seto et al.[12] performed laparoscopic local resection in 24 EGC patients using the principle of local resection of the stomach with 2 cm cancer-free margin, and there were no recurrences during 3 years of follow-up. The study of Ohgami et al.[13] showed that a resection line 1.5± 0.5 cm outside the tumor margin is enough to achieve a clear margin during laparoscopic wedge resection. In our study, local resection was made 2 cm outside the tumor margin, and no patient had microscopic invasion of the margin, indicating that local absence of tumor was achieved after local resection.

Before the advent of SN techniques, local resection was usually performed in EGC patients with lower risk of lymph node metastasis. In large retrospective studies, the prediction of lymph node status was mainly based on the depth of invasion, tumor size, and histology.[14] Thus, preoperative assessment of invasion depth and local lymph node involvement of EGC take on extreme significance. EUS allows relatively exact preoperative assessment of local invasion depth but cannot deliver the necessary diagnostic accuracy in every case. Therefore, the absence of lymph node metastasis in local resection, even with regional lymphadenectomy, was not completely certain.[15]

As a means to resolve this issue, the SN concept may become a major diagnostic tool for identification of clinically undetectable lymph node metastasis in patients with EGC. In the past 5 years, single institutional studies supporting the validity of the SN concept for GI cancers have been reported. Hiratsuka et al. reported 100% sensitivity in T1 lesions.[16] Isozaki et al.[17], in a regional multicenter clinical trial of SN mapping for gastric cancer using the conventional dye-guided method, as well as previous single institutional reports, supported the idea that dye-guided SN mapping may be feasible for T1N0 patients after the establishment of a standard procedure and the completion of the learning phase. In our study, retrospective analysis showed that the sensitivity, specificity, and accuracy of SN biopsy in EGC were 100%. These results, as well as others, indicate that lymphatic mapping using isosulfan blue is technically feasible in the setting of EGC. We then used the SN technique to indicate the absence of lymph node metastasis and to resort to minimally invasive

Table 3 Cases of Laparoscopic Local Resection Under Sentinel Node Guidance

Patient	Age/ sex	Location	Preoperative diagnosis	Gross type	Histology	Size (cm)	Depth of invasion	mLN/ rLN	mSN/ rSN
1	70/M	Lower 1/3	HGN	Iic	Differentiated	1.5	Mucosa	0/2	0/2
2	78/M	Lower1/3	HGN	Iic	Undifferentiated	3.5	Submucosa	0/6	0/2
3	52/F	Lower1/3	Mucosa	III	Undifferentiated	0.8	Mucosa	0/11	0/10
4	72/F	Lower1/3	HGN	Iic	Differentiated	3.0	Submucosa	0/7	0/4
5	48/M	Lower1/3	HGN	Iic	Differentiated	0.2	Mucosa	0/3	0/2

mLN/rLN Metastatic lymph nodes/retrieved lymph nodes; *mSN/rSN* metastatic sentinel nodes/retrieved sentinel lymph nodes; *HGN* high-grade neoplasia

surgery in five EGC patients. Four patients preoperatively diagnosed with high-grade neoplasia were intraoperatively confirmed to have EGC, and one of them had deep tumor invasion to the submucosa. SNs were identified in all patients. Laparoscopic local resection was successfully performed when SNs were confirmed to be negative for metastasis by frozen section in all patients. Thus, our preliminary results indicate that a combination of laparoscopic local resection and SN biopsy appears to be feasible, practical, and useful for some patients with EGC.

Based on our results, several issues need further discussion. We, as well as previous investigators, consider SN evaluation during open surgery as a useful tool in EGC. However, whether the same validity can be obtained during laparoscopic surgery needs further exploration. At the same time, lymph node micrometastases are not uncommon in EGC patients; in our study, one out of four patients had immunohistochemical evidence of SN micrometastasis. Therefore, improvements in the accuracy of intraoperative micrometastasis detection methods are needed to make SN-guided surgery more secure.

Conclusion

SN mapping using isosulfan blue is a feasible tool for early gastric cancer. SN-guided surgery promises to be a safe and promising approach for local resection in patients with EGC. However, more case studies are needed before an optimal strategy in laparoscopic local resection with SN biopsy for early gastric cancer can be established.

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Borrmann Type IV: An Independent Prognostic Factor for Survival in Gastric Cancer

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Abstract
Background Borrmann type IV gastric cancer has a poorer prognosis than other gastric carcinomas. This study compared the clinicopathological features of Borrmann type IV gastric cancer with those of other types of cancer and examined the significance of a Borrmann type IV carcinoma as a prognostic factor after gastrectomy.
Methods The clinicopathological features, tumor–node–metastasis (TNM) stage, and survival rates of 4,191 advanced gastric cancer patients, who had undergone a gastrectomy at the Samsung Medical Center between 1995 and 2005, were reviewed.
Results Borrmann type IV gastric cancer was found to be associated with more advanced and unfavorable clinicopathological features at diagnosis than the other cancers. The 5-year survival rate of the patients with Borrmann type IV cancer was 27.6%. In contrast, the 5-year survival rate of patients with the other types of cancer was 61.2%. The 5-year survival rate for each stage of Borrmann type IV gastric cancer and the other type gastric cancer was 61.0% and 88.8% for stage Ib ($P<0.001$), 49.8% and 76.1% for stage II ($P<0.001$), 36.4% and 55.1% for stage IIIa ($P<0.001$), 15.2% and 38.5% for stage IIIb ($P=0.001$), and 10.2% and 20.1% for stage IV ($P=0.008$), respectively. Multivariate analyses revealed a Borrmann type IV carcinoma, the surgical extent, curability, tumor stage, including T, N, and M status, and adjuvant therapy to be independent prognostic factors for survival.
Conclusion A Borrmann type IV carcinoma has unique clinicopathological features compared with other types of gastric carcinomas and is an important independent prognostic factor.

Keywords Gastric cancer · Bormann type IV · Prognostic factor · Gastrectomy

Introduction

The Borrmann classification system was developed in 1926 and is widely used to describe the endoscopic or gross findings. Borrmann type IV is characterized by poorly differentiated tumor cells that involve the diffusely infiltrative involvement of the stomach. The incidence of a Borrmann type IV gastric cancer is 11–13% of all advanced gastric carcinomas.[1,2] Although there have been substantial advances in various diagnosis and treatment modalities, which have resulted in the development of minimally invasive treatment techniques and the increased concern for the quality of life, most Borrmann type IV gastric cancers are not detected in the early stage and show a poor prognosis. Despite a curative resection increasing the survival rate up to 38.4 %, the overall 5-year survival rate of patients with Borrmann type IV gastric cancer ranges from 12.5% to 24.3%.[2–4]

Many studies have analyzed the clinicopathological features and prognosis of Borrmann type IV gastric cancer. However, the tumor stage including the depth of invasion, the number of lymph node metastases, the presence of a distant metastasis, and the therapeutic options need to be considered simultaneously when determining the prognostic impact of some variables. This is because these factors are the most important prognostic factors of gastric cancer. Therefore,

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there is some controversy regarding the role of Borrmann type IV gastric carcinoma as an independent factor for survival. More accurate tumor staging is possible when a gastrectomy was performed, which induces a feasible examination of the Borrmann type IV gastric cancer as a significant prognostic factor. In this study, we retrospectively compared the clinicopathological features of Borrmann type IV gastric cancer with other types of gastric cancer and analyzed the postoperative outcome at each stage of patients who underwent a gastrectomy, which could support establishing the therapeutic strategy of Borrmann type IV gastric cancer.

Patients and Methods

Between 1995 and 2005, 4,191 patients were diagnosed with a primary advanced gastric cancer after a gastrectomy. Patients with a prior gastric surgery history were excluded. Of the advanced gastric cancers examined, 136 (3.2%) were Borrmann type I, 831 (19.8%) were Borrmann type II, 2,618 (62.5%) were Borrmann type III, 555 (13.2%) were Borrmann type IV, and 51 (1.2%) were Borrmann type V. The patients with Borrmann type IV gastric cancer were compared with those with the other types of gastric cancer. The clinical features and variables for each group, including gender, age, tumor location, tumor size, type of operation, histological type, recurrence, and survival were analyzed from the patients' medical records.

A R0 resection was defined as a complete resection of the localized tumors without a distant metastasis. A R1 resection was defined as a gross removal of tumors with microscopic resection margin involvement. A R2 resection was defined as grossly residual tumors. An extended gastrectomy included a resection of the adjacent organs such as the spleen, colon, pancreas, small bowel, liver, and kidney in addition to a subtotal or total gastrectomy.

The tumors were staged according to the sixth edition of the International Union Against Cancer classification. The maximum diameter was recorded as the tumor size. The tumor histology was classified into two groups: the differentiated type, which included a papillary adenocarcinoma, well- or moderately differentiated adenocarcinoma; and the undifferentiated type, which included poorly or undifferentiated adenocarcinomas, signet ring cell carcinomas, and mucinous carcinomas.

The categorical variables were compared using a chi-square test. The continuous data is presented as a mean± SD, and a Mann–Whitney *U* test was used for the statistical comparisons. The Kaplan–Meier method was used to calculate the survival distribution (in months) of the two groups and was determined from the primary surgical treatment to the final follow-up or death. The univariate association between the various parameters and survival was determined using a log-rank test. Multivariate analysis of the parameters influencing the survival was carried out using the Cox proportional hazards regression model. In all statistical analyses, $P<0.05$ was considered significant.

Results

Comparison of Borrmann Type IV with the other Types of Gastric Cancer

Table 1 summarizes the clinicopathological findings of the 555 patients with Borrmann type IV gastric cancer and the 3,636 patients with the other type gastric cancer. There

Table 1 Clinicopathological Characteristics of Borrmann Type 4 Gastric Cancer

Factors	Bormann 1, 2, 3 (n=3,636; %)	Bormann 4 (n=555; %)	P value[a]
Age (years)	56.2±12.3	54.0±13.0	<0.001[b]
Gender			<0.001
Male	2567 (67.8)	289 (52.1)	
Female	1169 (32.2)	266 (47.9)	
Location			<0.001
Upper 1/3	512 (14.2)	109 (19.6)	
Middle 1/3	1132 (31.1)	238 (42.9)	
Lower 1/3	1973 (54.3)	166 (29.9)	
Entire	14 (0.4)	42 (7.6)	
Operation type			<0.001
Partial gastrectomy	2139 (63.8)	139 (25.0)	
Total gastrectomy	776 (21.3)	201 (36.2)	
Combined resection	541 (14.9)	215 (38.7)	
Curability			<0.001
R0	3326 (91.5)	438 (78.9)	
R1	277 (7.6)	106 (19.1)	
R2	33 (0.9)	11 (2.0)	
Tumor size (cm)	5.6±2.5	10.0±4.1	<0.001
Depth of invasion			<0.001
T2	2498 (68.7)	224 (40.4)	
T3	978 (26.9)	276 (49.7)	
T4	160 (4.4)	55 (9.9)	
Nodal status			<0.001
N0	1047 (28.8)	89 (16.0)	
N1	1331 (36.6)	142 (25.6)	
N2	761 (20.9)	130 (23.4)	
N3	497 (13.7)	194 (35.0)	
Distant metastasis			<0.001
M0	3437 (94.6)	485 (87.4)	
M1	198 (5.4)	70 (12.6)	
Stage			<0.001
Ib	889 (24.4)	50 (9.0)	
II	1504 (29.0)	95 (17.1)	
IIIa	739 (20.3)	98 (17.7)	
IIIb	233 (6.4)	57 (10.3)	
IV	721 (19.8)	255 (45.9)	

Table 1 (continued)

Factors	Bormann 1, 2, 3 (n=3,636; %)	Bormann 4 (n=555; %)	P value[a]
Histologic type			<0.001
Differentiated	1355 (37.3)	41 (7.4)	
Undifferentiated	2281 (62.7)	514 (92.6)	
Lauren classification			<0.001
Intestinal	1518 (41.7)	49 (8.8)	
Diffuse	1958 (53.9)	487 (87.7)	
Mixed	160 (4.4)	19 (3.4)	
Lymphatic involvement			<0.001
No	1836 (50.5)	232 (41.8)	
Yes	1800 (49.5)	323 (58.2)	
Venous involvement			0.758
No	3285 (90.3)	499 (89.9)	
Yes	351 (9.7)	56 (10.1)	
Neural involvement			<0.001
No	2598 (71.5)	288 (51.9)	
Yes	1038 (28.5)	267 (48.1)	
Adjuvant therapy			0.003
None	1802 (49.6)	233 (42.0)	
Chemotherapy	924 (25.4)	267 (30.1)	
Chemoradiotherapy	910 (25.0)	155 (27.9)	

[a] Chi-square test unless otherwise
[b] Mann–Whitney U test

were significant differences in the distribution of gender, age, tumor location, operation type, surgical curability, tumor size, depth of invasion, lymph node metastasis, distant metastasis, stage, differentiation, Lauren classification, lymphatic involvement, and perineural involvement between the two groups. All these factors suggest that Borrmann type IV gastric cancer is definitely associated with more advanced and unfavorable clinicopathological features at diagnosis.

Survival Analysis at Each Stage

During a median follow-up of 68.2 months, the overall 5-year survival rate in patients with Borrmann type IV and other types of cancer was 27.6% and 61.2%, respectively (P<0.001). The overall 5-year survival rate in patients with the other types of cancer was 74.2%, 64.6%, 59.0%, and 88.1% for types I, II, III, and V, respectively. Figure 1 shows the 5-year cumulative survival curves for each stage. The 5-year survival rate for each stage in the Borrmann type IV gastric cancer group and the other type gastric cancer group was 61.0% and 88.8% for stage Ib (P<0.001), 49.8% and 76.1% for stage II (P<0.001), 36.4% and 55.1% for stage IIIa (P<0.001), 15.2% and 38.5% for stage IIIb (P=0.001), and 10.2% and 20.1% for stage IV (P=0.008), respectively. The difference in the survival curve decreased considerably at stages IIIb and IV.

Prognostic Impact Analysis

The surgical extent, curability, Borrmann type, tumor stage including the tumor (T), node (N), and metastasis (M) status and adjuvant therapy were found to be independent prognostic factors for survival (Table 2). The relative hazard (RH) value of the extended resection and R1 group was 1.579 and 2.478 (P<0.001). The RH of R2 was 1.582, but there was no statistical significance possibly due to the small number of patients. The RH of Borrmann type IV was 1.443 (P<0.001). Patients who underwent chemotherapy only and no adjuvant therapy had a higher RH (1.423 and 2.313) than those who underwent chemoradiotherapy.

Prognostic Factors in Patients with Borrmann Type IV Gastric Cancer

The surgical extent, curability, tumor stage including T and N status, and adjuvant therapy were found to be independent prognostic factors for the survival of Borrmann type IV gastric cancer patients (Table 3). A combined adjacent organ resection with a gastrectomy showed a RH of 1.816 with statistical significance compared with the gastrectomy group. The RH of the R1 and R2 groups was 2.145 (P<0.001) and 1.981, respectively (P=0.252). The no adjuvant therapy group had a significantly higher RH value than the adjuvant therapy group including chemotherapy and chemoradiotherapy. Age, gender, tumor location, tumor size, histological type, and Lauren classification were not determined to be prognostic factors by multivariate analysis.

Discussion

Borrmann type IV gastric cancer has been reported to have characteristic clinicopathological features including a young female prominence, detection at an advanced stage, high frequency of peritoneal or distant metastasis at diagnosis, a low rate of curative surgery, and frequent peritoneal recurrence, even after a curative resection.[1–5] Therefore, many studies have been carried out to identify potential prognostic markers as well as adequate treatment strategies with a satisfactory outcome. In this study, only patients who underwent a gastrectomy were included for more accurate evaluation, and we found that Borrmann type IV gastric cancer showed markedly different features from other types of gastric cancer, and most variables indicated Borrmann type IV to be a detrimental factor. These findings are in agreement with previous reports and the generally described nature of the disease.[4,6]

Although Borrmann type IV gastric cancer is associated with a poor prognosis, the role of Borrmann type IV gastric carcinoma as an independent factor for survival is contro-

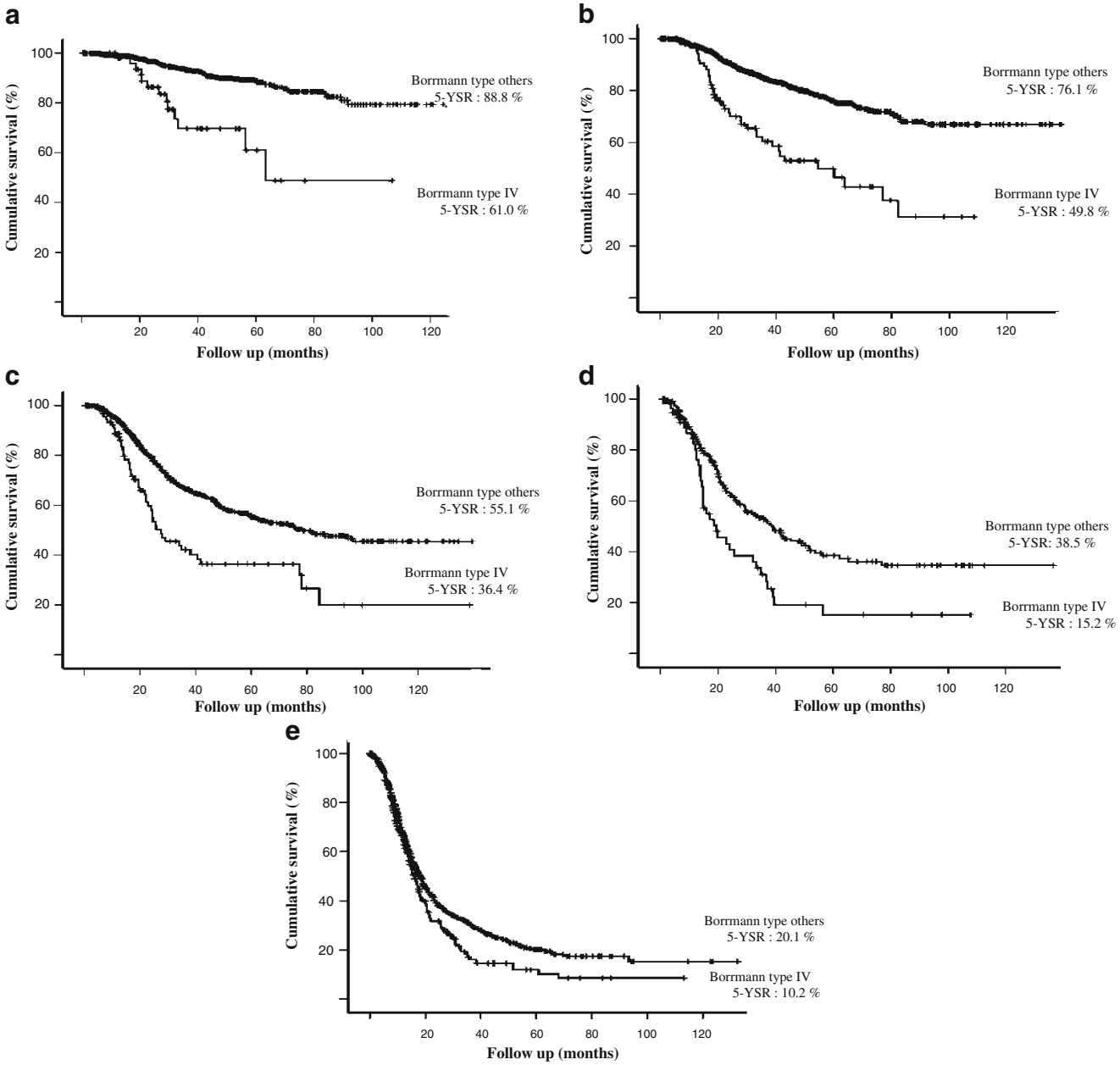

Figure 1 **a** Cumulative survival curves for stage Ib gastric cancer, **b** cumulative survival curves for stage II gastric cancer, **c** cumulative survival curves for stage IIIa gastric cancer, **d** cumulative survival curves for stage IIIb gastric cancer, **e** cumulative survival curves for stage IV gastric cancer.

versial. Some authors reported that Borrmann type IV is not an independent prognostic factor and suggested the TNM stage and curability to be the main determinant for survival.[3] They found postoperative survival curve showed significant difference between the Borrmann type IV and other type gastric cancer only at stage III. On the other hand, other studies reported that Borrmann type IV gastric carcinoma is an independent prognostic factor for survival.[13] In this study, multivariate analysis highlighted the importance of Borrmann type IV gastric carcinoma as a variable predicting the postoperative survival. The postoperative survival curves showed significant difference between the two groups at each stage. It seems that a large number of patients contribute to verify the difference in survival curves. The hazard ratio in Borrmann type IV patients, 1.443, also supports the prognostic impact of Borrmann type IV gastric cancer.

Otsuji et al. reported that the 5-year survival of patients with Borrmann type IV was 10.5% compared with 35.1% for patients with the other types of advanced gastric cancer.[13] Kim et al. reported that the 5-year survival rates of gastric cancer patients was 48.5%, 58.9%, 37.4%, and

Table 2 Multivariate Analysis of Prognostic Variables in Patients with Gastric Cancer

Variables	Hazard ratio	95% CI	*P* value
Operation type			
Subtotal gastrectomy			
Total gastrectomy	1.033	0.996–1.192	0.653
Extended resection	1.579	1.386–1.800	<0.001
Curability			
R0			
R1	2.478	2.005–3.062	<0.001
R2	1.582	0.821–3.046	0.170
Borrmann type			
Other type			
Borrmann type IV	1.443	1.258–1.656	<0.001
Depth of invasion			
T2			
T3	1.850	1.641–2.086	<0.001
T4	2.291	1.866–2.813	<0.001
Nodal status			
N0			
N1	2.530	2.803–3.076	<0.001
N2	4.512	3.689–5.519	<0.001
N3	7.474	6.080–9.187	<0.001
Distant metastasis			
M0			
M1	1.344	1.064–1.698	0.013
Adjuvant therapy			
Chemoradiotherapy			
Chemotherapy	1.423	1.215–1.666	<0.001
No	2.313	2.012–2.660	<0.001

Values are calculated by Cox proportional hazard model.
CI Confidence interval

21.8% for Borrmann types I, II, III, and IV, respectively.[2] In this study, the 5-year survival rates of patients with types I, II, III, and IV were 74.2%, 64.6%, 59.0%, and 27.6%, respectively, which are better than in other reports.[1,3,4,14] It is believed that the reason for this is that this study enrolled patients who had undergone a gastrectomy. In addition, the 5-year survival rate of stage Ib gastric cancer with Borrmann type IV was 61.0%, which are poorer than that of stage II gastric cancer with other type (76.1%). This result suggests that the necessity of aggressive adjuvant therapy in stage Ib gastric cancer with Borrmann type IV should be considered. Although there was a significant difference in the survival curve at each stage between Borrmann type IV cancer and the other types of cancer, the impact of Borrmann type IV on survival was considerably lower in stage IV gastric cancer, which means that stage IV gastric cancer is not a local disease but systemically propagative disease.

The prognostic factors in patients with Borrmann type IV gastric cancer were the surgical type, curability, depth of invasion, lymph node metastasis, and adjuvant therapy. These results are in agreement with other studies because both an extended adjacent organ resection and non-curative resection indicated an advanced disease stage including the tumor depth and lymph node metastasis.[3,4,13,14] Adjuvant therapy, either chemotherapy or chemoradiotherapy, can offer a similar survival benefit to patients with Borrmann type IV gastric cancer. This is in contrast to the results summarized in Table 2, which showed that adjuvant chemoradiotherapy is superior to chemotherapy or no adjuvant therapy in gastric cancer.

Because there is no appropriate therapeutic strategy for Borrmann type IV gastric cancer, various types of therapy including chemotherapy, hormone therapy, hyperthermia, and immunotherapy have been examined for their potential survival benefit.[15–18] Kunisaki et al. suggested that in scirrhous type gastric cancer, a gastrectomy with an extended lymph node dissection is justified only in patients with a limited lymph node metastasis, and a palliative gastrectomy should not be performed.[19] There are reports showing that a gastrectomy should be avoided in Borrmann type IV gastric cancer patients with a positive peritoneal washing cytology.[5,20] In this study, 78.9% of Borrmann type IV gastric cancer patients underwent a R0 resection, and they had a better survival than the non-curative resection patients, which can be applied to gastric cancer patients generally. Recently, chemotherapy including TS-1 was reported to have a greater anti-tumor effect and improve the survival time for Borrmann type IV gastric cancer than conventional chemotherapy including 5-FU, cisplatin, methotrexate, or mitomycin.[16] In

Table 3 Multivariate Analysis of Prognostic Variables in Patients with Borrmann Type IV Gastric Cancer

Variables	Hazard ratio	95% CI	*P* value
Operation type			
Subtotal gastrectomy			
Total gastrectomy	1.205	0.868–1.674	0.264
Extended resection	1.816	1.331–2.478	<0.001
Curability			
R0			
R1	2.145	1.605–2.867	<0.001
R2	1.981	0.616–6.373	0.252
Depth of invasion			
T2			
T3	1.539	1.182–2.003	0.001
T4	2.108	1.404–3.166	<0.001
Nodal status			
N0			
N1	1.596	1.045–2.436	0.031
N2	2.504	1.662–3.772	<0.001
N3	3.133	2.122–4.625	<0.001
Adjuvant therapy			
Chemoradiotherapy			
Chemotherapy	1.199	0.869–1.656	0.269
No	2.201	1.660–2.916	<0.001

Values are calculated by Cox proportional hazard model.

this study, adjuvant chemoradiotherapy based on leucovorin and 5-FU was performed on patients with a potentially curative resection. Patients with metastatic lesions received taxane and cisplatin-based chemotherapy. However, there have been changes to the adjuvant therapy regimen because there is no internationally accepted standard of care. The development of adjuvant therapy considering the biological behavior of cancer cells and the molecular interaction in a Borrmann type IV gastric carcinoma is essential for determining the therapeutic strategy and for achieving a favorable outcome.

The mechanisms for why Borrmann type IV gastric cancer has a poor prognosis have been investigated from a wide variety of pathologies. There are reports showing that transforming growth factor β1, which is secreted from scirrhous gastric cancer cells and is associated with excess collagen deposition in the tissue, up-regulates CD44H expression and stimulates the peritoneal metastatic ability of the scirrhous gastric cancer cells.[7,8] The low expression of the intercellular adhesion molecule, E-cadherin, in scirrhous gastric cancer cells suggests that scirrhous cancer cells might easily migrate to the peritoneal cavity and disseminate a peritoneal metastasis.[9,10] Yanagihara et al. proposed mouse models of human scirrhous stomach cancer for the development and biological analysis of a peritoneal metastasis.[11] Tanigawa et al. reported significantly higher vessel counts in Borrmann type IV gastric carcinomas than in other types of carcinomas and suggested that tumor angiogenesis might be closely related not only to the development of type 4 carcinomas but also to their poorer prognosis after surgery.[12] Because our study is a retrospective review and summarizes a 10-year experience of a single institute, it is insufficient to account for the causes of the poor prognosis of Borrmann type IV gastric cancer. We hope to present more advanced and unique results following this work before long.

In conclusion, compared with the other types of gastric carcinoma, a Borrmann type IV carcinoma has unique clinicopathological features and a poor survival outcome after a gastrectomy at each stage. Borrmann type IV is one of the important independent prognostic factors which also include the surgical curability, tumor stage, and adjuvant therapy. Overall, the suitable multimodal and intensive treatments are required in patients with Borrmann type IV gastric carcinoma.

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Salvage Gastrectomy Following a Combination of Biweekly Paclitaxel and S-1 for Stage IV Gastric Cancer

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Abstract
Background and aim We investigated the clinical benefits of salvage gastrectomy for stage IV gastric cancer patients whose distant lesions showed complete response after chemotherapy.
Methods We enrolled 18 stage IV gastric cancer patients whose distant metastases had disappeared or were controlled by a combination of biweekly paclitaxel (PTX) and S-1. After chemotherapy, these patients received gastrectomy with lymph node dissection. The postoperative outcome was analyzed with respect to both the histological effects of chemotherapy and tumor behavior.
Results Of the 18 patients, 8 had distant lymph node metastases, 9 had peritoneal dissemination, and five had multiple liver metastases prior to chemotherapy. Fourteen patients received curative surgery (R0). No severe postoperative complications were encountered. Pathological evaluation revealed grade 3 and grade 2 tumor regression in the primary lesion in one and five patients, respectively, and grade 3 and grade 2 tumor regression in the lymph nodes in one and six patients, respectively. Univariate analysis of the patients' prognosis identified R number, gross tumor type, histological grade of tumor regression, and gender as significant factors. Multivariate analysis showed that only the R number was an independent prognostic factor.
Conclusion R0 salvage gastrectomy following a combination of biweekly PTX and S-1 may have significant clinical efficacy for advanced gastric cancer patients.

Keywords Salvage surgery · Gastric cancer · Paclitaxel · S-1

Introduction

The prognosis of unresectable advanced gastric cancer has been very poor, despite the widespread use of anticancer drugs such as 5FU, MMC, adriamycine, and *cis*-diammine-dichloroplatinum (CDDP), because these drugs are not highly effective and chemotherapy alone does not prolong survival in gastric cancer patients.[1–3] At the beginning of the twenty-first century, new anticancer drugs, such as taxanes, S-1, capecitabine, and CPT-11, were introduced for the treatment of advanced gastric cancer. Combination regimens using these new anticancer drugs have been proposed and high efficacy for advanced gastric cancer has been reported by many clinicians.[4–7] Phase I and phase II clinical trials of combined biweekly paclitaxel (PTX) and S-1 for unresectable advanced gastric cancer were initiated at Kagoshima University Hospital in October 2001.[8] During these trials, we encountered patients whose distant metastases had apparently disappeared or were controlled by chemotherapy. For these patients, we aggressively performed salvage gastrectomy with curative intent. This salvage operation has been performed for various types of tumors, such as

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ovarian,[9] testicular,[10] and esophageal tumors,[11] and is widely accepted for these conditions; however, there is controversy regarding the clinical value of salvage surgery for advanced gastric cancer when performed after chemotherapy.[12–16] Since the advent of the latest generation of chemotherapeutic drugs for gastric cancer, there has been a paucity of information on the value of salvage surgery after chemotherapy in gastric cancer patients. The aim of the current study was to investigate the clinical value of salvage surgery for advanced gastric cancer.

Patients and Methods

Patients

The study included 18 patients with histologically confirmed, advanced gastric carcinoma with distant metastasis, who were treated with a combination of biweekly PTX and S-1 at Kagoshima University Hospital, Japan. Distant lesions were confirmed by computerized tomography (CT) or laparoscopy and all patients were confirmed as UICC stage IV. The median age of this patient population was 63 years (range 33–77 years), and the male/female ratio was 11:7. Nine had peritoneal dissemination, nine had distant lymph node metastases, and five had liver metastases (Table 1). Fourteen of the 18 patients received R0 salvage gastrectomy after the disappearance or control of distant metastases following chemotherapy.

Table 1 Patients' Information

Characteristic	Data
Gender	
Male	11
Female	7
Age	64 (33–77)
Gross type	
2	2
3	8
4	8
Histology	
Differentiated	6
Undifferentiated	12
Distant site	
Para-aortic lymph node	9
Liver	5
Peritoneum	9
Operation method	
Distal gastrectomy	6
Total gastrectomy	12
R classification	
R0	14
R2	4

R0, no residual tumor; R2, macroscopic residual tumor

Chemotherapeutic Regimen

Preoperative chemotherapy was performed according to a previous report.[8] A total of 120 mg/m^2 of PTX was administered by intravenous drip infusion over 1 h on days 1 and 14. S-1 at a dose of 80 mg/m2 was consecutively administered twice a day from days 1 to 14, followed by a 14-day recovery period.

Evaluation of the Clinical Response of Primary and Metastatic Lesions and Timing of Salvage Operation

Chemotherapy was continued until distant lesions were controlled or had disappeared. The clinical response of measurable disease was evaluated according to the response evaluation criteria of solid tumors.[17] Complete regression of immeasurable lesions such as malignant ascites and peritoneal seeding was confirmed by intraoperative laparoscopy before gastrectomy. To confirm the complete response (CR) of multiple liver metastases, hepatic lesions were reevaluated by enhanced magnetic resonance imaging. After confirmation of continuity of CR with two courses of chemotherapy, salvage gastrectomy with lymph node dissection, including previous metastatic sites, was performed with curative intent.

Histopathological Evaluation of Chemotherapeutic Efficacy and Grading

The resection specimens were prepared for pathological examination. Namely, after photographic documentation, lymph nodes were separated from the stomach and classified according to the designated lymph node number of the Japanese Classification of Gastric Carcinoma.[18] The stomach and lymph nodes were formalin-fixed. All areas of macroscopically identifiable residual gastric tumor were cross-sectioned serially at a thickness of 4 mm. The sections were stained with hematoxylin and eosin (HE), elastic von Gieson stain, and periodic acid Schiff stain and prepared for histopathological examination. Lymph nodes were cut and stained with HE. The histological grade of tumor regression was classified based on three categories according to the Japanese Classification of Gastric Carcinoma. Namely, grade 1, less than two-thirds of tumor shrinkage; grade 2, more than two-thirds of tumor shrinkage but incomplete; and grade 3, complete regression.

Postoperative Follow-up

Postoperatively, the patients received follow-up CT every 3 months at an outpatient clinic. Basically, six courses of

combination of PTX and S-1 were given to prevent the recurrence of gastric cancer. Prophylactic S-1 treatment was performed in the early postoperative course. Postoperative follow-up periods ranged from 3 to 38 months.

Postoperative Survival and Relapse Analysis

Patient outcomes were analyzed according to clinical pathological factors such as histological grade of tumor regression, gross tumor type, and relapse form of cancer. Survival curves were calculated by the Kaplan–Meier method from the initial date of treatment and were analyzed using the generalized Wilcoxson test. Univariate analysis was performed by the log-rank test, and clinical factors were evaluated as independent prognostic factors by multivariate analysis using the Cox proportional hazard model. A p value of less than 0.05 was regarded as significant.

Results

The 18 patients who received salvage gastrectomy following a combination of biweekly and S-1 had no critical chemotherapy-induced adverse effects. The average course of chemotherapy with S-1 and PTX was 6.5 courses (from 2 to 14, median=6 courses) before surgery. Prior to gastrectomy, nine patients who had peritoneal metastasis before chemotherapy received laparoscopy and, intraoperatively, eight were confirmed as peritoneal dissemination-negative during operation. All 14 R0 patients received more than D2 lymph node dissection. Three patients received D3 node dissection because they had positive para-aortic nodes prior to chemotherapy. Extensive resection of the colon (three cases), distal pancreas (two cases), spleen (six cases), lung (one case), and liver (one case) was performed. Four patients had overt residue of distant metastases (R2). One patient had minor anastomotic leakage, which was healed by conservative treatment. No patients died of perioperative complications.

Histological Effect of Chemotherapy in Primary Lesion and Lymph Node

Two and five patients achieved grade 3 and 2 tumor regression in the stomach, while four and seven patients showed grade 3 and grade 2 tumor shrinkage, respectively, in the lymph nodes (Table 2). Two of five patients whose multiple liver metastases were evaluated as CR preoperatively were confirmed histologically as grade 3.

Postoperative outcome according to histological grade of tumor regression, gross type, and R number. Multivariate analysis of clinical factors for patients' postoperative outcome

The median survival time (MST) of the 18 patients was 772 days, and the MST of the 13 patients who received R0 gastrectomy was 997 days. Univariate analysis of survival revealed that patients who received R0 gastrectomy had

Table 2 Surgical Treatment and Histological Evaluation

No.	Operation	LN dissection	R	Histological evaluation		Relapse	Outcome	
				Stomach	Lymph node			
1	Distal	D2	0	G2	G3	No	65M	Alive
2	Total	D2	0	G1	G2	Peritoneum	58M	Dead
3	Distal	D2	0	G2	G2	No	57M	Alive
4	Total	D3	0	G2	G2	No	56M	Alive
5	Total	D2	0	G1	G3	Peritoneum	38M	Alive
6	Total	D2	0	G1	G3	Peritoneum	33M	Dead
7	Total	D2	0	G2	G2	Peritoneum	29M	Dead
8	Distal	D3	0	G3	G2	No	26M	Alive
9	Total	D3	0	G1	G1	No	11M	Dead
10	Total	D3	0	G3	G3	No	13M	Alive
11	Distal	D2	0	G1	G1	No	9M	Alive
12	Distal	D2	0	G1	G2	Lymph node	30M	Dead
13	Distal	D1	2	G1	G1	Liver	15M	Dead
14	Total	D2	2	G1	G1	Peritoneum	8M	Dead
15	Total	D1	2	G1	G1	Lymph node	14M	Dead
16	Total	D2	0	G1	G1	Peritoneum	10M	Dead
17	Total	D1	2	G1	G1	Peritoneum	6M	Dead
18	Total	D2	0	G2	G2	No	18M	Alive

Total total gastrectomy, *Distal* distal gastrectomy, *G1* grade 1, *G2* grade2, *G3* grade3

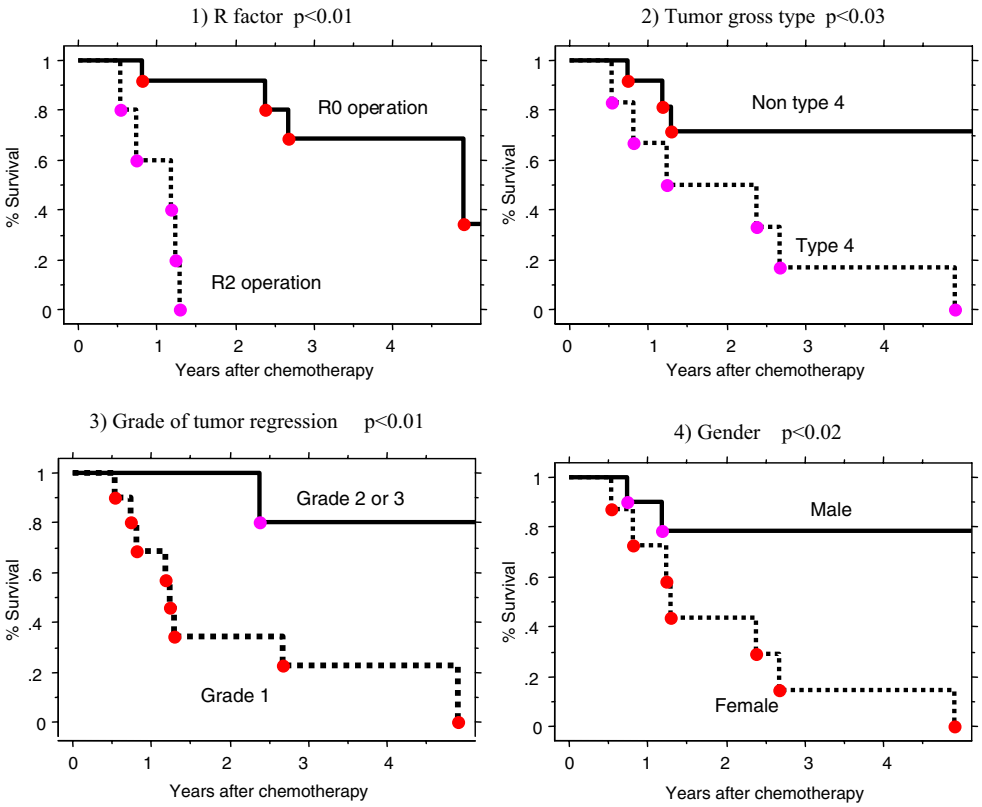

Figure 1 Univariate analysis of survival by the four clinical factors. Univariate analysis of survival revealed that patients who received R0 gastrectomy had significantly better postoperative outcomes than those with R2 gastrectomy ($p<0.01$). Furthermore, non-type 4 ($p=0.03$), histological grade of tumor regression ($p<0.01$), and gender ($p=0.03$) also affected postoperative outcome.

significantly better postoperative outcomes than those with R2 gastrectomy ($p<0.01$). Furthermore, non-type 4 ($p=0.03$), histological grade of tumor regression ($p<0.01$), and gender ($p=0.03$) also affected the postoperative outcome (Fig. 1). Multivariate analysis showed that the R number was the only independent prognostic factor for salvage operation (Table 3).

Discussion

Combination regimens of new anticancer agents have been reported to show high efficacy for unresectable gastric cancer and, as a result, surgeons now have a greater opportunity to treat gastric cancer patients whose distant metastases are controlled. Two published studies have investigated salvage gastrectomy after chemotherapy with a combination of new anticancer agents for gastric cancer. Satoh and associates treated 18 stage IV gastric cancer patients with a regimen of CDDP and S-1, achieved remarkable [8 (44%)] downstaging, and performed R0 gastrectomy.[19] Yoshida also performed salvage gastrectomy for 12 (25%) patients with curative intent.[6] The induction rate of R0 gastrectomy in that study was as high as ours; however, MST after gastrectomy was 1.8 years and no long-term survivors were documented; thus, the postoperative course was not better than in the current study. Our chemotherapeutic regimen, a combination of PTX and S-1, may be suitable for salvage gastrectomy among many chemotherapeutic regimens of new anticancer agents.

Most patients whose cancerous lesions were preoperatively diagnosed as CR had histological tumor residue in the stomach and lymph nodes. Among patients with residual tumor in the resected stomach, the volume of

Table 3 Multivariate Analysis of Survival with Clinical Factors

Factors	Univariate	Multivariate		
	p Value	*p* Value	Hazard ratio	95% CI
R factor	<0.01	0.02	0.03	0.01–0.58
Gender	0.02	0.18	8.1	0.4–170
Histological grade	0.01	0.86	0.7	0.05–11.8
Gross type	0.03	0.57	0.6	0.1–3.4

tumor shrinkage significantly correlated with survival. Becker and colleagues[20] demonstrated that the histological tumor regression grade was an objective measure of the effects of neoadjuvant chemotherapy in gastric cancer, which is in agreement with our results. In this context, patients with lower histological grades of tumor regression may need intensive chemotherapy after salvage gastrectomy.

With respect to tumor regression at different sites, the combination of PTX and S-1 was especially effective for lymph node metastases: 23% of grade 3 was achieved in lymph node metastases, and three of eight patients positive for para-aortic nodes showed complete regression. Chemotherapeutic effectiveness for lymph node metastases, which directly reflects downstaging of advanced gastric cancer, seems critical.[21] Thus, our combination regimen can be used for neoadjuvant chemotherapy for resectable advanced gastric cancer with high nodal involvement.

By univariate analysis of survival, significant differences were found between type 4 and non-type 4 tumors. All type 4 tumor patients showed recurrence in the peritoneum even though they underwent R0 gastrectomy or had a high grade of tumor regression; therefore, special attention should be paid to the gross tumor type when performing salvage gastrectomy with curative intent.

We often observed the immediate relapses of lesions that showed complete regression after chemotherapy; therefore, when we plan salvage gastrectomy with curative intent, we routinely add two courses of combination chemotherapy to confirm the continuity of CR status in distant lesions. Unlike with neoadjuvant chemotherapy for resectable gastric cancer, the number of courses of our chemotherapeutic regimen should not be predefined because of the variable response of distant lesions.

The question arises as to whether salvage gastrectomy is necessary for patients who achieve CR following chemotherapy. Among 14 patients who underwent salvage gastrectomy, 13 had residual cancer cells in the resected or dissected specimen ignoring anatomical lymphatic flow from the tumor, although preoperative imaging suggested complete regression of lymph node metastasis or the primary tumor. In this context, salvage surgery not only enabled confirmation of the histological evaluation of tumor regression but also the removal of minute residual tumor cells. Three patients who underwent R0 salvage gastrectomy and survived for more than 3 years have now discontinued anticancer drugs. R0 gastrectomy can free patients from needless intensive chemotherapy.

In the current study, we compared clinical variables that were strongly associated with patient survival, and multivariate analysis revealed only R factor as a significant variable. Although multivariate analysis by Yano and associates revealed that salvage surgery was an independent prognostic factor for noncurative gastric cancer patients, ours is the first report suggesting the significance of R0 salvage operation. Other factors, such as gross type, histological grade of tumor regression, and gender, were not significant; however, the sample size may not have been large enough to show significance.

In conclusion, R0 salvage gastrectomy following a combination of biweekly PTX and S-1 may have significant clinical efficacy for non-type 4 gastric cancer patients. R0 salvage gastrectomy after chemotherapy can be useful not only to confirm the histological grade of tumor regression but also to remove minute tumor residues from the stomach and lymph nodes. Further investigation in randomized clinical trials is warranted.

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Comparative Study on Three Types of Alimentary Reconstruction After Total Gastrectomy

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Abstract
Background More than 70 alimentary reconstruction procedures after total gastrectomy have been proposed to reduce the postoperative syndromes such as dumping syndrome, reflux esophagitis, and malnutrition. However, the optimal alimentary reconstruction method is still a matter of debate. The aim of the current study was to investigate the rationality of different alimentary tract reconstruction methods after total gastrectomy for gastric malignancy.
Methods Three types of digestive reconstruction methods were performed after total gastrectomy in 285 cases of gastric malignancy from May 1996 to December 2006, including Orr-type Roux-en-Y reconstruction (Orr-type), P-type Roux-en-Y reconstruction (P-type), and Moynihan-type reconstruction (Moynihan-type) methods. The operative time, early postoperative complications and mortality, food intake, alimentary symptoms, Visick scores, nutritional status at 1 and 3years after surgery, and cumulative survival at 1, 3, and 5years were comparatively analyzed.
Results There were no significant differences among the three methods in early postoperative complications and mortality, postoperative food intake and nutritional status (hemoglobin, total proteins and albumin), and incidence of diarrhea and dumping syndrome at 1 and 3years ($p > 0.05$). The overall 1-, 3-, and 5-year cumulative survival rate were 75.30%, 39.86%, and 21.48%, respectively, without significant differences among the three groups ($p > 0.05$). However, the average operative time used in the Orr-type reconstruction method (2.9 ± 0.1h) was comparatively shorter than that used in the P-type (3.4 ± 0.2h) and the Moynihan-type (3.2 ± 0.1h). The incidences of reflux esophagitis after the gastric reconstruction with the Moynihan-type method at 1 and 3years (72% and 65%) were significantly higher than that with the Orr-type (3% and 0%) and P-type (5% and 0%; $p < 0.01$). Constituent ratio of Visick scores I–II of the Moynihan-type method at 1 and 3years (54% and 73%) were smaller than that of the Orr-type (94% and 96%) and the P-type (93% and 96%) methods ($p < 0.01$).
Conclusion Orr-type Roux-en-Y reconstruction method can avoid reflux esophagitis, and the procedure is simpler than the other two methods. Therefore, Orr-type Roux-en-Y reconstruction can be recommended as an adoptable method of digestive reconstruction after total gastrectomy for gastric cancer.

Keywords Gastric cancer · Total gastrectomy · Alimentary reconstruction

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Introduction

Gastric carcinoma is one of the most common malignant tumors in China, and the number of cases treated by total removal of the stomach (total gastrectomy) is increasing yearly.[1] However, total gastrectomy may result in early satiety, dumping syndrome, reflux esophagitis, malabsorption, malnutrition, and weight loss. The increase incidence of these postoperative syndromes thus leads to the continuous study of different alimentary reconstruction methods. During the past decades, over 70 alimentary reconstruction methods have been proposed.[2] However, the

optimal digestive tract reconstruction procedure after total gastrectomy is still a matter of debate.

From May 1996 to December 2006, three types of alimentary reconstruction methods had been performed on 285 patients having gastric malignancy after total gastrectomy. The methods include Orr-type Roux-en-Y esophagojejunostomy (Orr-type), P-type Roux-en-Y esophagojejunostomy (P-type; the preference used before year 2000), and Moynihan-type reconstruction (Moynihan-type). A retrospective study was carried out to evaluate the operative time, postoperative complications, and food intake, digestive tract symptoms, and nutritional status at 1 and 3years after surgery.

Materials and Methods

Patients

A total of 285 patients (150 male and 135 female, with a mean age of 57years ranging from 26 to 75) who had undergone total gastrectomy with either one of the three types of reconstruction methods were studied (Orr-type, P-type, and Moynihan-type). All patients were diagnosed to have gastric cancer with clinical, barium meal, endoscopic, and histological examinations. Concerning tumor location, tumor diffused in thee sections of stomach in 45 cases, mainly in cardia and body in 95 cases, mainly in body and antrum in 93 cases, and only in body of stomach in 52 cases. In the pathological examination, the tumors of 263 patients were diagnosed as adenocarcinoma, nine as malignant lymphoma, and 13 as leiomyosarcoma. The stage of the growing tumors in 27 patients was classified as stage II, 143 patents as stage IIIa, 86 patients as stage IIIb, and 29 patients as stage IV. All subjects received a curative total gastrectomy.

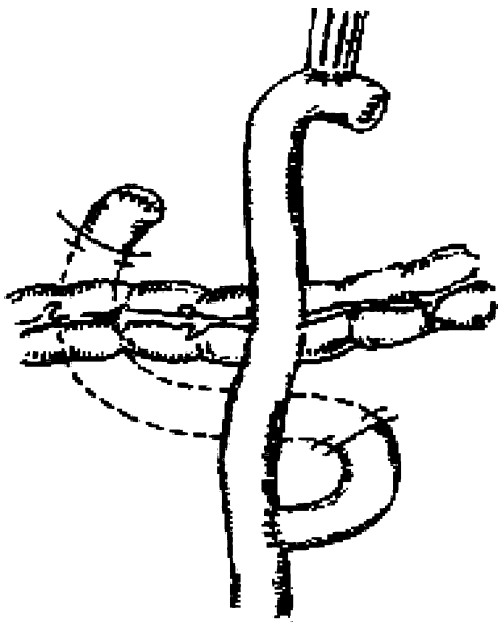

Figure 1 Illustration of Orr-type Roux-en-Y reconstruction.

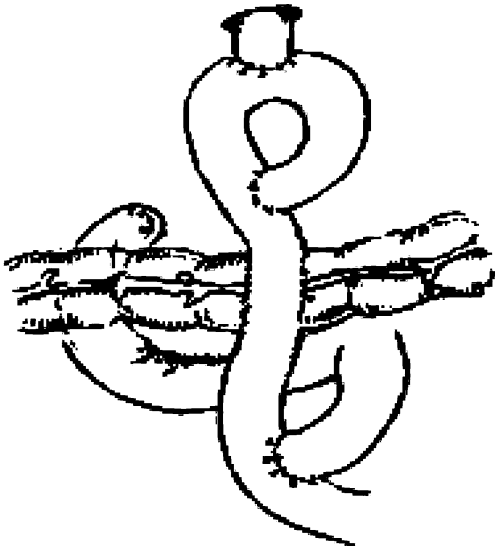

Figure 2 Illustration of P-type Roux-en-Y reconstruction.

Reconstruction After Total Gastrectomy

Three types of alimentary reconstruction methods were performed by the same operation team. Most of the reconstruction methods performed before year 2000 were P-type and Moynihan-type, and Orr-type has become the main procedure since 2000:

(1) Orr-type Roux-en-Y esophagojejunostomy was performed in 155 cases. After total gastrectomy, the distal end of the duodenum was closed. The jejunum was separated 15–20cm distal to the Treitz's ligament, and an end-to-side esophagojejunostomy was done at the distal

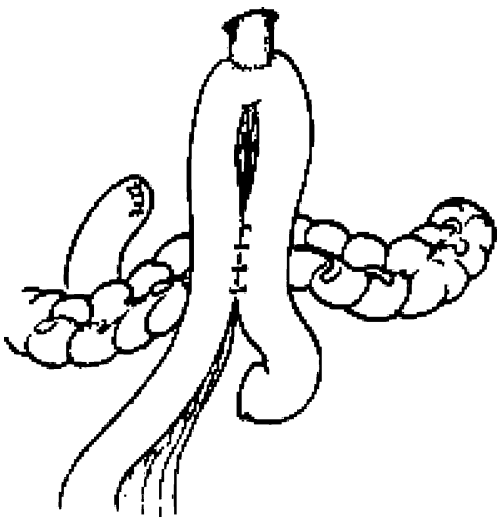

Figure 3 Illustration of Moynihan-type reconstruction.

Table 1 Clinicopathologic Characteristics of the Patients

Operation procedures	Number of patients	Mean age (years)	Sex (M/F)	Clinicopathologic stage [*n* (%)]			
				II	IIIa	IIIb	IV
Orr-type	155	56.8 (26–73)	82:73	15 (10)	76 (49)	49 (31)	15 (10)
P-type	63	58 (30–75)	33:30	6 (10)	32 (51)	18 (28)	7 (11)
Moynihan-type	67	57.4 (29–72)	35:32	6 (9)	35 (52)	19 (28)	7 (11)

No significant differences among the procedures ($p>0.05$)

side of the jejunum. Then, the continuity of the jejunum was reconstructed with side-to-end jejunojejunostomy at 40–45cm distal to esophagojejunostomy. The operative design in this procedure is shown in Fig. 1 (Orr-type group, $n = 155$).

(2) P-type Roux-en-Y esophagojejunostomy was performed in 63 cases. This type of reconstruction method was done in a way similar to the Orr-type except that a "P" type jejunum loop was made in the proximal jejunum before doing the end-to-side esophagojejunostomy, as shown in Fig. 2 (P-type group, $n = 63$).

(3) Moynihan-type reconstruction was performed in 67 cases. After the distal end of the duodenum was closed, an end-to-side esophagojejunostomy was made at 40–45cm distal to the Treitz's ligament, and then a 10-cm side-to-side jejunojejunostomy was made between the afferent jejunal loop and the transposed jejunal loop, which was also called Braun anastomosis. See Fig. 3 (Moynihan-type, $n = 67$).

All the esophagojejunal end-to-side anastomoses were performed using a circular stapler, and other types of anastomoses were sutured by hand. There were no significant differences among the three groups of patients in age, sex, and clinicopathologic stage classification, as summarized in Table 1.

Postoperative Following Up

For the purpose of this comparative study, the case files were reviewed by two of our staff members to obtain the following data: the operative time, the early postoperative complications, and the mortality. The patients were followed up regularly by one of our experienced staff through out-patient visit, telephone interview, and letter contact.

A standardized questionnaire concerning the postgastrectomy symptoms was distributed to the patients and was collected 1 and 3years, respectively, after surgery. The questions included several items relating to eating habits and alimentary symptoms. The patients needed to estimate their craving for eating in each meal and the number of meals they had each day. The items of alimentary symptoms included heartburn, diarrhea, and dumping syndrome, and Visick scores were calculated. Postgastrectomy symptoms were classified into either good/fair (I–II) or poor (III–IV) based on the Visick scores.

These evaluations of nutritional status also had comprised various nutritional parameters on laboratory examinations (serum albumin, hemoglobin, and serum proteins) at 1 and 3years after surgery. Endoscopy was performed with interval of 6 to 12months.

Statistic Analysis

All values were expressed as mean ± SE. The data were analyzed by chi-square test, Student's t test, and the analysis of variance; postoperative survival was analyzed by Kaplan–Meier. The statistical calculations were carried out using Statistical Package for the Social Sciences (SPSS) 11.0 statistical software package. The level of significance was defined at $p < 0.05$.

Results

Operative Time and Postoperative Complications

Early postoperative complications occurred in 27 cases, including pulmonary infection in 14 cases, anastomotic straightness in five cases, anastomotic bleeding in seven cases, and anastomotic leakage in one case. Six patients died, one because of anastomotic leakage, four because of adult

Table 2 Operative Time, Early Postoperative Complications, and Mortality of the Three Procedures

Operation procedures	Number of patients	Operative time (h)	Complications [*n* (%)]	Mortality [*n* (%)]
Orr-type	155	2.9±0.1	12 (8)	3 (2)
P-type	63	3.4±0.2	7 (11)	1 (2)
Moynihan-type	67	3.2±0.1	8 (12)	2 (3)
p value		<0.01	>0.05	>0.05

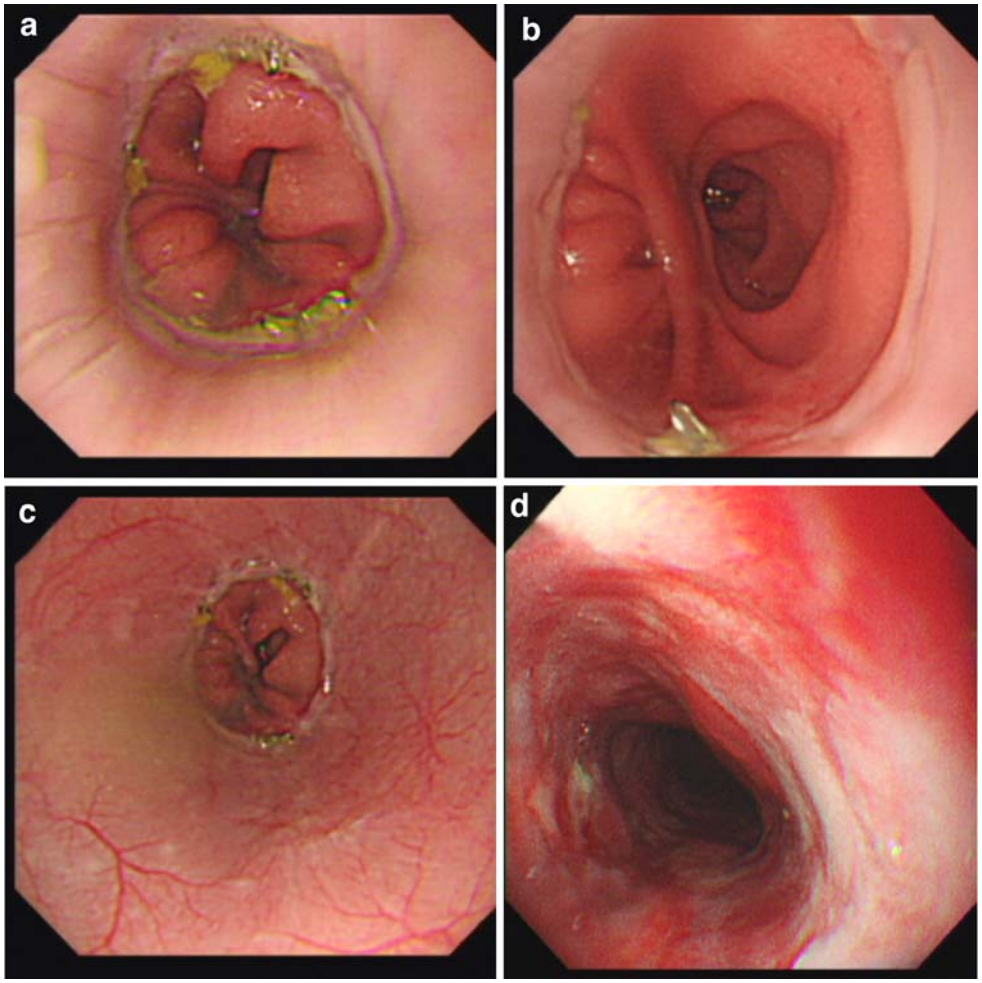

Figure 4 Illustration of endoscopic manifestations. **a** The representative endoscopic image of the Orr-type group. **b** A patient's endoscopic image of the P-type group. **c**, **d** The representative endoscopic images of the Moynihan-type group.

respiratory distress syndrome, and one because of myocardial infarction. No significant ($p > 0.05$) intergroup differences of early postoperative complications and mortality were found. The average operative time required for the Orr-type reconstruction method (2.9 ± 0.1h) was shorter than that required for the P-type (3.4 ± 0.2h) and the Moynihan-type (3.2 ± 0.1h) reconstruction methods. The differences were significant ($p < 0.01$; Table 2)

Table 3 Alimentary Symptoms of the Three Procedures [*n* (%)]

Operation procedures	Number of patients	Eating capacity (per meal)		Eating frequency (per day)		Diarrhea	Dumping syndrome	Reflux esophagitis	Visick scores	
		>300 ml	<300 ml	3–5	>5				I–II	III–IV
Orr-type										
1 year	154	140 (91)	14 (9)	150 (97)	4 (3)	15 (10)	8 (5)	5 (3)	145 (94)	9 (6)
3 years	56	53(95)	3 (5)	55 (98)	1 (2)	0	0	0	54 (96)	2 (4)
P-type										
1 year	54	50 (93)	4 (7)	51 (95)	3 (5)	5 (9)	3 (6)	3 (5)	50 (93)	4 (7)
3 years	23	22 (96)	1 (4)	23 (100)	0	0	0	0	22 (96)	1 (4)
Moynihan-type										
1 year	52	47 (91)	5 (9)	50 (96)	2 (4)	5 (10)	4 (8)	37 (72)	28 (54)	24 (46)
3 years	26	24 (95)	2 (5)	26 (100)	0	0	0	17 (65)	19 (73)	7 (27)
p value		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.01	<0.01	<0.01

Table 4 Serum Protein of the Three Procedures (Mean±SE)

Operation procedure	Hemoglobin (g/l)		Total protein (g/l)		Albium (g/l)	
	1 year	3 year	1 year	3 year	1 year	3 year
Orr-type	110.20 ± 11.80	111.30 ± 10.15	69.58 ± 4.96	67.08 ± 5.20	39.32 ± 3.30	37.75 ± 4.86
P-type	111.45 ± 11.33	110.10 ± 11.98	68.32 ± 5.45	66.21 ± 6.24	38.76 ± 3.41	36.66 ± 5.37
Moynihan-type	109.87 ± 10.92	107.63 ± 12.14	67.94 ± 4.86	65.86 ± 6.78	38.56 ± 3.50	36.12 ± 5.74

No significant differences among the procedures ($p>0.05$)

Nutritional Status and Alimentary Symptoms

The patients were followed up over 12months; the data were collected 1year after total gastrectomy. The following up rate was 91.6%, with 24 dropped-out cases because of the lost of contact with the patients.

There were no significant differences among the three methods in postoperative food intake and nutritional status (hemoglobin, total protein, and albumin), and the incidence of diarrhea and dumping syndrome at 1 and 3years ($p > 0.05$). However, the incidence of reflux esophagitis of the Moynihan-type group at 1 and 3years (72% and 65%) was higher than that of the Orr-type (3% and 0) and P-type (5% and 0) groups. The endoscopic manifestations of the three groups were shown in Fig. 4. The differences were significant ($p < 0.01$). Constituent ratio of Visick scores I–II of the Moynihan-type group at 1 and 3years (54% and 73%) were less than that of the Orr-type (94% and 96%) and P-type (93% and 96%) groups ($p < 0.01$). Otherwise, the ratio of Visick scores III–IV was larger than the other two, as shown in Tables 3 and 4.

Postoperative Survival

The mass postoperative cumulative survival at 1, 3, 5years was 75.30%, 39.86%, and 21.48% respectively, and no significant ($p > 0.05$) differences were discovered among the three procedures, as shown in Fig. 5.

Discussion

Total gastrectomy is indicated when a radical subtotal gastrectomy cannot widely encompass a malignant gastric lesion. It is estimated that 20% to 40% of gastric cancers necessitate a total gastrectomy. Since Schlatter performed the first successful total gastrectomy in 1897, more than 70 different kinds of digestive tract reconstruction methods after total gastrectomy have been described, and the number is continuously increasing.[3]

It has been generally accepted that the optimum procedure of alimentary reconstruction after total gastrectomy must fulfill the following requirements: (1) maintain the fluency of duodenal food; (2) a good digestive and absorptive function of the gastric substitute; (3) minimal or no "non-gastric syndromes" (e.g., reflux esophagitis, dumping syndrome, lack of appetite, feeling of gull and being bloated, and indigestion); (4) keep the patients in good postoperative nutritional status and have better quality of life; and (5) safe, simple, and less postoperative complications and mortality. However, no reconstruction procedures have been reported to meet all the above requirements.[2,4–8].

It is well known that food chyme moving along the duodenal passage can promote the secretion of cholecystokinin and secretin, and this also has an advantage for maintaining the normal digestion and absorption functions of the digestive tract.[3,6,9,10]. Although the importance of keeping the duodenal passage was widely investigated, very few studies had successfully reported the practical value of this procedure. Many investigations indicated that preservation of the duodenal passage was difficult and the operation procedures were complex, as there were more anastomoses needed to be reconnected. Moreover, there were more

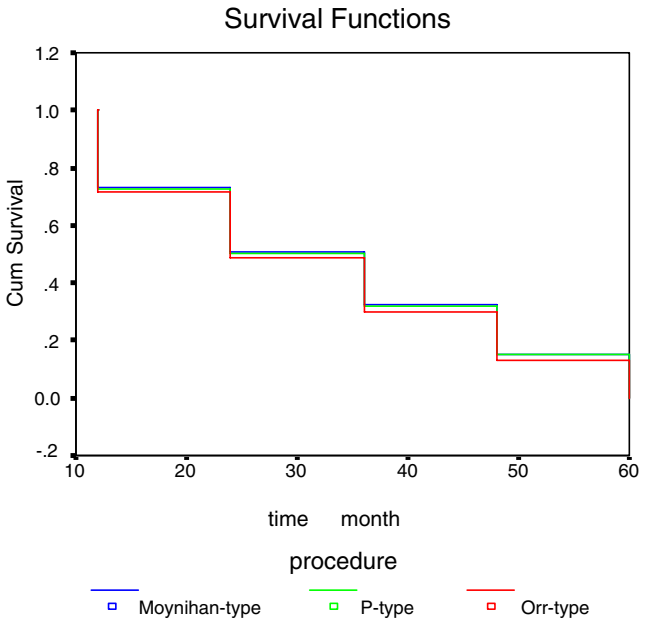

Figure 5 Survival curve of the three procedures.

postoperative complications and higher mortality rate and no significant difference in body weight and nutritional status from the Roux-en-Y esophajejunostectomy.[11,12].

The optimal procedure of forming a substitute stomach is also a matter of debate.[2,13–17] Some scholars even questioned on the functions of the gastric substitute, arguing that this procedure not only contributes very little to the long-term food intake and the recovering of nutritional status but also increases the operation's complexity, operative time, and postoperative complications.[18,19] For example, Hunt– Lawrence pouch construction has been widely accepted in western countries because it can restore a large food reservoir and consequently improve the nutritional status of the patients. However, there is a potential hazard as ischemia may occur at the upper acute angle while suturing the 180-degree rotated jejunum to form a pouch, not to mention the three anastomoses in the procedure. Additionally, if the length of the jejunum or the mesentery is insufficient for pouch construction, it may cause undue tension or difficulty in forming a Hunt–Lawrence pouch.[20] In this point, Orr-type Roux-en-Y reconstruction is safer and technically less demanding.

The current comparative study indicates no significance in postoperative complications, mortality and food intake, nutritional status, and cumulative survival rate among the three procedures. However, both the Orr-type Roux-en-Y reconstruction and the P-type Roux-en-Y reconstruction were superior to Moynihan-type anastomosis in the Visick scores because both of them can play a role in anti-esophageal reflux. The original purpose of Moynihan-type procedure was to reduce the incidence of reflux esophagitis by means of Braun anastomosis. However, in fact, the side-to-side jejunojejunostomy between the afferent and transposed jejunal loop failed to transfer bile and pancreatic secretions, so the incidence of reflux esophagitis in Moynihan-type anastomosis still reached up to 33–70%. As the lower esophageal sphincter is resected at total gastrectomy, the synperistaltic function of the afferent jejunal loop can transport the alkaline digestive juice to the distal part of the esophagus in Moynihan-type reconstruction. Alkaline reflux esophagitis occurs because the chronic reflux of bile and pancreatic secretions into the esophagus may cause serious injury to its mucosa. Sometimes, the food chyme or alkaline digestive juice can circulate through the Braun anastomosis, thus causing lesions to the esophagus mucosa. However, in the Orr- or P-type reconstruction, with a 40- to 50-cm distance between the esophagus and the Roux-en-Y anastomosis, the interposed jejunual "Y" limb with can restrain the esophagus from damaging by the alkaline juice. Thus, both the Orr- and P-type reconstruction methods can decrease reflux esophagitis and improve the long-term quality of life. Furthermore, when compared the Orr-type with the P-type esophagojejunostomy, the operation procedures of the Orr-type esophagojejunostomy was simpler and shorter operative time was needed (2.9±0.1 h vs. 3.4±0.2 h).

In conclusion, the data of our study suggest that the Orr-type Roux-en-Y esophagojejunostomy is safe and technically less demanding and can contribute to the avoidance of reflux esophagitis effectively. Therefore, Orr-type Roux-en-Y reconstruction can be recommended as an adoptable method of alimentary reconstruction after total gastrectomy for gastric cancer.

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Comparison of Two Methods of Selective Hepatic Vascular Exclusion for Liver Resection Involving the Roots of the Hepatic Veins

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Abstract
Background Selective hepatic vascular exclusion (SHVE) is an effective hepatic vascular exclusion in controlling both inflow and outflow without interruption of caval flow, as it combines Pringle maneuver with extrahepatic selective occlusion of hepatic veins. But SHVE has not been widely used due to difficulty in extrahepatic dissection of hepatic veins. When the tumor is very close to the roots of the hepatic veins, dissecting the posterior wall of the hepatic vein may lead to rupture and massive bleeding of the hepatic vein. With our experience, clamping hepatic veins with Satinsky clamps is a safer and easier occlusion method by which the posterior wall of the hepatic veins does not need to be separated and encircled. In this report, we compared the results of selective hepatic vascular occlusion with tourniquet and Satinsky clamp for major liver resection involving the roots of the hepatic veins.
Methods Between January 2003 to June 2006, 180 patients who underwent major liver resection with SHVE were divided into two groups according to different methods of hepatic vascular occlusion: occlusion with tourniquet (tourniquet group, n=95) and occlusion with Satinsky clamp (Satinsky clamp group, n=85). In the tourniquet group, the hepatic veins were encircled and occluded with tourniquet. In the Satinsky clamp group, the hepatic veins were not encircled and clamped directly by Satinsky clamp.
Results Intraoperative and postoperative consequences of the patients were analyzed. The dissecting time for each hepatic vein was significantly shorter in the Satinsky group (6.2±2.4 min vs 18.3±6.2 min) than in the tourniquet group. In the tourniquet group, five hepatic veins (one right hepatic vein and four common trunk of left-middle hepatic veins) could not be dissected and encircled because the tumors involved the cava hepatic junction, and another common trunk of the left-middle hepatic vein had a small rupture during the dissection. These six patients then received successful occlusion with Satinsky clamp. There was no difference between the two groups regarding the operation duration, ischemia time, intraoperative blood loss, and postoperative complication rate.
Conclusion Both methods of the hepatic vein occlusion have the same effect on controlling hepatic vein bleeding, but occlusion with Satinsky clamp is safer, easier, and consumes less time in dissecting.

Keywords Liver neoplasm · Hepatic vein · Exclusion · Hepatectomy

Li Ai-Jun And Pan Ze-Ya contributed equally to this work.

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Introduction

In hepatectomy, massive hemorrhage during the operation remains a potentially lethal problem.[1–3] Total hepatic vascular exclusion (THVE), which is occlusion of the liver inflow combined with occlusion of the inferior vena cava (IVC) above and below the liver, has been used as the common solution during the major hepatectomy at present.[4,5] This method, however, may cause hemodynamic changes and may not be tolerated by some patients.[6–8] Selective hepatic vascular exclusion (SHVE) is a newly developed

technique,[8–11] which is only occlusion of inflow and outflow blood of the liver with preservation of caval flow so that general hemodynamics remains stable.[12,13] However, SHVE is not widely used due to the difficulties in extrahepatic dissection of the hepatic veins. Between January 2003 and June 2006, we performed major liver resection in 180 patients with the tumors involving the hepatic veins with the use of SHVE. Two methods of hepatic vein occlusion (with tourniquet or with Satinsky clamp) were used for controlling bleeding. In this report, we compared the feasibility and safety of these two methods of occlusion.

Material and Methods

Clinical Data

From January 2003 to June 2006, in our department, 180 consecutive patients with tumors adhering to the major hepatic veins underwent liver resection with the use of SHVE. According to different ways of hepatic veins exclusion, these patients were divided into two groups: a tourniquet group (95 cases) from January 2003 to January 2005 and a Satinsky clamp group (85 cases) from February 2005 to June 2006. The preoperative data and operative indications of two groups are shown in Tables 1 and 2, respectively. Routine preoperative assessment and a series of imaging including ultrasonography (US), computer tomography (CT), and/or magnetic resonance imaging (MRI) were undertaken in all patients. Various types of hepatectomy performed in two groups are shown in Table 3.

Table 1 Clinical Data for Undergoing Hepatectomy of Different Hepatic Vein Occlusion

Clinical data	Tourniquet group (n=95)	Satinsky clamp group (n=85)
Age (years)[a]	50.4±8.1	51.6±9.5
Gender(male/female)	60/35	52/33
Tumor size (cm)[a]	10.8±6.2	11.4±5.6
HBsAg(+)	61	52
With cirrhosis	53	44
Child grade(A/B)	90/5	81/4
Hepatic vein invaded	95	85
RHV	10	12
RHV + MHV	25	23
RHV + MHV + LHV	24	18
RHV + LHV	30	25
LHV	6	7
IVC invaded	47	44

RHV right hepatic veins, *MHV* middle hepatic veins, *LHV* left hepatic veins, *IVC* inferior vena cava

Table 2 Indication for Liver Resections

Indication	Tourniquet group (n=95)	Satinsky clamp group (n=85)
Hepatocellular carcinoma	60	50
With cirrhosis	52	43
Without cirrhosis	8	7
Hepatocholangio carcinoma	4	3
Hepatoblastoma	2	2
Metastatic carcinoma	5	4
Hepatic sarcoma	1	1
Hemangiomas of liver	21	18
Focal nodular hyperplasia	1	0
Angioleiomyolipoma	0	1
Hepatic adenoma	1	1

Preoperative chemoembolization was carried out in 22 patients.

Surgical technique

A bilateral subcostal incision was used to enter the abdomen, and the intraoperative US was used to determine the position, size of the liver tumor, and its relationship to the surrounding vascular structures and to assess the tumor resectability. The liver was fully mobilized after transection of the entire ligament. In the tourniquet group, the liver was isolated and the right wall of IVC exposed. The minor hepatic veins were dissected and ligated. On the right side, the hepatocaval ligament (Makuuchi ligament) was dissected to expose the right and the inferior wall of the right hepatic veins. The connective tissue in the space between

Table 3 Types of Liver Resection

Procedure	Tourniquet group (n=95)	Satinsky clamp group (n=85)
Right hepatectomy	21	17
Right extended hepatectomy	12	10
To segment 4	9	7
To segment 1	3	3
Left hepatectomy	17	14
Left extended hepatectomy	15	12
To segment 5 + 8	11	9
To segment 1	4	3
Segments hepatectomy	30	32
4 + 5 + 8	12	12
7 + 8	4	5
6 + 7	5	6
4 + 1	2	1
8	7	8

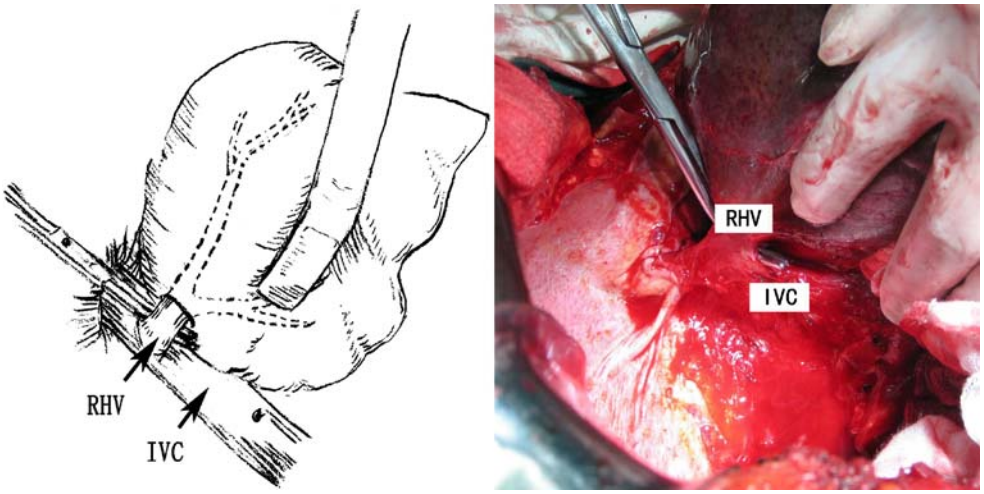

Figure 1 A clamp is inserted the space between the right hepatic vein and IVC. *RHV* right hepatic vein, *IVC* inferior vena cava.

the right and common trunk of the left-middle hepatic vein was dissected downward. The right hepatic veins were separated and encircled with the vessel loop (Figs. 1 and 2). On the left side, the lesser omentum and venosum ligaments were dissected to expose the junction of the left hepatic vein and IVC, then the clamp was used to dissect between the common trunk of the left-middle hepatic vein and the vena cava, and the common trunk was divided and encircled (Figs. 3 and 4). If the left hepatic vein and middle hepatic vein joined the IVC separately, they were encircled individually. A tourniquet was tightened to occlude the hepatic veins. In the Satinsky clamp group, the right lobe of the liver was mobilized until the right wall of the IVC. Hepatocaval ligament was dissected 3–4 cm downward along the space between the right- and left-middle hepatic vein, and the right, left, and superior wall of the right hepatic vein was exposed. A Satinsky clamp was used to occlude the root of the right hepatic vein parallel to the longitudinal axis of the IVC, ensuring that the tip of the clamp exceed the inferior margin of the right hepatic veins (Figs. 5 and 7). When the tumor is large and/or invaded the hepatocacal junction, the liver is difficult to be mobilized and rotated, and the poor exposure of the liver therefore may lead to the very dangerous isolation of the hepatocaval ligament. In this condition, the hepatocaval ligament was not necessary to be dissected. On the left side, the left coronary ligament was dissected and the left wall of the left hepatic vein exposed. It was not necessary to dissect the ligament venosum and the tip of the caudate lobe. After the space being dissected downward between the right and left-middle hepatic vein at a length of 3–4 cm, the right, left, and superior wall of the common trunk of the left-middle hepatic vein was exposed. Another Satinsky clamp was used to occlude the root of common trunk parallel to the longitudinal axis of the IVC (Figs. 6 and 7). Pringle maneuver was applied to occlude blood inflow of the liver. The porta hepatis was continuously clamped for 30–40 min if with liver cirrhosis the porta hepatis was intermittently clamped for 15–20 min with a 5-min interval. IVC clamp bands were pre-placed in 15 patients in the tourniquet

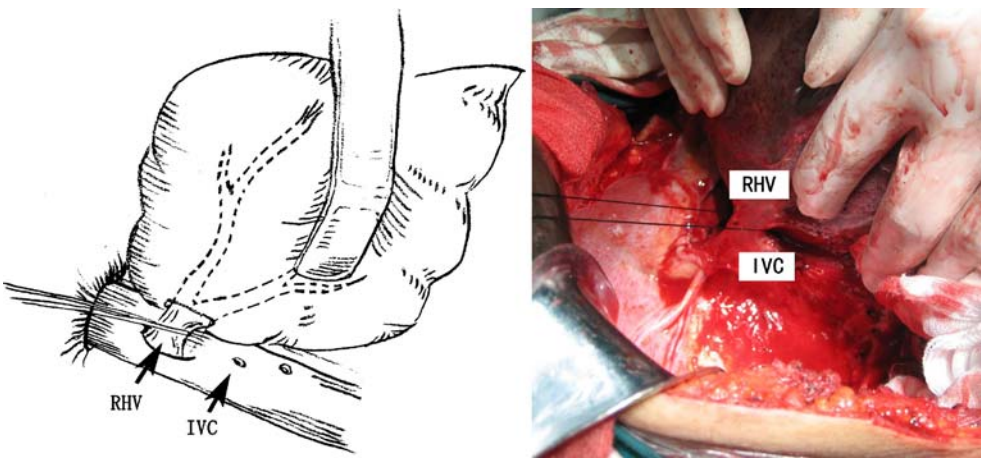

Figure 2 The right hepatic vein is *encircled with a loop*. *IVC* inferior vena cava, *RHV* right hepatic vein.

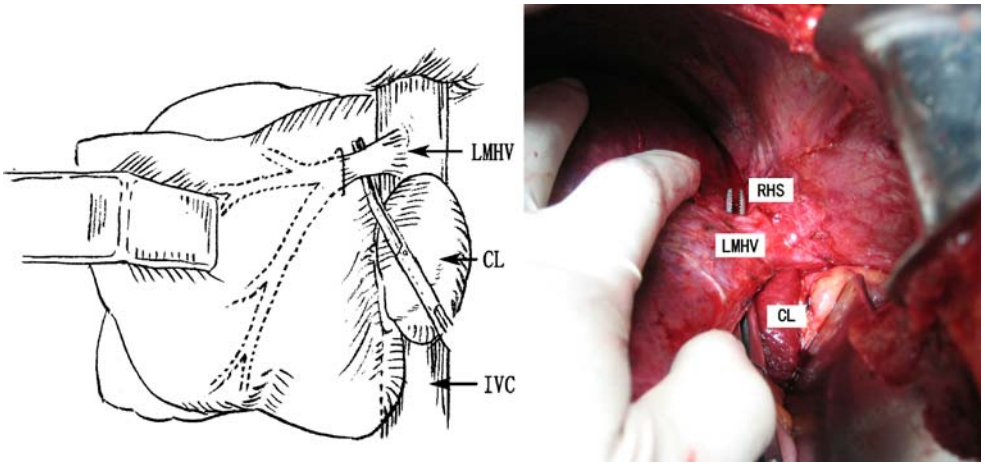

Figure 3 A clamp is inserted the space between the common trunk of the left-middle hepatic vein and IVC. *LMHV* left-middle hepatic vein, *CL* caudate lobe, *IVC* inferior vena cava.

group and 17 patients in the Satinsky clamp group, and no occlusion was used in both of the groups. Liver resection was carried out by a clamp-crushing manner in all patients. Cholecystectomy was performed in 84 patients and right kidney resection in one patient.

Anesthetic management was accomplished by general anesthesia. A Swan–Ganz catheter and a radial arterial line were inserted. The central venous pressure (CVP) was maintained from 1–8 mmHg during the liver resection.

Postoperative data were collected daily until discharge. Complications were recorded. Hepatocellular injury was monitored by serum alanine aminotransferase (ALT), bilirubin, prothrombin time and albumin on postoperative days 1, 3, 7.

In the case of malignancy, incision was 1 cm bigger than tumor to remove whole tumor. However, when located close to the root of the hepatic vein, inferior caval vein, or trunk of the portal vein, tumor was resected closely to the margin of tumor. Benign tumor was resected closely to the margin of tumor no matter the location.

Statistical Analysis

The data of two groups were collected and compared. Results were expressed as $x \pm s$ difference. Student's t test was used to compare continuous variables, and χ^2 test was used to deal with discrete variables. $P<0.05$ was considered statistically significant.

Results

The patients in two groups were similar in terms of age, gender, preoperative hepatic function, tumor size, and malignant /benign tumor ratio. There was no difference between the two groups regarding type of hepatectomy, operation duration, intraoperative warm ischemia time, intraoperative blood loss, and transfusion volume (Tables 3 and 4). Hepatic veins rupture occurred in ten patients in the tourniquet group and 12 patients in the Satinsky group

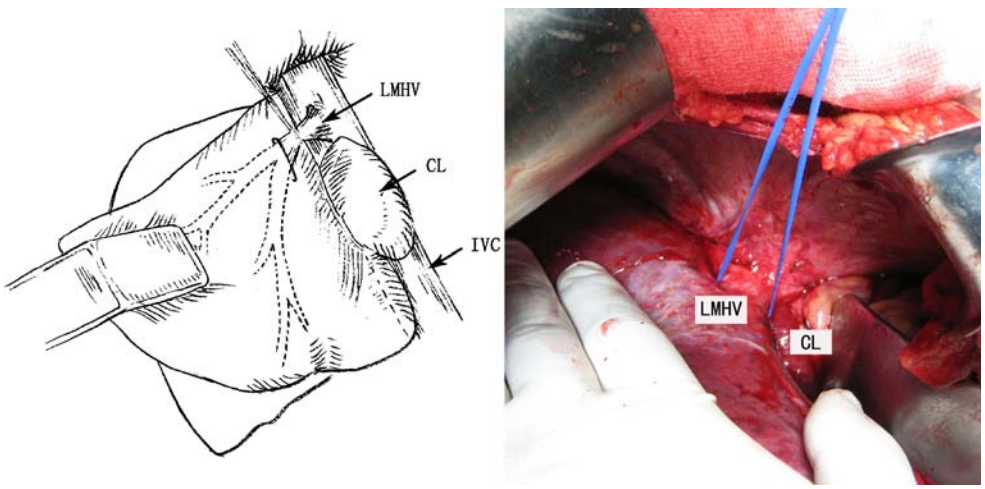

Figure 4 The loop is tensioned and clamped with a small clamp. The outflow of the left-middle hepatic vein is occluded. *LMHV* left-middle hepatic vein, *CL* caudate lobe, *IVC* inferior vena cava.

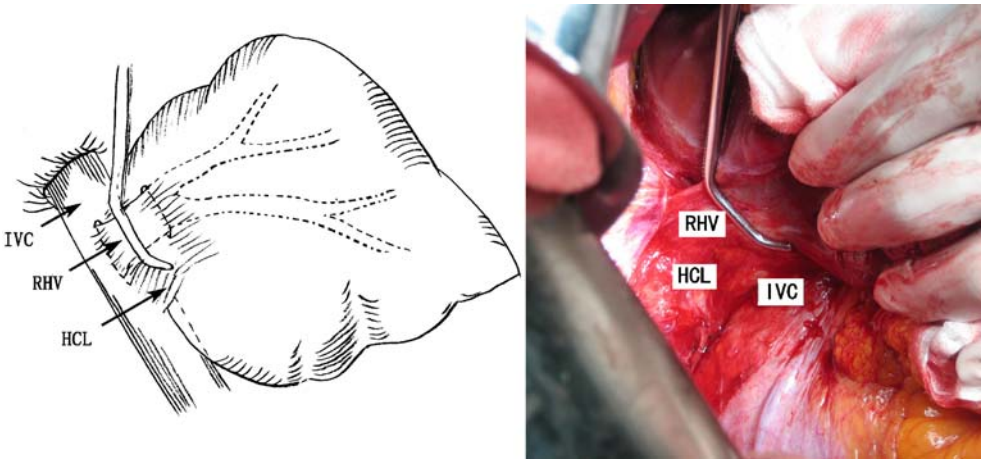

Figure 5 Occlusion of the RHV with a Satinsky clamp. The hepatocaval ligament is not dissected and ligated. *IVC* inferior vena cava, *RHV* right hepatic vein, *HCL* hepatocaval ligament.

during the parenchymal transection of the liver, but no massive bleeding or air embolism occurred because hepatic veins had been already occluded before liver resection. No hemorrhage of IVC occurred. Compared with Satinsky clamp group, the time of dissecting hepatic veins in tourniquet group was longer with significant difference (t=16.90, P<0.01). In the tourniquet group, five patients' hepatic veins (one right hepatic vein and four common trunk of left-middle hepatic veins) could not be dissected and encircled because the tumors involved the hepatocaval junction and the liver was difficult to mobilize and rotate. Another patient's common trunk of the left-middle hepatic vein had a small rupture during dissection. These six patients then received successful occlusion with Satinsky clamp. No rupture of hepatic veins occurred in the Satinsky clamp group during dissecting the hepatic veins. During dissection, tumor rupture occurred in four cases, and diaphragmatic was partially injured in seven cases. There was no operative mortality in both groups. Postoperative complication rates were 30.5% in the tourniquet group and 30.6% in the Satinsky clamp group. In the tourniquet group, hemorrhage from the liver raw surface during operation occurred in one patient was controlled with gauze parking. Seven days after the operation, the gauze parking was removed and hemorrhage stopped (Table 5).

There was no significant difference between two groups on liver function (ALT, bilirubin, prothrombin time, and albumin) on 1, 3, 7 days after operation (Table 6).

Discussion

With the increase of resection rate of the liver tumor involving the cava hepatic junction, complications involving hepatic veins are also on the rise, among which, massive bleeding and venous air embolism remain the potentially lethal problems.[6,14,15] As we know, Pringle

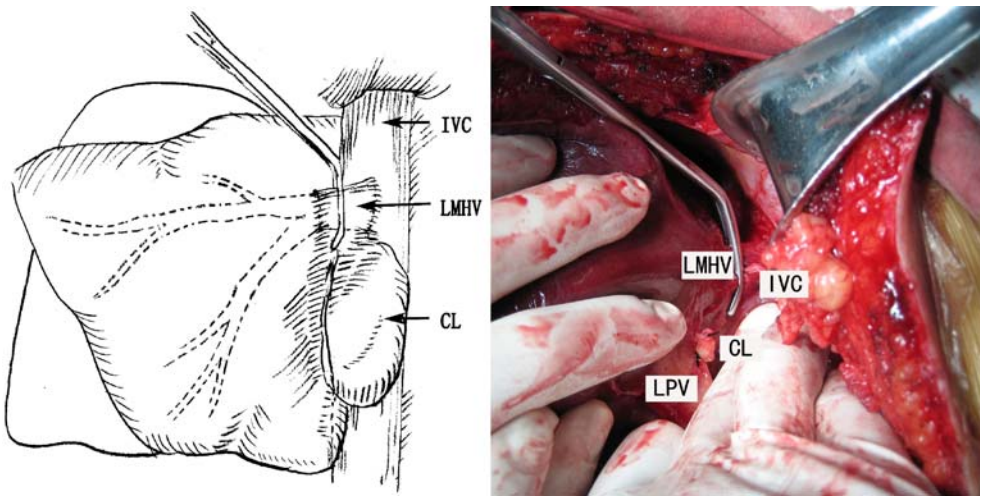

Figure 6 Occlusion of the common trunk of the left-middle hepatic vein with a Satinsky clamp. The tip of the caudate lobe and lesser omentum is not dissected. *IVC* inferior vena cava, *LMHV* left-middle hepatic vein, *CL* caudate lobe.

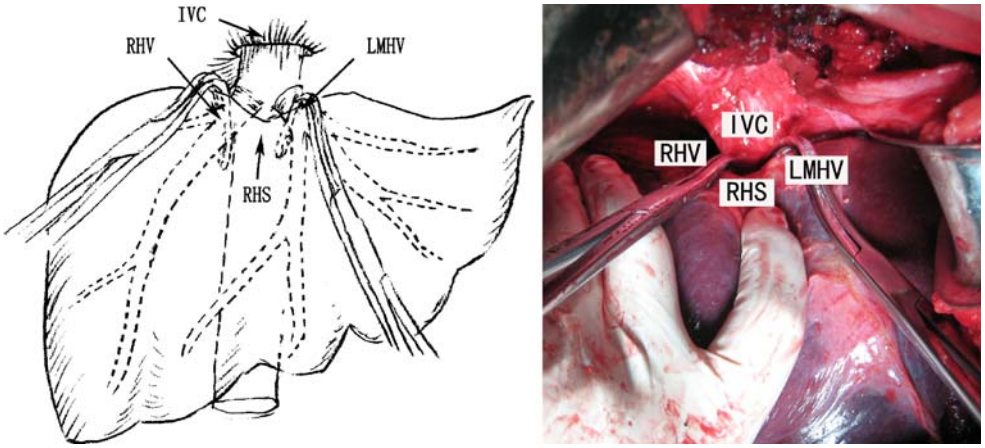

Figure 7 The bases of right hepatic vein and the common trunk of left-middle hepatic vein were clamped parallel to the IVC with Satinsky clamp. *IVC* inferior vena cava, *RHV* right hepatic vein, *LMHV* left-middle hepatic vein, *RHS* retrohepatic space.

maneuver cannot prevent hepatic vein bleeding. Although THVE can occlude the blood inflow and outflow of the liver to prevent bleeding of the hepatic veins, it leads to hemodynamic disturbance because of occlusion of IVC, and it is not tolerable in some patients.[10,11] SHVE occludes the blood inflow and outflow of the liver with preservation of inferior caval flow so that generally stable hemodynamics is maintained during the operation.[16] Therefore, it combines the Pringle maneuver with extrahepatic selective occlusion of the hepatic veins, providing an appropriate way of hepatic vascular occlusion. However, SHVE is not widely used in hepatic resection mainly because hepatic venous rupture may cause massive bleeding during dissecting the hepatic vein.[3,17] Therefore, many surgeons prefer THVE to SHVE in resecting the tumor involved the cava hepatic junction. However, with the anatomy progress of hepatic veins, dissection of hepatic veins is no longer contraindicated.[18] Three critical anatomic landmarks should be emphasized[17,19–21]: (1) hepatocaval ligament (Fig. 5): a landmark for root of the right hepatic veins, which can be revealed when hepatocaval ligament is dissected; (2) the space between the right and common trunk of the hepatic vein (Fig. 7): a landmark for medial wall of the right and middle hepatic veins, which is 0.5–1.5 cm wide and 4–7 cm long. Minor veins are rare in the anterior wall of the vena cava; (3) left hepatic veins-inferior vena gap: a landmark for posterior wall of the common trunk of left-middle hepatic veins and anterior wall of IVC. Along this gap, the common trunk of the left-middle hepatic veins can be divided.

Despite the great improvement in dissection of the conventional method, the hepatic veins demands full mobilization and disconnection of the liver from the IVC by dividing liver ligament and short hepatic veins, and the trunk of the hepatic veins need to be totally dissected and encircled with vessel loops. It may cost relatively longer time to dissect the hepatic veins. When the tumor is large and/or invades the hepatocaval junction, the liver is difficult to be mobilized and rotated, and the poor exposure of the liver therefore may lead to the hazardous isolation of the posterior side of the hepatic veins.[2,22–24] In this condition,

Table 4 Intraoperative Data of Patients

Intraoperative data[a]	Tourniquet group (n=95)	Satinsky clamp group (n=85)	T value	P value
Dissecting time (min)[a,b]	18.3±6.2	6.2±2.4	16.9	0.001
Warm ischemia time (min)[a]	20.4±8.7	21.2±7.9	0.64	0.5
Operating duration (min)[a]	130±45	123±47	1.02	0.3
Intraoperative blood loss(ml)[a]	520±310	540±270	0.72	0.45
Intraoperative transfusion (packed RBCs)[a]	1.1±0.4	0.9±3.0	1.79	0.07
No transfusion patients	60/95	52/85	0.07	0.81
Failure for hepatic vein dissecting	6/95	0/85	1.93	0.18
Hepatic vein rupture during resecting	10/95	12/85	0.54	0.40
Hemorrhea of hepatic veins	0/95	0/85		
Air embolism	0/95	0/85		

[a] Values were expressed as the $\bar{x} \pm s$
[b] Each hepatic vein dissecting time

Table 5 Mortality, Morbidity, ICU, and Hospital Stay after Operation

Complication	Tourniquet group (n=95)	Satinsky clamp group (n=85)	T value	P value
Intraoperative no coagulopathy	1	0		
Reoperation	0	0		
Bile leak	6	6		
Liver function failure after operation	0	0		
Wound infection	2	3		
Pleural effusion	20	17		
Subphrenic collection	8	7		
Death	0	0		
ICU stay(days)[a]	1.1±0.7	1.2±1.1	0.74	0.45
Postoperative hospital stay(days)[a]	9.2±2.6	8.8±3.0	0.93	0.3
Overall complication rate	30.50%	30.60%		

ICU intensive care unit
[a] Values were expressed as the $\bar{x} \pm s$

the conventional method seems to be impossible, so we use a new technique to exclude the hepatic veins. In our experience, after liver mobilization, the upper surface of the liver is exposed up to the anterior surface of the supra-hepatic IVC and the hepatocaval junction. The space between the right and middle hepatic veins is dissected on 3–4 cm download. The fibrous tissue near the hepatocaval ligament is exposed on the right side, but it is not necessary to be isolated and tied. On the left side, the bare area near the IVC is dissected, and the left lateral wall of the common trunk of the left-middle hepatic vein is exposed. After clamping the portal triad, the right hepatic vein and the common trunk of the left-middle hepatic vein are clamped with two Satinsky clamps on the basis of the hepatic vein parallel to the longitudinal axis of IVC. With this technique, the posterior wall of the hepatic veins is not necessary to be dissected and encircled with vessel loop.

Compared with the conventional method, the "superior approach" provides shorter time for dissecting the hepatic veins. The causes of this difference are as follows: in the tourniquet group, the hepatocaval ligament on the right side and lesser omentum and ligament venosum on the left side must be dissected and tied, and the posterior wall of the hepatic veins also must be dissected and encircled. While in the Satinsky group, it is not necessary to dissect and ligate the hepatocaval ligament, lesser omentum, and ligament venosum and isolate to posterior wall of the hepatic veins. This step may be dangerous and may spend a long time. In the early stage of the study (January 2003 to January 2005), conventional maneuver (tourniquet method) was used in most patients for dissecting the hepatic veins, while in the late stage (February 2005 to June 2006), "superior approach" (Satinsky technique) was used in most of the patients. With the accumulation of our experience for hepatic vein dissection, the duration of hepatic veins isolation in the late stage was shorter than that in the early stage. The total operating time was longer in the tourniquet group than in the Satinsky group, but there was no statistically significant difference.

In the tourniquet group, the hepatic vein could not be isolated and encircled in five patients, and the hepatic vein ruptured in one patient because the tumor invaded the hepatocaval junction. These hepatic veins could not be occluded with tourniquet. While in the Satinsky group, all of the hepatic veins could be controlled with Satinsky clamp and no hepatic vein rupture occurred. This result shows that "superior approach" is easier and safer than conventional method.

Hepatic vein rupture occurred in ten patients (10.5%) in the tourniquet group and in 12 patients (14.1%) in the Satinsky group during the parenchymal transection, but no massive bleeding or air embolism occurred because the hepatic veins had been occluded already before liver resection, and the incidence of the hepatic vein rupture was not statistically significant. It suggests that both of the methods can successfully control hepatic vein bleeding during hepatectomy.

Table 6 Liver Function after Operation

Postoperative time (days)	Tourniquet group (n=95)			Satinsky clamp group (n=85)		
	1	3	7	1	3	7
ALT (U/l)	540.0±170.0	210.0±70.0	65.0±15.0	570.0±160.0	195.0±65.0	55.0±10.0
Bilirubin (μmol/l)	30.1±7.2	23.6±5.3	16.4±4.4	28.7±9.4	22.5±6.1	17.3±5.2
Prothrombin time (s)	12.1±2.1	15.6±4.2	13.6±3.3	12.6±3.1	16.1±5.2	13.9±3.5
Serum albumin (g/l)	38.4±5.1	32.1±3.2	35.6±4.0	39.1±4.4	33.2±3.7	36.6±4.3

[a] Values were expressed as the $\bar{x} \pm s$

Conclusion

Compared with the tourniquet method, the Satinsky clamp method provides shorter time for dissecting and occluding the hepatic veins and provides a safer and easier approach to clamp the hepatic veins because the posterior wall of the hepatic vein is not necessary to be dissected. It is particularly useful when the liver tumor is large and is extremely difficult to be mobilized.

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Interval Period Tumor Progression: Does Delayed Hepatectomy Detect Occult Metastases in Synchronous Colorectal Liver Metastases?

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Abstract
Background Rapid remnant liver recurrence in patients with synchronous colorectal liver metastases (CRLM) is occasionally experienced after simultaneous colorectal and liver resection. We evaluated the tumor progression during interval periods to determine whether delayed hepatic resection detects occult metastases.
Methods One hundred thirty-seven patients underwent hepatectomy for synchronous CRLM. Up to 2003, 116 patients underwent simultaneous colorectal and hepatic resection. From 2004 onward, we identified 21 patients undergoing delayed hepatectomy for synchronous CRLM. The tumor progression during interval was determined by a dynamic computed tomography scan.
Results Median/mean interval between the two evaluations prior to the first and second surgery was 2/2.4 months. The median/mean number of metastases detected at each evaluation was 2/3.3 and 3/4.6, respectively. Nine of the 21 (43%) patients had new detectable metastatic lesions after reevaluation. For 11 of the 21 patients, it was necessary to reconsider planned surgical procedure which was determined prior to colorectal surgery. Hepatic disease-free survival was significantly different between patients undergoing delayed and simultaneous hepatectomy. Multivariate analysis showed that the delayed hepatectomy was a significant independent prognostic factor in hepatic disease-free survival.
Conclusion Tumor progression was recognized and occult metastases were detected after the interval reevaluation. Delayed hepatectomy may be a useful approach to reduce rapid remnant liver recurrence in synchronous CRLM.

Keywords Timing of metastases · Occult metastases · Prognostic factors · Liver resection

Abbreviations

CRLM	colorectal liver metastases
CEA	carcinoembryonic antigen
CA19-9	carbohydrate antigen 19-9
CT	computed tomography

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Introduction

Hepatic resection for colorectal liver metastases (CRLM) is the only potentially curative option, and the 5-year survival rate has been reported to range between 26% and 45%.[1–5] Previous studies have shown that there are several prognostic factors, such as the timing of metastases, the number of nodules, tumor size, a higher stage of primary cancer, extrahepatic metastases, and hepatoduodenal lymph node involvement.[2,5,6–14] Approximately 60% of the patients undergoing hepatic resection for CRLM experienced disease recurrence.[2] While aggressive surgery can prolong survival even in patients with synchronous and multiple CRLM, rapid remnant liver recurrence is occasionally experienced in patients with synchronous CRLM after simultaneous colorectal and hepatic resection. Patients with rapid remnant liver

recurrence may gain no survival benefit from hepatectomy, which may be one reason of their survival being worse than that of patients with metachronous CRLM. Thus, the most appropriate timing for hepatic resection for synchronous CRLM remains to be determined.

Previous studies demonstrated that the survival benefit of hepatic resection is determined by the biological features of the tumor.[10] Scheele et al. demonstrated that the micrometastases are likely to remain unresected in the remnant liver in patients undergoing synchronous surgery.[9] This suggests that the interval hepatic resection is a possible means of assessing tumor biological features for the identification of occult metastases. The only previous study of which we are aware, by Elias, reported only a 5% to 10% chance of new metastases being detected during the interval between separate operations (3–6 months).[15] However, preoperative radiological techniques such as multidetector row computed tomography (CT) have recently been developed and could define the extent of metastases more accurately.[16] Overall, the biological features of tumors, including tumor progression and the effect of interval resection on survival, have not been well-defined in patients with synchronous CRLM. In the present study, we reviewed our cases of patients who had undergone hepatic resection for CRLM to examine the changes in metastatic lesions during the interval period and to determine whether the delayed hepatic resection reduces the hepatic recurrence in patients with synchronous CRLM.

Materials and Methods

Patients

Between March 1985 and December 2006, a total of 137 patients underwent initial liver resection for synchronous CRLM in our institution. The medical records of these patients were retrospectively reviewed. In this study, synchronous metastases were defined as metastases diagnosed before colorectal surgery or at the time of surgery. Up to 2003, 116 patients with synchronous CRLM underwent simultaneous colorectal and hepatic resection. We previously reported that the synchronous metastasis is a significant factor by univariate analysis but is not independently significant prognostic factor by multivariate analysis.[4] However, the timing of metastases (synchronous) has become an independent significant prognostic factor defined by the multivariate analysis.[17] We also previously reported that 31% of the patients who underwent hepatectomy for CRLM had micrometastases that had not been detected.[4] Thus, we hypothesized that the rapid remnant liver recurrences in synchronous CRLM may originate from the occult hepatic metastases that cannot be detected at the time of hepatic resection. Therefore, from 2004 onward, our strategy for the treatment of synchronous liver metastases has been changed to perform separate operations for primary cancer and CRLM. From 2004 onward, 21 consecutive patients with synchronous CRLM underwent separate colorectal and hepatic resection.

Preoperative Evaluation

All patients were preoperatively evaluated by colonoscopy, abdominal ultrasonography, and thoracoabdominal enhanced CT. Identical thoracoabdominal-enhanced CT was performed for reevaluation prior to the delayed hepatectomy in patients undergoing delayed hepatectomy. In this study, hepatic metastasis was defined by an abdominal CT scan. Multidetector row CT has been used for the evaluation from 2002 onward. Remnant liver functional reserve was predicted from the indocyanine green retention rate at 15 min. Hepatectomy was performed regardless of the number of tumors whenever a remnant functional hepatic reserve was predicted to be preserved. Future remnant liver volume was predicted by CT volumetry in every patient from 1998 onward. Patients with a predicted remnant liver volume $\geq$35% of the total liver volume underwent liver resection without preoperative portal vein embolization from 1998 onward. The hepatectomy procedure did not differ whether the patients underwent simultaneous or delayed hepatectomy. With reference to synchronous extrahepatic metastases, patients with resectable pulmonary metastases at the time of diagnosis of liver metastases were surgical candidates. Based on this decision-making, three patients in the interval resection group underwent irinotecan-based chemotherapy (CPT-11+S-1) because they were diagnosed as irresectable at the time of reevaluation after colorectal surgery. Sixty-eight of the 116 patients in the simultaneous resection group received either hepatic arterial or systemic (oral) 5-fluorouracil-based chemotherapy. Twelve of the 21 patients in the interval resection group received systemic oral 5-fluorouracil-based chemotherapy.

Follow-up

After hepatic resection, all patients were followed up. Tumor markers such as carcinoembryonic antigen and carbohydrate antigen 19-9 were determined every 3 months. Ultrasonography, thoracoabdominal CT, or total colonoscopy was performed to examine recurrence. Patients were followed for survival until death or October 1, 2007.

Statistical Analysis

Data were expressed as median/mean, and variables were analyzed using the Mann–Whitney *U* test, Fisher's exact test, or chi-square test as appropriate. Hepatic disease-free

survival was calculated by the Kaplan–Meier method, and comparisons were performed using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model. We analyzed survival on an intention-to-treat basis. For all evaluations, a p value <0.05 was considered significant.

Results

Patient Characteristics

A total of 137 patients with synchronous CRLM who underwent hepatic resection were identified, of whom 83 were men and 54 were women. Up to 2003, 116 patients with synchronous CRLM underwent simultaneous colorectal and hepatic resection. Twenty-one consecutive patients with synchronous CRLM underwent delayed hepatic resection from 2004 onward. Median age was 66 years old (range, 41–75 years old). Twelve were men and nine were women.

Reevaluation of Tumor Progression during Interval Periods

The primary tumor was T3 or T4 in 21 patients and there were lymph node metastases in 15 of the 21 (71%). The median/mean duration of each evaluation by CT scan which was performed prior to the first (primary tumor resection) and second (hepatic resection) operations was 2/2.4 months (Table 1). The median/mean number of metastases was increased after reevaluation (from 2/3.3 to 3/4.6; Table 1). Nine of the 21 (43%) patients had new detectable metastatic lesions in different segments defined by Couinaud's classification after reevaluation, and eight patients had new metastases in the future remnant liver as determined by hepatectomy procedure which had been planned prior to primary colorectal tumor resection (Table 2). One patient (case 16), who had major complications associated with primary surgery, showed a significant increase in the number of metastases after reevaluation (Table 2). Unilateral metastases became bilateral in two patients. For 11 of the 21 patients, it was necessary to reconsider the planned surgical procedure of hepatectomy which was determined before colorectal surgery. At reevaluation after primary colorectal surgery, 3 patients underwent preoperative irinotecan-based chemotherapy (CPT-11+S-1) because they were diagnosed as irresectable as a result of a lack of liver volume due to the increased numbers of multiple bilateral metastases in one, an ill-located huge tumor in another, and an extrahepatic disease defined prior to colorectal resection (bilateral multiple lung metastases) in the third. All of the three patients underwent curative hepatic resection after chemotherapeutic downstaging. Two patients underwent preoperative portal vein embolization because of a lack of hepatic volume.

Table 1 Characteristics of Metastatic Lesion before or after Colorectal Resection (n=21)

Category	Resection of primary carcinoma	Number of cases	
		Before	After
Maximum diameter	median/mean (mm)	29.8/32.8	34.8/43.8
5 cm or less		19	17
More than 5 cm		2	4
Number of tumors	median/mean	2/3.3	3/4.6
Solitary		5	4
2–3 nodules		9	8
4 or more		7	9
Location			
Unilateral		8	6
Bilateral		13	15
Extrahepatic metastasis			
Lung		1	1

Survival and Prognostic Factors in Synchronous Metastases

To determine whether interval reevaluation identified a subgroup of patients with improved prospects of survival, the hepatic disease-free survival of all 21 patients undergoing delayed hepatic resection was compared with that of all the 116 patients undergoing simultaneous colorectal and hepatic resection. Patient characteristics are shown in Table 3. Forty-eight percent of patients who underwent simultaneous resection for synchronous CRLM had hepatic recurrence within 12 months (Fig. 1) and 61% of patients had recurrence within 24 months. To identify a subset of patients who could undergo either simultaneous or interval hepatic resection, we examined tumor-related factors. In patients who underwent simultaneous hepatic resection, multiple liver metastases and positive nodal involvement of the primary colorectal cancer were significant factors indicative of early recurrence within 12 months after hepatectomy (Table 4). Multiple liver metastases were a significant factor in early recurrence within 24 months after hepatectomy (data not shown). However, there was no significant predictive factor for interval recurrence in patients who underwent interval hepatic resection (Table 4). Hepatic disease-free survival was significantly different between the patients undergoing delayed hepatic resection (n=21) and simultaneous hepatic resection (n=116) based on an intention-to-treat analysis (p=0.0028; Fig. 1a). Hepatic recurrence within 12 months after hepatectomy was reduced in the delayed resection group (13%) compared with the simultaneous resection group. We examined hepatic disease-free survival in subgroups strat-

Table 2 Tumor Progression during Interval Period (n=21)

No.	Age	Sex	Tumor location (before)	Tumor location (after)
1	63	F	S5 (1), S6 (3), S7 (1), S8 (2)	S5 (1), S6 (3), S7 (1), S8 (2)
2	63	F	S3 (2), S6 (3), S7 (1), S8 (3)	S3 (2), **S4 (2)**, **S5 (2)**, S6 (3), S7 (1), S8 (3)
3	70	M	S3 (1), S6 (1), S8 (1)	S3 (1), **S4 (2)**, S6 (1), **S8 (2)**
4	74	F	S8 (1)	S (1)
5	65	F	S4 (2), S7 (1), S8 (1)	S4 (2), S7 (1), S8 (1)
6	70	F	S1 (1), S4 (1), S6 (1), S7 (1), S8 (1)	S1 (1), S4 (1), S6 (1), S7 (1), S8 (1)
7	70	M	S4 (1), S5 (1), S6 (1), S8 (1)	S4 (1), S5 (1), **S6 (2)**, S8 (1)
8	66	M	S4-5 (1), S6 (1), S7 (2)	**S2 (1)**, S4–5 (1), S6 (1), S7 (2), **S8 (1)**
9	41	F	S3 (1), S8 (1)	S3 (1), **S4 (1)**, **S4–8 (1)**, S8 (1)
10	71	M	S4 (1), S5 (1)	S4 (1), S5 (1)
11	73	F	S7 (1)	S7 (1), **S4 (1)**, **S6 (1)**
12	61	M	S2 (1), S4 (1)	S2 (1), S4 (1)
13	75	M	S6 (1), S7 (1)	S6 (1), S7 (1), **S4 (1)**
14	67	M	S3 (1), S8 (1)	S3 (1), S8 (1)
15	75	F	S2 (1), S3 (1), S6 (1)	S2 (1), S3 (1), S6 (1)
16	50	M	S1 (1), S2 (2), S4 (2), S6 (2), S7 (1), S8 (1)	S1 (1), S2 (2), **S3 (1)**, S4 (2), **S5 (2)**, S6 (2), **S7 (3)**, **S8 (3)**
17	67	M	S5 (1)	S5 (1)
18	61	M	S2, 3, 4, 5, 8 (1)	S2, 3, 4, 5, 8 (1)
19	58	F	S3 (1)	S3 (1)
20	46	M	S3 (1), S5 (1), S6 (1), S7 (2)	S3 (1), **S5 (2)**, **S6 (2)**, S7 (2), **S8 (1)**
21	50	M	S4 (2)	S4 (2)

Number of metastases is shown in parenthesis. A new metastatic lesion is indicated by bold characters and a new lesion appeared in different segments is underlined.
S Segment (defined by Couinaud's classification)

ified by the number of metastases (solitary/multiple). Hepatic disease-free survival was significantly different between patients undergoing delayed hepatic resection (n=17) and simultaneous hepatic resection (n=75) in multiple CRLM (p=0.0014; Fig. 1b). Hepatic disease-free survival was not significantly different between patients undergoing delayed hepatic resection (n=4) and simultaneous hepatic resection (n=41) in solitary CRLM (p=0.2767; Fig. 1c),

Table 3 Characteristics of Patients who Underwent Interval Hepatic Resection (n=21) or Synchronous Resection (n=116)

Category	Interval (n=21)	Synchronous (n=116)	p value
Sex			
Man	12	71	0.914
Woman	9	45	
Primary site			
Colon	11	67	0.827
Rectum	10	49	
Histology (primary)			
Well	4	31	0.092
Moderately	13	81	
Poorly, mucinous	3	4	
Primary tumor			
T1	0	2	0.645
T2	0	6	
T3	11	61	
T4	10	47	
Regional lymph nodes			
Negative	6	34	0.848
Positive	15	82	
Number of metastatic nodules			
Solitary	4	41	0.226
Multiple	17	75	

T factor is defined by TNM classification.

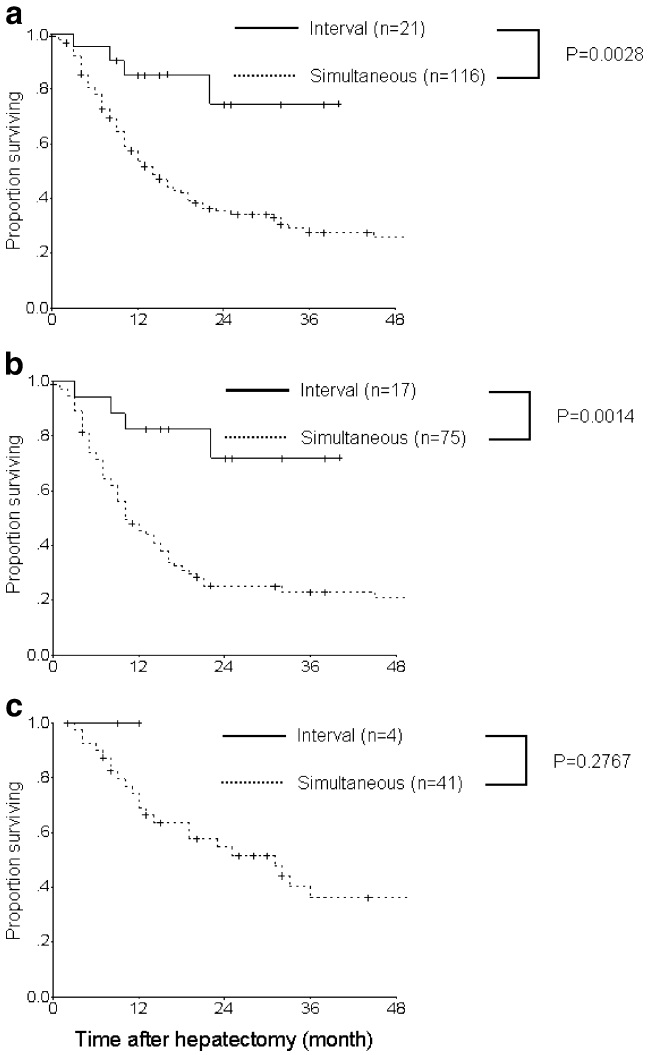

Figure 1 Hepatic disease-free survival of patients after hepatectomy was calculated by the Kaplan–Meier method. **a** Patients with synchronous colorectal liver metastases were divided into simultaneous hepatectomy (n=116) and interval hepatectomy (n=21) groups. **b** Patients with synchronous and multiple colorectal liver metastases were divided into simultaneous hepatectomy (n=75) and interval hepatectomy (n=17) groups. **c** Patients with synchronous and solitary colorectal liver metastases were divided into simultaneous hepatectomy (n=41) and interval hepatectomy (n=4) groups. A comparison was performed using the log-rank test. A p value <0.05 was considered significant.

although the number of patients in delayed hepatic resection was small. Interval resection was demonstrated by multivariate analysis to be a significant independent prognostic factor in hepatic disease-free survival in patients with synchronous CRLM (Table 5).

Discussion

The indication of hepatectomy for CRLM has recently been extended to include patients with multiple bilobar metastases, synchronous pulmonary metastases, and the invasion of major vasculatures.[18–20] The timing of metastases was reported to be a significant prognostic factor for survival.[9] Our previous report indicated that the timing of metastases was not a statistically significant independent prognostic factor, although more recently, it has become so.[4,17] One reason for this may be that the patients with more advanced synchronous CRLM have become candidates for hepatic resection. The other reason for this may be that the synchronous CRLM are likely to include occult micrometastases which cannot be detected preoperatively. We previously reported that 31% of the patients who underwent hepatectomy for CRLM had micrometastases,[4] and Yokoyama et al. showed that micrometastases are detectable by immunohistochemistry in 68% of the resected specimens and suggested that the micrometastases may be associated with a intrahepatic recurrence.[21]

Table 4 Association between Tumor (Primary and Metastatic)-Related Factors and Early Recurrence (within 12 months after Hepatectomy) in Simultaneous Resection Group (n=116) or Tumor Progression during Interval in Interval Resection Group (n=21)

Category	Recurrence (yes)	Recurrence (no)	p value
Within 12 months after simultaneous hepatectomy (n=116)	n=56	n=60	
Primary site			
Colon	35	32	0.418
Rectum	21	28	
Regional lymph nodes			
Negative	45	37	0.045
Positive	11	23	
Number of metastatic nodules			
Solitary	14	27	0.032
Multiple	42	33	
Tumor			
More than 5 cm	20	15	0.292
5 cm or less	36	45	
Iinterval recurrence in delayed hepatectomy (n=21)	n=9	n=12	
Primary site			
Colon	5	6	1.000
Rectum	4	6	
Regional lymph nodes			
Negative	2	4	0.659
Positive	7	8	
Number of metastatic nodules			
Solitary	1	5	0.178
Multiple	8	7	
Tumor size			
More than 5 cm	0	2	0.486
5 cm or less	9	10	

Table 5 Multivariate Analysis of Predictive for Hepatic Recurrence in Patients with Synchronous Metastases

Factors	Relative	95% confidence intervals		*p* value[a]
		Lower	Upper	
Primary nodal involvement (positive to negative)	1.49	0.90	2.46	0.124
Tumor (5 cm or less to more than 5 cm)	1.14	0.70	1.86	0.604
Number of metastases (solitary to multiple)	1.49	1.02	2.78	0.043
Time of resection (synchronous to interval)	4.74	1.72	13.1	0.003

[a] Cox proportional hazards model

The current study showed that nine of the 21 (43%) patients had new lesions at the time of reevaluation, an incidence greater than that reported by Elias,[15] and similar to our previous finding for the recurrence rate in the remnant liver after hepatectomy for CRLM.[4] One reason for this discrepancy might be the fact that the preoperative radiological techniques such as multidetector row CT have recently been developed and could define the extent of metastases more accurately.[16] However, the current findings suggested the possibility of occult micrometastases in patients with synchronous CRLM. Furthermore, during interval periods, new detectable lesions appeared in preoperatively planned future remnant liver which was determined prior to colorectal surgery, suggesting that occult metastases might cause rapid remnant liver recurrence after simultaneous hepatectomy. In fact, as shown in Fig. 1, a higher rate of rapid remnant liver recurrence was found in patients undergoing simultaneous colorectal and hepatic resection for synchronous CRLM. The current data showed that delayed hepatic resection reduced hepatic relapse and was a significant prognostic factor in hepatic disease-free survival in patients with synchronous CRLM. Taken together, we proposed that delayed hepatic resection for synchronous CRLM may detect the occult micrometastases and may allow curative hepatectomy, thus reducing hepatic recurrence. The results of the current study are consistent with those of Lambert et al.[22] demonstrating that interval resection may be a beneficial strategy for patients with synchronous CRLM.

Although this study was retrospective, there may have been some bias in relation to the variability of surgical approaches. In our study, there is likely to have been little substantial difference in surgical approach between the two groups, except for the development of radiological techniques. One concern regarding this strategy is that additional metastases at the time of reevaluation represent new lesions that have appeared during the interval period. Lambert et al.[22] considered it unlikely that new nodules would develop during interval periods; rather, it likely that occult metastases become detectable. In the current study, interval resection for synchronous CRLM, at least, did not impair survival. Rather, interval resection could allow the detection of occult metastases which might be associated with rapid liver recurrence after hepatectomy. A further advantage of this strategy is the minimization of surgical morbidity and mortality related to resection of the primary tumor, such as anastomotic breakdown which leads to infectious complications and secondary hepatic failure after hepatectomy.[18,23] Overall, from the standpoint of biological features, interval reevaluation may be a beneficial strategy.

Although our surgical indication for hepatectomy is to proceed to surgery regardless of the number of metastases whenever the remnant functional liver volume is preserved and a potentially curative resection can be performed, surgical procedures planned prior to resection of the primary tumor were reconsidered for the 11 patients. There may thus be some debate regarding the timing of resection. One requirement is to clarify how long duration between the first and second operations would be appropriate; another is to determine whether all patients with synchronous CRLM should undergo interval hepatectomy; the third is to determine whether chemotherapy is required during interval periods. Our current strategy was to perform separate surgery for synchronous CRLM, and the median/mean duration between the first and second operations was 2/2.4 months. Allen et al.[24] reported that for patients who underwent the second surgery immediately after recovery from colorectal surgery, the median interval was 7 weeks, similar to that in our study. In another study, it was reported that the interval period was up to 6 months.[25] Since no new extrahepatic metastases were found after colorectal surgery, the current strategy of interval resection may be appropriate. Although we examined predictive factors for early recurrence after simultaneous hepatectomy and interval recurrence during interval periods, we were unable to identify specific predictive factors for the two groups. The real need is to be able to identify which patients will not progress and to operate on them as early as possible, while sparing those who will progress an operation. As we do not have the ability to do this yet, delayed hepatectomy may be a reasonable approach.

Chemotherapy for colorectal carcinoma with agents such as oxaliplatin or irinotecan has greatly improved recently, and the median survival of patients with irresectable

advanced colorectal carcinoma has been reported to be over 20 months to date.[26–28] It has been reported that neoadjuvant chemotherapy could downstage irresectable CRLM in selected patients and prolong survival.[29] Such a strategy seems favorable for some patients with irresectable CRLM. In contrast, in patients with resectable CRLM, neoadjuvant chemotherapy still remains controversial. Allen et al.[24] reported that patients who received neoadjuvant chemotherapy experienced similar overall survival as patients who did not. They also demonstrated that subgroups of the patients with disease that did not progress with chemotherapy experienced significantly improved survival.[24] Similarly, Adam et al. reported that responsiveness to neoadjuvant chemotherapy prolongs survival and neoadjuvant chemotherapy may thus be beneficial for patient selection.[30] In the current study, three patients underwent preoperative chemotherapy because of irresectability after reevaluation. Fortunately, our 21 consecutive patients could undergo curative hepatectomy. It is important to note that irresectability is determined by an insufficient hepatic functional volume due to the multiple metastatic nodules, huge ill-located tumors which invade the hepatic hilus of portal pedicles or invade hepatic veins draining the remnant liver, and combined irresectable extrahepatic metastases. Thus, it seems likely that chemotherapy will be needed during interval periods if the observation periods are longer and if patients have either a huge tumor, ill-located tumor, or extrahepatic metastases. Since multiple CRLM was also a significant prognostic factor in our series, patients with synchronous multiple CRLM may need to undergo chemotherapy during interval periods. These data are consistent with the findings of Capussotti et al. indicating that patients with more than three metastases should receive neoadjuvant chemotherapy before liver resection.[31] Additionally, the number of lymph node metastases has been reported to be a survival indicator.[32] Interval resection could allow the evaluation of pathological findings prior to hepatectomy, and thus, patients with N2 in the tumor–node–metastasis (TNM) classification might need to undergo chemotherapy during interval periods. It might remain controversial to receive neoadjuvant chemotherapy instead of interval resection. In our study, rapid recurrence was found in patients with multiple metastases or primary nodal involvement after simultaneous resection. Thus, when patients with multiple metastases or suspicious primary nodal involvement are planned to undergo simultaneous hepatectomy instead of interval resection, neoadjuvant chemotherapy may be required. Rather, in the future, neoadjuvant chemotherapy appears to play an important role in this setting by diminishing occult micrometastases and allowing curative resection. However, care should be taken in administering neoadjuvant chemotherapy for resectable CRLM, since the incidence of surgical morbidity after hepatectomy increases in some patients due to liver damage after neoadjuvant chemotherapy.[33,34]

In conclusion, tumor progression was recognized and occult metastases were detected after interval reevaluation. Delayed hepatic resection may be a useful approach that allows the detection of occult metastases in synchronous CRLM and may reduce rapid remnant liver recurrence after hepatic resection for synchronous CRLM.

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Survival of Patients with Synchronous and Metachronous Colorectal Liver Metastases—is there a Difference?

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Abstract
Background The aim of this study was to compare outcomes in patients with synchronous and metachronous colorectal liver metastases, with special emphasis on prognostic determinants.
Study design We analyzed prospectively collected data on 101 patients with synchronous metastases (group A) who were treated surgically during the time period from April 1998 to December 2006 in regard to overall and disease-free survival, impact of chemotherapy, as well as several serum parameters. A group of patients with metachronous colorectal liver metastases (group B) was considered for baseline comparison.
Results Twenty-three patients in group A received only an explorative laparotomy. Surgical treatment included right hepatectomy (n=7), left hepatectomy (n=5), right trisectionectomy (n=10), left trisectionectomy (n=1), left lateral resection (n=11), and sectionectomy (n=44). Thirty-day mortality was 3%. Morbidity was observed in 10% of the patients. One-, 3-, and 5-year overall survival rates for synchronous metastases were 86%, 68%, and 47%, respectively. The corresponding rates for metachronous metastases were 94%, 68%, and 39% (p>0.05). Disease free survival was 74%, 42%, and 33% in group A versus 84%, 62%, and 13% in group B (p=0.28). There was no difference in survival between patients receiving neoadjuvant chemotherapy and no chemotherapy (p>0.05). Out of all serum parameters, carcinoembryonic antigen levels were a negative predictor for overall and disease-free survival only.
Conclusions Patients with synchronous colorectal liver metastases had a similar 5-year overall and disease-free survival, which corresponds to patients with metachronous metastases. The impact of neoadjuvant chemotherapy in patients with synchronous metastases needs to be further clarified.

Keywords Colorectal carcinoma · Liver metastases · Resection · Metachronous · Synchronous

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Introduction

Approximately 20% of patients with colorectal cancer have synchronous metastases at the time of diagnosis.[1,2] Several authors have stated that the seemingly more aggressive tumor biology of synchronous metastases is responsible for the worse prognosis compared to patients with metachronous metastases.[3–5] Others found no decrement in 5-year survival in patients with synchronous metastases.[6]

The optimal surgical therapy, though for synchronous metastases, is still a matter of debate. Similar surgical outcome after simultaneous or staged hepatectomy has led to a tendency toward simultaneous resection, even though this has included mostly minor liver resections.[7–10] Others advocate a delayed hepatic resection to be able to administer chemotherapy before liver resection.

Nevertheless, the modern oncologic multimodal concepts, like adjuvant or neoadjuvant chemotherapeutic regimen and their impact on patients with synchronous metastases, remain unclear.

The aim of our case control study was to evaluate disease-free and overall survival rates in patients with synchronous metastases and to compare them with a control group of patients with metachronous metastases.

Patients and Methods

Between April 1998 and December 2003, 101 consecutive patients with synchronous metastases were treated for metastases from colorectal cancer at the Department of General, Visceral and Transplantation Surgery at the University Hospital Essen, Germany. An equal number of patients with metachronous metastases were chosen (in the context of a retrospective case-control study) from a group of 551 patients with colorectal liver metastases. Selection of patients of the control group was done (by stratified randomization) to ensure a balanced distribution of confounding factors[11] by defining the following confounding variables: age, site of primary tumor (right –left side), grade of primary tumor, stage of primary tumor, carcinoembryonic antigen (CEA) levels, number and distribution of metastases, resection margins, size of maximum metastasis, portal lymph node involvement, and type of resection. Age and CEA levels were categorized by decimal *c* values and size of maximal metastasis classified by a cutoff value of 6 cm. Classification of hepatic metastases were performed according to Gayowski et al.[12] Characteristics of patients of both groups were compared to establish the baseline indicators (Table 1).

Timely selection of patients was done to make certain at least a 4-year follow-up (according to bibliography, patients with liver resections have a median survival of approximately 3 years). We considered metastases as synchronous when detected before colon resection, intraoperatively at the time of the primary tumor surgery or by imaging studies within 12 months after the primary operation. Criteria for liver resection included (a) removal of the primary lesion with curative intent, (b) technical feasible resection by preoperative workup, and (c) no evidence of unresectable extrahepatic disease.

Almost half of all patients with synchronous metastases received chemotherapy in a neoadjuvant fashion based on the recommendations of the medical oncology services.

Follow-up was obtained from outpatient records, letters, and telephone interviews. Follow-up ranged from 7 to 104 months, with a median of 40 months. Deaths within hospital were considered as perioperative mortality.

Disease free- and overall survival rates were calculated using the Kaplan–Meier method and comparisons were made using the log-rank test. A multivariate Cox proportional hazard regression analysis was performed to identify potential prognostic survival factors in the group of patients with synchronous metastases (not in the control group to avoid a type 2 error). The p values<0.05 were considered statistically significant. Statistical analyses were performed using SPSS 11.5 and SAS 8.

Results

Patient and Tumor Characteristics

There were no significant differences in mean age, gender, primary tumor site, CEA levels, modified tumor–node–metastases stage of metastatic disease, or comorbidities between patients with synchronous (group A, $n=101$) and metachronous (group B, $n=101$) liver metastases (Table 1). Twenty-nine patients in each group had a single metastasis, 47 had two to three, and 25 had four or more. Seventy metastases were unilobar and 31 bilobar (Table 1).

Table 1 Patients and Primary Tumor Characteristics of Patients with Synchronous (Group A) and Metachronous (Group B) Colorectal Liver Metastases

	Synchronous liver metastases (group A)	Metachronous liver metastases (group B)	*p* Value
Patients *N*	101	101	
Female/male	48/53	40/61	NS
Median age	60 (23.6–81.2)	62.7 (27.5–81.2)	NS
Primary tumor stages I, II, III, IV	2, 25, 43, 31		
Metastases classification[12] I, II, III, IVa, IVb	14, 30, 26, 24, 7	15, 32, 23, 23, 7	NS
Comorbidity	61	61	NS
Cardiovascular	43	53	
Nephrological	7	4	
Pulmonary	11	4	
Size of metastases (cm)	5 (1.5–7)	4.7 (1.5–8)	NS
CEA (μg/l)	51	20	0.012

Preoperative Serum Parameters

CEA, CA 19-9, and alkaline phosphatase were found to be of prognostic value for 5-year overall survival ($p<0.001$, 0.008, and 0.040). Only CEA was of predictive value for disease-free survival ($p<0.012$).

Preoperative Chemotherapy

Forty-seven ($n=47$, 47%) of the 101 patients in group 1 received preoperative chemotherapy. Chemotherapy consisted mostly of a fluorouracil-based regimen complemented by additional agents such as leucoverin, irinotecan, or oxaliplatin. Within the group of patients who received chemotherapy, 12 different chemotherapy strategies could be found with varying numbers of cycles. We were unable to identify a clear treatment regimen as to why the patients received chemotherapy in the first place and what was the reason to consult a surgeon after several cycles of chemotherapy. Within the group of patients receiving chemotherapy, we found several patients with regression under chemotherapy, as well as several patients with a progression and others with no change of tumor volume at all.

Surgical Results

Median time between primary operation and liver resection was 6 months (range, 1–30). The time interval between primary operation and liver resection was 2.3 months (range, 0–7) for patients who did not receive chemotherapy vs. 8.5 months (range, 2–30) in patients who received chemotherapy. Of the 101 patients with synchronous metastases (group A), 23 underwent only exploration because of unresectable extrahepatic ($n=7$) or hepatic ($n=16$) disease. The remaining 78 patients received the following procedures: right hepatectomy ($n=7$), left hepatectomy ($n=5$), trisectionectomy right ($n=10$), trisectionectomy left ($n=1$), left lateral sectionectomy ($n=11$), wedge resection ($n=12$), and segmentectomy ($n=32$). None of the operations were concurrently performed with resection of the primary cancer.

In the group with metachronous metastases (group B), 19 patients received an explorative laparotomy only due to extrahepatic tumor in eight and non-resectable liver manifestations in 11 cases. The surgical procedures were as follows: right hepatectomy ($n=15$), left hepatectomy ($n=12$), trisectionectomy right ($n=3$), trisectionectomy left ($n=3$), left lateral sectionectomy ($n=5$), and sectionectomy ($n=44$; Table 2).

Mortality, Morbidity, and Feasibility

Operative time, units of blood transfused, and length of hospital stay associated with the hepatectomy were 196 min (range, 35–480), 2 IU (range, 0–13), and 17 days (range, 3–61), respectively, for those with synchronous metastases. The corresponding values for patients with metachronous metastases were 202 min (range, 60–450), 1 U (range, 0–15), and 18 days (range, 3–102; p=ns).

Morbidity rates were 10% for group A and 9% for group B ($p=0.8$). Seventeen patients in group A developed 17

Table 2 Perioperative characteristics of Patients with Synchronous (Group A) and Metachronous (Group B) Colorectal Liver Metastases

	Synchronous liver metastases (group A)	Metachronous liver metastases (group B)	*p* Value
Operative Procedure			NS
Explorative laparotomy	23	19	
Right hepatectomy	7	15	
Left hepatectomy	5	12	
Trisectorectomy right	10	3	
Trisectorectomy left	1	3	
Left lateral resection	11	5	
Sectorectomy	44	44	
Operative time	196 (35–480)	202 (60-450)	NS
Units of blood	1.7 (0–13)	1 (0-15)	
Units of GFP	1 (0–12)	0.3 (0-16)	
Hospital stay (days)	17.3 (3–61)	18.2 (3-102)	
Complications	11 in 17 patients (10%)	9 in 17 patients (9%)	NS
Abscess	4	3	
Ileus	1	1	
Bile leakage	6	9	
Bleeding	2	1	
Transient liver insufficiency	4	3	
Reoperation	6 (6%)	9 (9%)	NS
Hospital mortality	3 (3%)	2 (2%)	NS

postoperative complications, including subphrenic abscesses (n=4), ileus (n=1), bile leakages (n=6), bleeding (n=2), and transient liver insufficiency (n=4) defined as international normalized ratio below 1.5. This was statistically insignificant to the 9% of postoperative complications in group B. Six (n=6) patients (5%) required reoperations to address such complications. Mortality rates within 30 days postoperatively were 3% (n=3) in group A and 2% (n=2) in group B (p=ns; Table 2).

Patient Outcome

Overall 1-, 3- and 5-year survival rates for group A patients (n=63) who underwent R0 resections were 86%, 68%, and 47%, respectively, compared to 94%, 68%, and 39% for the R0 resected patients in group B (n=63; p=0.78; Fig. 1a). Disease-free survival was 74%, 42%, and 33% in group A versus 84%, 62%, and 13% in group B (p=0.28; Fig. 1b). Among the factors studied, univariate analysis showed significant differences in survival and recurrence rates associated with the presence of extrahepatic disease and R0 resection margins (Fig. 1b). Statistical significance was observed within each group when comparing R0 vs. R1 resections (p=0.04 in group A; p=0.02 in group B). As long as an R0 resection was achieved, there was no difference between synchronous and metachronous metastases (p=0.78). Outcome was not influenced either by the temporal relationship of synchronous versus metachronous metastases or by the use of chemotherapy among group A patients (Fig. 2a,b).

Discussion

The benefit of liver resection for patients with colorectal metastases has been previously established.[1,8,9,13–16] The treatment regimen of choice for synchronous metastases though is not as clear. Several authors have stated that

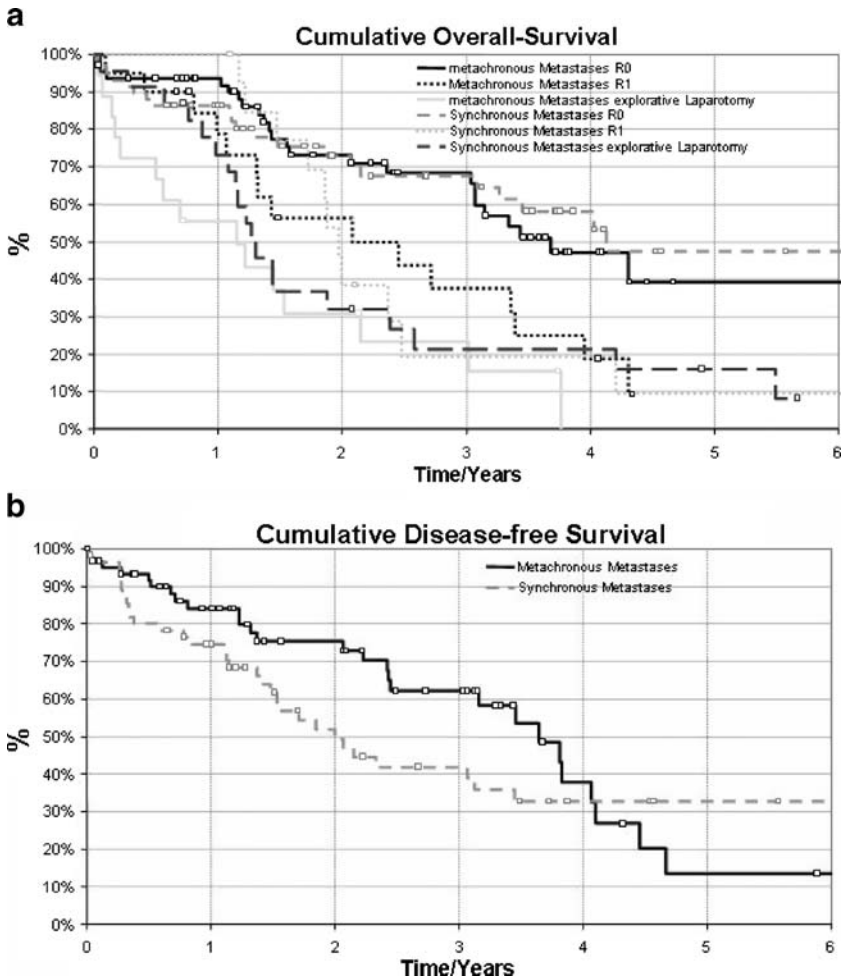

Figure 1 **a** Cumulative 5-year overall survival of patients with synchronous or metachronous colorectal liver metastases according to tumor-free margins. There was a significant difference in patient survival between tumor-free margins and those with no tumor-free margins (p<0.03 to <0.001). **b** Cumulative disease-free survival of patients with synchronous or metachronous colorectal liver metastases after R0 resection.

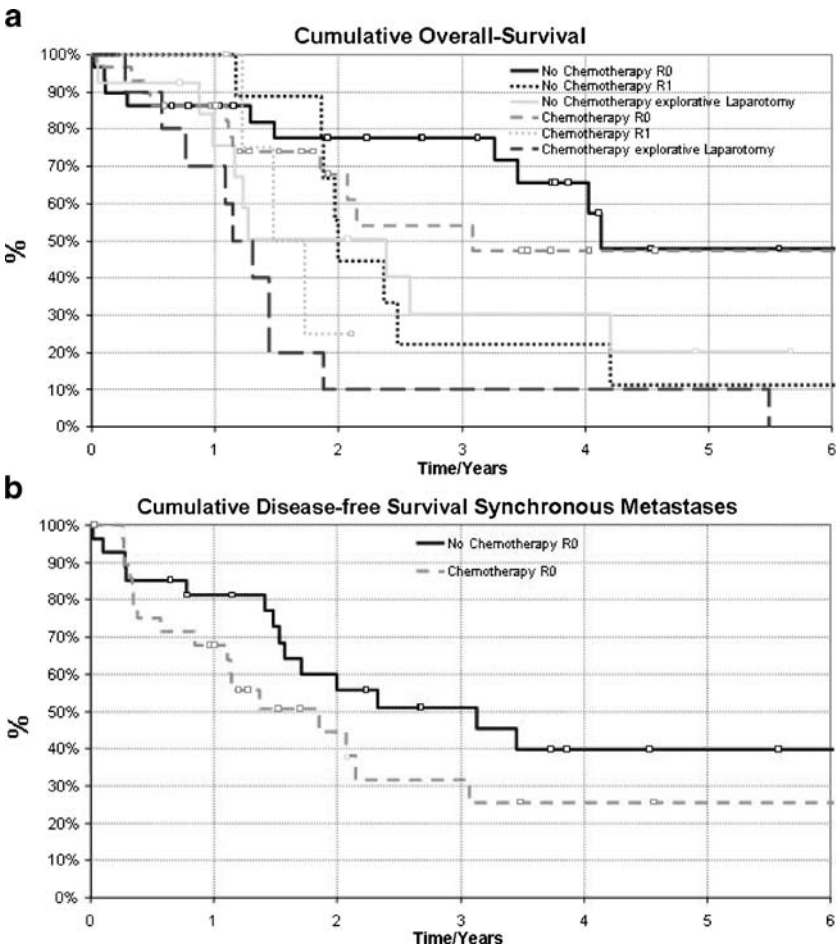

Figure 2 **a**, **b** Cumulative 5-year overall survival of patients with synchronous or metachronous colorectal liver metastases receiving chemotherapy and those who did not. We were unable to show a statistical significance in 5-year overall and disease-free survival.

synchronous metastases have a worse prognosis compared to patients with metachronous metastases.[3–5] This might be the reason that a high percentage of patients with synchronous metastases are presented to the oncology service first, before the respectability of the metastases has been judged by a surgeon. Our study refutes the widespread assumption that patients with synchronous have a worse prognosis than those with metachronous metastases.

Much thought has been given to the timing of the hepatectomy. While Nordlinger et al. and Bolton et al. reported higher complication and mortality rates for patients with simultaneous resection of colon and liver lesions,[17,18] others showed that in selected patients, simultaneous resections have morbidity and mortality rates similar to those of staged procedures.[8–10] Despite these pros and cons, in our own experience as a tertiary referral center for hepatobiliary surgery, most patients are sent to us after the decision to proceed with a delayed resection has been already made by the referring physician.

Various clinical and pathological factors have been evaluated and identified as important prognostic determinants of survival in patients with colorectal liver metastases, including sex, age at hepatectomy, stage of the primary tumor, number, size and distribution of hepatic metastases, extrahepatic lesions, type of hepatectomy, and surgical resection margins.[18–21] Nevertheless, none of these seemingly prognostic factors has been considered an absolute contraindication to liver resection. Of all the factors evaluated, we identified CEA to be a negative prognostic factor for 5-year overall and disease-free survival only. This might be due to the fact that larger tumors produce more CEA and are therefore associated with a higher percentage of greater resections or explorative laparotomies only.

Univariate analysis in our series identified a positive pathological margin as a significant prognostic factor. Wray et al. have stressed the importance of a clear resection margin of at least 1 cm, others though were not able to confirm this and showed that the margin does not matter or that even a positive margin was not associated with an increased risk of recurrence either at the surgical margin or elsewhere.[22–24] In our own study, even R1-resected patients have a statistically significant better overall survival than patients with no resection at all. Our results prompt us to recommend that every measure should be undertaken to resect the metastases, even if only an R1 situation can be achieved. Our aggressive approach is also the explanation for the high

percentage of explorative laparotomies. Nevertheless, similar percentages of explorative laparotomies have been described in the literature from high-volume centers.

New agents and drug combinations have led to improved response and survival rates.[25] Interestingly, we could not see in our series any survival benefit associated with the use of chemotherapy. Variations in chemotherapy indications, choice of agents, and timing prevented us from obtaining firm conclusions. It seemed that the preference for chemotherapy or resection was more dependent on the choice of our referring physician rather than on available clinical evidence. Standard guidelines, regular tumor board meetings, and the availability of an experienced hepatobiliary surgeon are indispensable to provide every patient with the best treatment options possible.

From a technical point of view, we have seen tremendous developments in surgical outcomes with the use of devices such as intraoperative ultrasonography,[26] vascular clamping techniques supplemented with low central venous pressure,[27] availability of novel transection devices,[28] controlled anatomic hepatectomies,[29] portal vein embolization leading to hypertrophy of future liver remnants, and staged hepatic resections,[30] as well as pre-procedure virtual tumor resections with computer-assisted risk analyses.[31] By pushing the frontiers of resection, we have certainly helped patients, but the number of patients with primarily unresectable colorectal liver metastases remains high and has not been decreased significantly for over a decade.[19,32,33] The development of new therapeutic drugs and multimodal concepts is necessary to increase the percentage of resectable patients.[34]

In conclusion, we have demonstrated that synchronous metastases can be safely resected with survival rates similar to those of metachronous metastases.

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ORIGINAL ARTICLE

The Effects of Scolicidal Agent Propolis on Liver and Biliary Tree

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Abstract
Background This study was designed to examine the effects of propolis on the liver and biliary system when used as a scolicidal agent.
Materials and Methods Thirty Wistar–Albino rats were divided into two groups. Propolis and 0.9% saline (NaCl) were injected into the biliary tract of the rats. Three rats from control group and four rats from propolis group died within 5 days after the procedure. Blood samples of remaining 23 rats were obtained 1 week after and at the end of the experimental study for liver function tests. Six months after the procedure, retrograde and magnetic resonance cholangiography were performed and liver, common bile duct, and duodenum were excised en bloc for histopathological examination.
Results Liver function tests were slightly elevated 1 week after the procedure and were found to be normal at the end of the sixth month in both groups. No stricture in the biliary tree was found on the retrograde and magnetic resonance cholangiograms. The tissue samples of the propolis group showed no histomorphological difference from the control group.
Conclusions Propolis may be used as a scolicidal agent even in the case of cystobiliary communication with no side effects on liver and biliary tree.

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Keywords Propolis · Scolicidal agent · Hepatobiliary system · Hydatid disease · Sclerosing cholangitis

Introduction

Echinococcosis is a parasitic tapeworm infection, caused by the larval cestode *Echinococcus* granulosus. Hydatid disease is endemic in many countries, and the disease remains endemic in Mediterranean countries, the Middle and Far East, South America, Australia, New Zealand, and East Africa. Historically, management of hydatid cysts in the liver typically involved an open surgical approach with meticulous operative site packing and employed a variety of conservative and radical operative techniques. Dissemination of protoscolex-rich fluid during surgery is a major cause of recurrence. Various scolicidal solutions have been used for surgical and percutaneous approaches.[1,2] Caustic sclerosing cholangitis is a dreadful complication after surgical treat-

ment. The development of sclerosing cholangitis had been attributed to the passage of the scolicidal solutions into the biliary tree through a cystobiliary fistula.[3,4]

Propolis is a resinous material collected by bees from various plants. Once collected, this material is enriched with salivary and enzymatic secretions of bees. Propolis is used by bees to cover hive walls, fill cracks or gaps, and embalm killed invader insects.[5] Propolis contains a variety of flavonoids, phenols, alcohols, terpenes, sterols, vitamins, and amino acids.[6] In a previous study, we found that propolis was totally effective on protoscolices in low concentrations and short exposure time.[7]

This study was conducted to investigate the effects of propolis on the liver and biliary tree by direct injection of propolis into the common bile ducts of the rats.

Materials and Methods

Animals

Thirty Wistar–Albino female rats, weighing 200±25 g, were included in this study. Animals were deprived of food 12 h before anesthesia but had free access to water 2 h before operation. No enteral or parenteral antibiotics were administered at any time. Rats were housed under constant temperature (21±2°C) individually in wire cages with 12-h light–dark cycle. The rats that died during the study were excluded. The procedures in this experimental study were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals, and ethical approval was obtained from the Animal Ethics Committee of Ankara Research and Training Hospital.

Surgical Procedure

The rats were randomly divided into two equal groups of 15 rats each. Rats were anesthetized by intramuscular injection of 40 mg/kg body weight ketamine HCl (Ketalar, Parke-Davis, Eczacibasi, Istanbul, Turkey) and 5 mg/kg body weight xylazin (Rompun, Bayer, Leverkusen, Germany). All animals were allowed to breath spontaneously during the experiments. After the abdomen was shaved and cleaned with povidone iodine, a midline laparotomy was carried out, and the intestines were covered with sterile gauze pads soaked with isotonic saline at 36–38°C. In addition, 5 ml Ringer's lactate solution was given subcutaneously to prevent dehydration in animals during the experimental period. A 3-mm duodenotomy was performed and 0.15 ml of test solutions, either sterile isotonic saline solution (control group) or 1% dilutions of propolis (study group) (Anzer propolis, Rize, Turkey), were injected without pressure into the common bile duct with a 27-gauge syringe. Immediately after the injection, the common bile duct was clamped with an atraumatic vascular clamp. The catheter was then withdrawn. The clamp was removed 5 min later, and the duodenotomy was closed with a 6-0 polypropylene suture. There was no operative mortality. The study animals were kept for 6 months, during which time they were fed with rat chow ad libitum and tap water and kept at room temperature (18–20°C) in separate cages.

Blood samples were obtained 1 week after the surgical procedure and at the end of the experimental study (6 months after the procedure) for liver function tests including bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transferase.

Six months after the procedure, 0.3 ml of radiopaque solution was injected from the tail vein of the rats, and magnetic resonance cholangiograms were performed under ketamine hydrochloride anesthesia. After this procedure, retrograde cholangiography was performed, midline laparotomy was carried out, and a 3-mm duodenotomy was performed. Radiopaque solution 0.15 ml per rat was injected without pressure into the common bile duct with 27-gauge syringe. Immediately after the injection, anteroposterior cholangiograms were obtained. After cholangiography, blood samples for determination of liver function tests were obtained by cardiac puncture. Liver, common bile duct, and duodenum were excised en bloc for histopathological examination.

Biochemical and Histopathological Examinations

The biochemical analyses were made by an autoanalyzer (Olympus AU 640, Japan) using commercial kits.

The liver specimens of the right and left lobes and common bile duct were taken and immediately fixed in 10% neutral-buffered formaline solution for 1 week. Tissues were washed in flowing water and dehydrated with rising concentrations of ethanol (50%, 75%, 96%, and 100%). After dehydration, specimens were put into xylene to obtain transparency and were then infiltrated with and embedded in paraffin. Histological sections of the specimens in thickness of 6 μm from all the groups were stained with hematoxylin and eosin. The whole tissue blocks were sectioned, and histopathological examinations were performed on systematically randomly sampled preparations by a blinded researcher. Liver specimens were evaluated to assess the morphology of the hepatocytes, portal areas, sinusoidal lesions, cellular infiltration in the lobule or portal spaces, and parenchymal lesions. Histopathological examination of the common bile duct was performed to assess the histomorphology of the epithelium, connective tissue, inflammation, fibroblastic proliferation, and necrosis.

Table 1 Mean values of liver function tests at the end of first week

GROUPS	ALT	AST	ALP	GGT
Control (group I)* (n=12)	56.66±6.86	58.91±7.02	62.08±9.30	41.25±4.31
Propolis (group II)* (n=11)	60.81±2.22	61.81±2.71	64.63±5.86	41.04±4.47

ALT Alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transferase
*p>0.05

Statistical Analysis

Differences between the groups were analyzed with Mann–Whitney *U* test. Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows (SPSS Inc., Chicago, USA). *p* values less than 0.05 were considered to be significant.

Results

General

Three rats from control group and four rats from propolis group died within 5 days after the procedure. Three of seven died in the early postoperative period possibly due to anesthesia, and the others died because of trauma to the common bile duct and leakage into the peritoneum. The remaining 23 rats were alive until the end of the study without any problem.

Biochemical and Radiological Results

Liver function tests were slightly elevated 1 week after the procedure in both groups, and this might be due to the cannulation and injection of common bile duct. There was no difference between the groups (Table 1). At the end of the sixth month, liver function tests were found to be normal in both groups (Table 2).

No stricture in the biliary tree was found on the retrograde and magnetic resonance (MR) cholangiograms (Fig. 1).

Histopathological results

Both control and the propolis groups did not show any difference from normal lobul structure of liver tissue. There was no enlargement of hepatocytes or/and dilatation of canalicular spaces in both groups. In addition, there was no bile pigment accumulation in the tissue sections of both groups (Fig. 2A,C). Tissues from the both groups presented no morphological alterations in the portal tract. A typical portal tract containing terminal branch of the hepatic portal vein, terminal branches of the hepatic artery with the structure of arterioles, and bile ductules were in regular architecture in propolis and control groups (Fig. 2B,D). The infiltration with mononuclear cells was not seen in the liver parenchyma of the propolis group, while it was visualized around the terminal hepatic venules of the control group (Fig. 2A,C). The mononuclear cell infiltration in the portal areas was recognized in the liver specimens of control group, while it could be neglected in the propolis group (Fig. 2B,D). When we examined the ductus choledochus specimens of the propolis group, we clearly observed the lining epithelium consisted of a single layer of tall columnar cells above with the connective tissue. The lumen was wide-opened, as it was in the control group. The sacculi of Beale, the appearance of the infoldings of the surface epithelium, were observed in common aspect. We did not notice any recognizable mononuclear cell infiltration in both groups. As a result, the tissue samples of liver and ductus choledochus of the propolis group showed no histomorphologic difference from the control group, and also, we did not see any adverse effect of the treatment (Fig. 3).

Table 2 Mean values of liver function tests at the end of sixth month

GROUPS	ALT	AST	ALP	GGT
Control (group I)* (n=12)	47.08±2.74	46.33±3.93	48.91±4.77	37.83±3.83
Propolis (group II)* (n=11)	47.72±2.93	46.63±3.64	49.54±5.35	37.95±3.44

ALT Alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transferase
*p>0.05

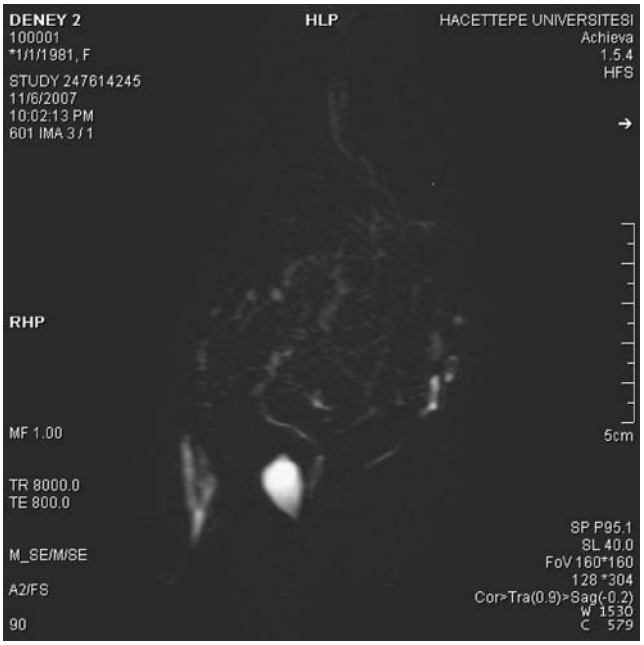

Figure 1 The magnetic resonance cholangiogram of a rat from propolis group.

Discussion

Propolis, or bee glue, is a brownish resinous material collected by honeybees from various plant sources. The chemical composition of propolis has been clarified to some extent in recent years. Hundreds of chemical compounds have been identified in propolis samples. The main chemical classes present in propolis are flavonoids, phenolics, and various aromatic compounds. Other compounds in propolis are volatile oils and aromatic acids (5–10%), waxes (30–40%), resins, balms, and pollen grains, which are rich sources of essential elements such as magnesium, nickel, calcium, iron, and zinc. However, its chemical composition varies depending on the site of its collection.[8] Antimicrobial properties of propolis seem attributable mainly to the flavonoids, pinocembrin, galangin, and pinobanksin. Pinocembrin also exhibits antifungal properties. Other active compounds are ester of coumaric and caffeic acids. Prenylated *p*-coumaric and diterpenic acids possess antibacterial and cytotoxic activities. Caffeoylquinic acid derivates show immunomodulatory and hepatoprotective actions and furofuran lignans inhibit the growth of some bacteria.[6]

The ideal treatment for hepatic hydatid disease should completely eliminate the parasite and prevent recurrence of

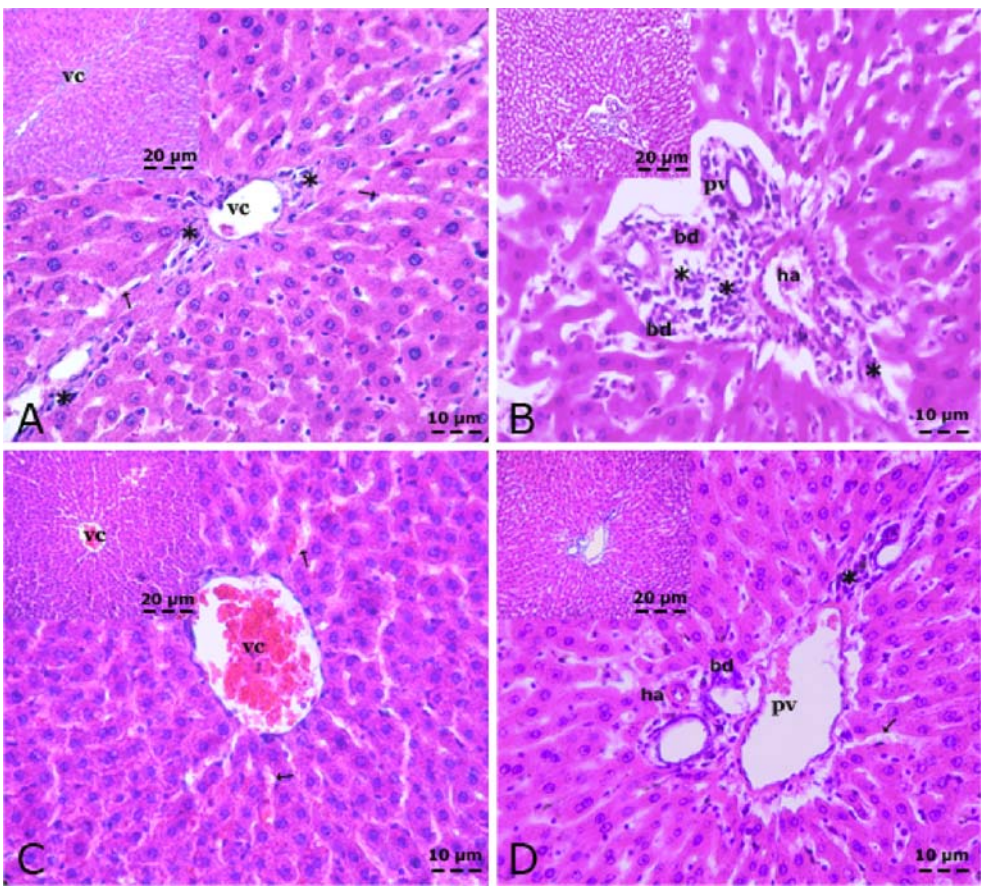

Figure 2 Liver sections stained with hematoxylin and eosin. **A**, **B** Control group showing the regular architecture. **C**, **D** Propolis group. *vc* Central vein, *pv* portal vein, *ha* hepatic arter, *bd* bile ductules; *arrow*, hepatic sinusoids, *asterisk* cell infiltration.

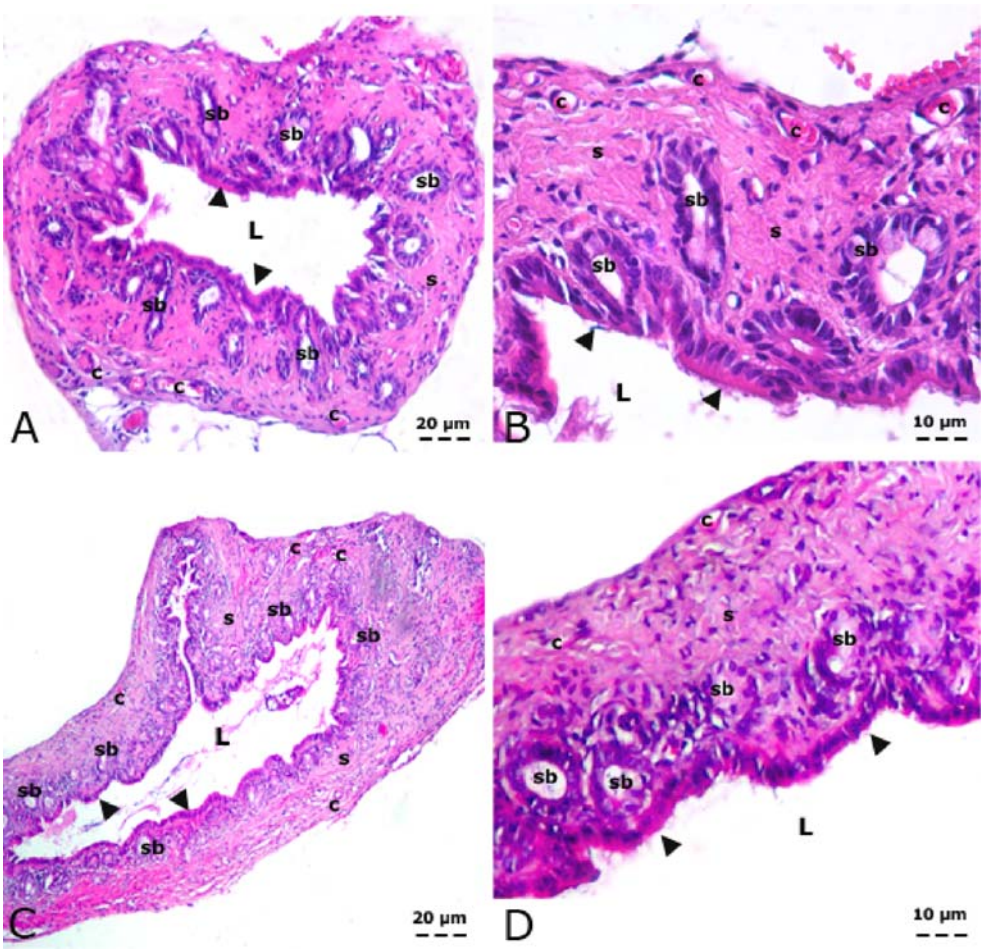

Figure 3 Common bile duct (ductus choledochus) sections stained with hematoxylin and eosin. **A**, **B** Control group. **C**, **D** Propolis group. *L* Lumen, *sb* sacculi of Beale, *c* capillary, *s* stroma; *arrowhead*, simple columnar epithelium.

the disease with minimum morbidity and mortality. There are three available therapeutic modalities for hepatic hydatid cysts: systemic chemotherapy, surgery, and percutaneous treatment.[9] Meticulous packing of the operative field is necessary irrespective of the surgical technique employed. In conventional or minimally invasive hydatid disease surgery, disinfection of the cyst cavity is very important, justifying the routine use of scolocidal solutions. In the presence of cystobiliary communications, the passage of these solutions may cause spotty necrosis in the liver parenchyma, widening of the sinusoids, regenerative changes in hepatocytes, Kupffer cell hyperplasia, pigment accumulation, periductal fibrosis, inflammation, fibroplastic proliferation, and necrosis in the extrahepatic biliary ducts.[1,4] Various scolicidal agents such as 95% alcohol, 10% povidone iodine, hypertonic saline, hydrogen peroxide, 5% formalin, silver nitrate, cetrimide, and albendazole have been evaluated for scolicidal effects and hepatobiliary complications in the presence of cystobiliary communications.[10–13] Sahin et al.[12] evaluated the effects of hypertonic saline (20%), povidone-iodine (1%), and silver nitrate (0.5%) on liver and biliary tree and found that the use of these agents resulted in chronic low-grade biliary inflammation. The intensity of the lesions was more remarkable in the silver nitrate group. In an experimental study, Houry et al.[3] showed that injection of 20% hypertonic saline or 2% formaldehyde solution into the biliary tracts of the rats was followed by lesions of the biliary epithelium. As compared with 20% hypertonic saline solution, the 2% formaldehyde solution caused more severe lesions of the biliary epithelium and, in addition, induced the development of sclerosis. They concluded that that intracystic injection of 2% formaldehyde solution should be abandoned. Belghiti et al.[14] reported five cases of caustic sclerosing cholangitis with the use of 2% formaldehyde and 20% sodium chloride solutions, and because of the risk of this complication, they recommended that intracystic injection of a scolicidal solution should be abandoned in the surgical treatment of hydatid disease of the liver. In conclusion, sclerosing cholangitis is a serious complication that develops with the use of some scolicidal agents in the presence of cystobiliary communications, and an ideal scolicidal agent should not cause such a serious side effect.

Sclerosing cholangitis may be due to immunological, infectious, vascular, or chemical factors. In patients with hydatid disease of the liver, various factors, including

injection of scolicidal agent into the cyst cavity, a communication between the cyst and biliary tree, and a particular sensitivity to the scolicidal agent, seem to be necessary to promote caustic sclerosing cholangitis.[12]

In a previous study, we evaluated the scolicidal effect of propolis and found that it was totally effective in low concentrations (1%) and short exposure time (3 min). Propolis did not cause any systemic side effects when it was applied intraperitoneally. We concluded that propolis might be used as a potent scolicidal agent if it would not cause caustic sclerosing cholangitis when injected into the biliary tree.[7] We also designed another study that would compare propolis with other scolicidal agents for the side effects on liver and biliary tree.

Conclusion

We designed the present study to evaluate the effects of propolis on liver and biliary tree. Propolis did not cause any side effects that have been documented with radiological evaluation, histopathological examinations, and biochemistry analysis in liver functions tests in 6 months of follow-up period. According to the results of this experimental study, we concluded that propolis might be used as a scolicidal agent even in the case of cystobiliary communication with no side effects on liver and biliary tree.

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Long-term Outcome and Risk Factors of Failure after Bile Duct Injury Repair

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Abstract
Background The real long-term outcome of a hepaticojejunostomy (HJ) to repair bile duct injury (BDI) is unclear, and the risk factors for repair failure are partially defined.
Study Design A retrospective, nonrandomized study of the long-term outcome of biliary reconstructions after major BDIs. All injuries occurred in association with cholecystectomy.
Results Twenty-nine patients were referred with complete transection of the common ($n = 16$), right ($n = 5$), or right sectoral ($n = 4$) hepatic ducts or of >1 major duct ($n = 4$) between October 2002 and January 2007. Mean follow-up was 24 months, range 12–60 months. Original repairs were "immediate" in 14, "delayed" (within 24–72h) in 5, and "elective" (after >8 weeks) in 10, and strictures developed in 9, 5, and 1 of those HJs, respectively. The surgical outcomes were significantly better when the intervention took place electively ($p = 0.003$). Original HJ repairs were done by a hepatobiliary surgeon ($n = 23$) or by a general surgeon ($n = 6$): the outcome was significantly better for the former ($p < 0.001$).
Conclusions The 51.7% incidence of strictures after BDI repair in this study was higher than reported in the literature, probably because of selection bias secondary to the referral pattern. The timing of repair and the surgeon's expertise are significant risk factors of failure.

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Keywords Bile duct injury · Cholecystectomy · Hepaticojejunostomy · Outcome

Introduction

Laparoscopic cholecystectomy is one of the most frequently performed surgical interventions and the treatment of choice for cholelithiasis. Over 750,000 cholecystectomies are performed annually, making gallstone disease one of the most common digestive health problems.[1] A serious complication of cholecystectomy is bile duct injury (BDI). The advent of laparoscopic cholecystectomy has resulted in an inevitable continuous interest in BDI and its subsequent management. Previous studies suggested that injury incidence ranged between 0.3% and 1.3% in the 1990s.[2–4] BDI after cholecystectomy is an iatrogenic catastrophe associated with significant perioperative morbidity and mortality,[5,6] reduced long-term survival and poorer quality of life.[7,8] In the United States and British Columbia, a reported 12–49% of surgeons

have caused a major BDI with an individual experience of one to two such cases.[9–13]

Complex BDIs are defined as injuries of the common bile duct or its tributaries that require a circumferential anastomosis as a presumed definitive repair.[14] Strasberg et al.[1] published an algorithm outlining the approach to the management of these patients that includes four components: injury classification, sepsis control and drainage from all parts of the biliary tree, diagnosis of the complete extent of the injury to the biliary tree, and patient preparation for surgery with insertion of biliary stents into each isolated branch of the biliary tree. The optimal waiting period before the performance of an elective repair has not been established, but Strasberg et al.[1] advise a wait of 3 months, given that bilomas, collections, and concomitant inflammation typically resolve within this period or time. Furthermore, once the injury has reached its maximum level of ischemia, the lesion is stable and ready for repair.

The aim of the current study was to determine the specific factors that influence long-term outcome after BDI and to describe our experience in treating BDI after cholecystectomy.

Methods

Setting

The study was done in a tertiary referral center with a dedicated high-volume HPB unit performing more than 150 major HPB cases annually including liver and pancreatic transplantations, resections, and reconstructions.

Study Population

We conducted a retrospective, nonrandomized, single-center study of the long-term outcome of biliary reconstructions after major BDIs. These included injuries of the common hepatic duct, common bile duct, or major segmental ducts at the porta hepatis. Cases of bile leaks from the cystic duct or gallbladder bed were excluded. All injuries were incurred in association with cholecystectomy, which was predominantly laparoscopic, and irrespective of whether the operation was entirely laparoscopic or converted to an open procedure. Biliary reconstructions that followed planned biliary interventions for cases of non-injuries, such as choledocholithiasis, malignant causes, and after liver transplantation or trauma were also excluded. BDI cases were referred to our service either at the time of the primary diagnosis or after the failure of a previous repair. To achieve long-term outcome, we included cases with minimal follow-up of 12 months.

Data Collection

Patient charts were reviewed for demographics, the specifics of the referring surgeon's management, type and level of biliary tract injury, diagnostic procedures, therapeutic interventions before and after referral, and our group's perioperative surgical management and outcomes.

Diagnosis and Treatment

Diagnosis and anatomical definition of the BDI was determined by intraoperative cholangiography (IOC) if recognition was at the time of cholecystectomy or by combined data from endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC) if the original injury was missed. Treatment included temporary percutaneous drainage of the biliary tree and of perihepatic bilomas. The repair of choice was always a wide Roux-en-Y hepaticojejunostomy (HJ) to a bile duct, depending on the type and level of the BDI.

Timing of Repair

The timing of repair depended on when the patient was referred and the presence of complications, such as cholangitis, jaundice, and abscess formation. For outcome analysis, we defined three periods: immediate, delayed, and elective. If the BDI was identified during the primary procedure, an immediate reconstruction was performed. Missed injuries recognized and corrected within 3 days of the cholecystectomy were categorized as delayed. All other corrections were elective and performed after waiting until most of the inflammatory process had subsided. In elective cases and in the presence of cholangitis, abscess, or biliary fistula, ERCP or PTC with stent placement was utilized to stabilize and improve local conditions, and surgical reconstruction was deferred for a minimum of 8 weeks.

Technique

Immediate repairs included fashioning of the injured duct and a duct to intestinal mucosa anastomosis with interrupted, usually 5/0 or 6/0 absorbable monofilament, sutures. In the immediate setting, there was no routine insertion of indwelling biliary stents. In all elective and redo cases, anastomosis (or reanastomosis) were done over the external–internal PTD catheter, which was left in place 1–2 weeks after the repair. This practice also enables a postoperative cholangiography to confirm a sealed and patent connection.

Follow-up clinical and laboratory assessments were scheduled every 3 months in the first postoperative year and every 6 months thereafter. Special attention was attributed to

episodes of cholangitis and cholestatic abnormalities in liver function tests. In such cases, initial imaging was by hepatic sonography, followed by a diagnostic and therapeutic PTC.

Management of Strictures

HJ anastomosis strictures were defined anatomically (level of the biliary tree, length of stricture) and characterized functionally (elasticity, response to balloon dilatation, and contrast dye clearance from the biliary tree) by PTC. Based on this data, the patients were treated with repeat PTCs or they were referred for corrective surgery.

Outcome

The outcome after the primary HJ was graded according to objective criteria, i.e., abnormal liver function tests, episodes of cholangitis, and a need for reintervention. Patient outcome was classified into three categories: "good"—no biliary complications and normal liver function tests, "fair"—symptomatic, cholestatic abnormalities requiring intervention, but managed successfully by interventional radiology (IR), i.e., successful balloon dilatation and long-term independence from tubes, and "poor"—failure of IR treatment and need for surgical revision of the strictured HJ.

Analysis

Basic frequencies and percentages were calculated using Microsoft Excel. The association between the risk factors and outcome was examined by the Fisher's exact test. A two-tailed p value of <0.05 was considered significant.

Results

Between October 2002 and January 2007, 29 patients (9 males, 20 females; mean age 52 years, range 20–82) were referred after BDIs and are included in the study. Of the 29 BDIs, 23 occurred during laparoscopic cholecystectomy and 6 occurred during open cholecystectomy. Thirteen of the primary operations were performed at our institution, whereas 16 were performed at other institutions in Israel or in the Palestinian authority (Table 1). Mean follow-up was 24 months, range 12–60 months.

BDI was detected during the primary surgical procedure in 14 cases (9/23 or 39% during laparoscopic operations and 5/6 or 83% during open intervention) and undetected in 15 cases (14 during laparoscopic operations and 1 during open intervention). Indications for the primary surgical intervention included cholecystitis, biliary colic, and choledocholithiasis (Table 1).

BDIs included complete transections or severe lacerations at various levels of the ductal system, detailed in Table 1. In five cases, major BDI was associated with vascular injury (portal vein laceration in one case and right hepatic artery injury in four cases; Table 1). The patients and bile duct injuries were divided into three groups based on the timing of recognition and principle management. Group 1 was comprised of the BDIs that were diagnosed

Table 1 Characteristics of the Initial Operation (Cholecystectomy)

Initial cholecystectomy		Number	Percentage
Approach	Laparoscopic	23	79
	Open	6	21
Setting	Elective	21	72
	Urgent	8	28
Indication	Biliary colic	16	55
	Acute cholecystitis	5	17
	S/P acute cholecystitis	6	20
	Choledocholithiasis	2	7
Recognition of BDI at surgery	Laparoscopic	9	39
	Open	5	83
	Total	14	48
Level of injury	Common hepatic duct	16	55
	Right hepatic duct	5	17
	Right posterior hepatic duct	3	10
	Right anterior hepatic duct	1	3
	Combined, CHD and RHD	2	6
	Combined, CHD and LHD	1	3
	Multiple, RPHD, RAHD, and LHD	1	3
Concomitant vascular injury	Portal vein laceration	1	
	Right hepatic artery transection	4	

BDI: bile duct injury, *CHD*: common hepatic duct, *RHD*: right hepatic duct, *LHD*: left hepatic duct, *RPHD*: right posterior hepatic duct, *RAHD*: right anterior hepatic duct

and treated immediately (14 cases: 9 laparoscopic and 5 open cholecystectomies). Group 2 included cases of delayed repair (five cases in which diagnosis was within 24–72h of the primary cholecystectomy) that were treated surgically after recognition. In the nine cases of group 3, diagnosis was late and treatment included interim drainage and elective repair.

There was a higher rate of early postoperative bile leaks in group 1 (4/14 vs. no leaks in the other two groups). Fifteen of the 29 study patients (51%) developed a stricture in the HJ anastomosis. The incidence of anastomotic stricture was higher in groups 1 and 2 compared to group 3 (Table 2). The mean interval to stricture presentation was longer in group 2 (14.7 months, range 6–24 months) than in group 1 (4.8 months, range 2–12 months). The clinical presentation of anastomotic stricture formation was an episode of cholangitis in half of the cases (8/15, 53%) and jaundice or abnormal liver function tests in the other half (Table 2).

All delayed repairs developed strictures (5/5) compared to 9/14 (64%) of immediate repairs and only 1/9 (11%) of elective repairs. However, strictures after delayed repair were more amenable to IR treatment compared to those in the immediate and elective repair groups (Table 2).

All strictures, once diagnosed, were initially managed by IR. Percutaneous transhepatic cholangiography, balloon dilatation, and temporary external–internal stenting achieved good long-term results in 7/15 (46%) cases. The remaining eight stricture cases required surgical revision indicated by the nature of the stricture as demonstrated by PTC or because of early restenosis after IR treatment.

Primary BDI repairs were performed by hepatobiliary surgeons (HPB) in 23 cases and by general surgeons in six cases. All repairs by the non-HPB surgeons took place immediately. Overall, patients who underwent reconstructions by HPB surgeons developed fewer anastomotic leaks (1/23, 4% vs. 3/6, 50%) and/or strictures (9/23, 39% vs. 6/6, 100%). Strictures developing after repair by HPB surgeons were significantly more amenable to IR treatment ($p = 0.007$). As a result, patients who were operated on by HPB surgeons required fewer surgical revisions of their primary HJ (2/23, 8.6% vs. 6/6, 100%; Table 3).

Patient outcomes were categorized as detailed above including: good, fair, and poor. Long-term outcome was significantly affected by the timing of repair ($p = 0.003$). Group 1 outcome was poor in 42% of cases. Group 2 outcome was fair in 60% and poor in 40% of cases. Group 3 outcome was good in 90% of cases (Table 4). When stratified by surgeon's expertise, the results were poor in all cases in the non-HPB surgeon group, whereas the outcome was good in 61%, fair in 30%, and poor in 9% of cases for the HPB surgeons ($p < 0.001$ non-HPB vs. HPB surgeons; Table 4). The number of cases in each category is not sufficient for multivariate analysis.

Discussion

The initial management of a BDI includes control of sepsis and the ongoing bile leak. Definitive reconstruction of the BDI and the operation of choice involve bilioenteric anastomosis. The primary goal of biliary reconstruction is a high-quality bilioenteric anastomosis that will not malfunction over time. Although the incidence is declining, BDIs sustained during laparoscopic cholecystectomy continue to present a problem. Combined bile duct and hepatic arterial (right or common hepatic) injuries carry a particularly poor prognosis for postoperative morbidity, mortality, and successful outcome after remedial surgery.[15] Despite technological improvements, BDI continues to pose a significant clinical challenge. Several series have reported the long-term outcomes after the repair of laparoscopic BDI, the largest of which was a retrospective analysis of 200 patients from the Johns Hopkins Medical Institution.[16] The aim of our study was to investigate the factors influencing the long-term outcomes of correction BDI and the results after appropriate management.

Our series revealed several interesting trends in the outcome of correction BDI. Nearly half of the BDIs (51%)

Table 2 Anastomotic Stricture Formation, Presentation, and Course

	Group 3 "elective" (n=10)	Group 2 "delayed" (n=5)	Group 1 "immediate" (n=14)
Stricture formation, n (%)	1/10 (10)	5/5 (100)	9/14 (64)
Time to stricture, in months; mean (range)	–	14.7 (6–24)	4.8 (3–12)
Clinical presentation			
Cholangitis		3	5
Jaundice		–	2
Abnormal liver function tests	1	2	2
Invasive radiology treatments (n=15)			
Successful, n (%)	1/1	3/5 (60)	3/9 (33)
Failed, n (%)	–	2/5 (40)	6/9 (66)
Redo hepaticojejunostomy	–	2	6

Table 3 Results: HPB Surgeons vs. General Surgeons

Surgeon	HPB surgeon (n=23)	General surgeon (n=6)
Timing of repair		
Immediate	8	6
Delayed	5	–
Elective	10	–
Complications, n (%)		
Leak	1/23 (4)	3/6 (50)
Stricture	9/23 (39)	6/6 (100)
Results of interventional radiology treatment, n (%)		
Successful	7/23 (30)*	–
Failed	2/23 (8)*	6/6 (100)*
Need for surgical revision, n (%)	2/23 (8.6)	6/6 (100)

*p<0.05; significant (Fischer's exact test)

were not recognized immediately during the laparoscopic procedure but rather at a later time. Other reports have noted this as well. In our study, the most common BDIs that occurred during cholecystectomy were transections of the common hepatic ducts.

According to our results, the outcome of BDI corrections depends upon several factors, specifically timing of the intervention and performing surgeon. Optimal results were achieved when elective correction was performed after the resolution of the local inflammatory process. The mean time until the performance of the elective operation was 4.5 months (range 2–12 months) after BDI and these patients (group 3) developed fewer postoperative complications, significantly fewer strictures, and had less need for re-HJ than the others.

Patients who underwent immediate correction BDI by a HPB surgeon clearly had better results, developed significantly fewer strictures, and required significantly fewer re-HJ than those operated by non-HPB surgeons. Several other studies have provided evidence for the advantage of an experienced HPB surgeon in the management of these complex repairs.[16,17] Nevertheless, a major Medicare database search indicated that 75% of primary surgeons attempt to repair the injury themselves,[18] and the consequences, according to Stewart and Way,[19] are that only 17% of primary repair attempts and no secondary repair attempts performed by general laparoscopic surgeon are successful. Patients treated by the injuring surgeon have an increased risk of death of 11% at 9 years.[20]

Table 4 Long-term Outcome of BDI Repairs

	Good	Fair	Poor
Immediate repair	5/14 (36%)*	3/14 (21%)*	6/14 (42%)*
Delayed repair	0/5 (0%)*	3/5 (60%)*	2/5 (40%)*
Elective repair	9/10 (90%)*	1/10 (10%)*	–
HPB surgeon	14/23 (61%)*	7/23 (30%)*	2/23 (9%)*
Non-HPB surgeon	–	–	6/6 (100%)*

*p<0.05; significant (Fischer's exact test)

Our data suggest that most of the failures of early repairs could be attributed to the lack of expertise (non-HPB surgeons). About our performance, the critical observation is related to the patients who underwent delayed correction of BDI (i.e., 24–72h after the primary operation). Although treated by HPB surgeons, all developed some degree of anastomotic stricture. Nevertheless, within this group, there were more successful IR interventions, requiring fewer re-HJs compared to group 1. The ideal management in this group of early, but not immediate, diagnosis of BDI has not been fully addressed and there is no data from prospective or comparative studies. Our findings show a clear advantage for patients undergoing late elective repair demonstrating good results in 90% of cases without the need for reoperation. This success rate corresponds to the Johns Hopkins Medical Institution report in which 94% of 109 patients with laparoscopic BDI, elective repair, and a follow-up approaching 5 years had a successful outcome.[21] It is our definitive recommendation to avoid any attempt of primary reconstruction if the diagnosis is delayed more than 24h after the BDI occurrence.

Hepaticojejunostomy for postcholecystectomy benign bile duct strictures offers the best possible long-term results.[21,22] Recurrence of the stricture is a known complication of the procedure,[23,24] and Pitt et al.[24] reported that 68% of recurrences occur within 3 years of the most recent repair and 80% within 5 years. In another series, two thirds of patients became symptomatic within 2 years of the last repair and 90% within 7 years.[22] It is interesting to note that we observed a significantly shorter time to stricture development after immediate repair versus delayed repairs. Furthermore, strictures after immediate repairs were less amenable to balloon dilatation than in delayed repairs. This may suggest the various roles that mechanisms such as ischemia, inflammation, and fibrosis contribute to the development of strictures.

There are several limitations to this study. Firstly, our patient sample is small. Therefore, we were unable to do a multivariate analysis of the data, thus precluding generalizable and firm statistical conclusions. In addition, all of the repairs undertaken by non-HPB surgeons were carried out immediately, so we cannot directly compare the results of the two groups of surgeons with patients who had undergone elective surgery after inflammatory processes had subsided.

In conclusion, the two major interrelating factors that seem to predict the success of BDI reconstruction are the optimal timing of surgery and its performance by experienced HPB surgeons.

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Evaluation of Liver Enzymes Following Elective Laparoscopic Cholecystectomy: Are They Really Elevated?

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Abstract
Background Studies made on a small number of patients have demonstrated significant modifications in liver function tests (LFT) following laparoscopic cholecystectomy.
Aim To assess retrospectively, post-operative LFT in a large group of patients undergoing elective uneventful laparoscopic cholecystectomy.
Methods Between 1999 and 2007, 1,997 patients were scheduled for laparoscopic cholecystectomy. In 1,034 patients (the study group), the surgery was elective and normal LFT were measured a day prior to surgery. Exclusion criteria included acute cholecystitis, acute pancreatitis, pre-operative endoscopic retrograde cholangio-pancreatography, medication that may affect liver metabolism, and intra-operative complications. Liver function tests were evaluated pre-operatively and 20–24 h post-operatively.
Results The mean post-operative value of liver function tests and amylase were well within normal limits, although mild increase was inspected in part of it. We observed post-operative mild hepatic enzyme increase only in 41 patients (3.9%), in nine of these, choledocholithiasis was found.
Conclusions In contrary to previously published data, we have validated, in light of our broad sampling, that the induction of CO_2 pneumoperitoneum does not cause deranged liver function tests.

Keywords Liver function test · Laparoscopic cholecystectomy · Pneumoperitoneum

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Introduction

Apart from the well-known advantages of the laparoscopic approach for various abdominal operations, there is awareness concerning possible hemodynamic side effects following induction of positive pressure pneumoperitoneum (PP). Many studies have demonstrated decreased cardiac output and stroke volume as well as increased systemic vascular resistance during laparoscopic operations.[1–5] Creation of PP can also lead to decreased hepatic and gastrointestinal (visceral) perfusion.[6–12] Recently, consequences of oxidative stress (by an ischemia/reperfusion mechanism) were detected following laparoscopic cholecystectomy.[13–15] By far, such pathophysiologic changes do not carry clinical significance in most patients undergoing elective laparoscopic operations.

Usually, changes in post-operative liver function test reflect hemodynamic instability, anesthetic hepatotoxicity, or iatrogenic bile duct injury. In an attempt to analyze the effects of increased intra-abdominal pressure and reduced

splanchnic circulation on hepatic tissue, several studies were carried out, detecting the change in liver function tests following uneventful and elective laparoscopic cholecystectomy.[16–21] Those studies, done on a small number of patients, revealed significant increased liver enzyme levels 24 h following surgery. Such data was not confirmed according to our experience, hence, we have decided to evaluate routinely post-operative liver function tests in all patients undergoing such operation in our department.

The aim of our study was to assess the significance and the possible correlation between changes in liver function tests and the hemodynamic consequences of PP during elective and uneventful laparoscopic cholecystectomy in a significantly larger group of patients.

Patients and Methods

During an 8-year period (1999 to 2006), 1,997 patients were scheduled for laparoscopic cholecystectomy in our department due to symptomatic cholecystolithiasis. Of these 1,997 patients, 475 were admitted on an emergency basis, mainly due to acute cholecystitis, and were excluded from the study. Among the remaining elective 1,522 patients, we could not obtain liver function tests 1 day prior to operation in 436 patients (a prerequisite inclusion criteria, for unity of the study population). Most of those patients had already had previous normal laboratory results that were not repeated in our department, thus excluding them from the study. In 56 patients, mild elevation (above normal limits) of at least one liver enzyme was inspected pre-operatively, thus leaving 1,034 patients to be included in the study group. Thus, inclusion criteria were elective laparoscopic cholecystectomy with normal blood tests a day prior to surgery. Exclusion criteria included emergency admission, mainly for acute cholecystitis, acute pancreatitis, recent retrograde endoscopic cholangio-pancreaticography, medication that may affect liver enzymes, obstructive jaundice, and intra-operative complications such as iatrogenic bile duct injury.

Blood was repeatedly measured 20–24 h post-operatively, including hemoglobin, WBC, glucose urea and electrolytes, gamma-glutamyl transpeptidase (γ-GGT), transaminases (ALT, AST), alkaline phosphatase (Alk Phos), bilirubin, amylase, and lactate dehydrogenase (LDH) levels. Every patient served as his own control. A chart review was conducted to analyze retrospectively changes in those parameters.

Laparoscopic cholecystectomy was performed in a supine reversed Trendelenburg position and the intra-abdominal insufflation pressure was set on 14 mmHg. Intra-operative cholangiogram was not performed in the vast majority of the study group. An experienced senior laparoscopic surgeon was always involved in the operation (five surgeons). The average length of operation was less than 1 hour. We used four trocars to accomplish the operative procedure. An intermittent sequential pneumatic compression device was not routinely used during elective laparoscopic cholecystectomy.

Statistical analysis Quantitative parameters such as laboratory data were defined by mean and standard deviation, median, and range. Qualitative parameters like gender and medical history were detailed by frequency and percentage. We used the *paired T-test* to analyze the change between pre- and post-operative liver function tests. We used the *binominal* and *one sample proportion tests (one sided)* to evaluate the significance of the number of patients (percentage) who exhibited increased post-operative liver enzyme levels above upper normal limit. We considered an increase of 10% or above to be significant. A P value less than 0.05 was considered statistically significant.

Results

Among the study group (1,034 patients), 718 (69.5%) were women. The average age was 54.1±15.6 (19 to 96) years. The ratio of the Arab to Jew population was 47 to 53%. Mild controlled hypertension was noted in 23.4% of the study group, type II diabetes in 10.5%, stable cardiac disease in 15.4%, and state post mild recent pancreatitis in 0.4% (including overlapping medical problems). The mean post-operative blood levels of liver enzymes were well within normal limits, although mild increase was recorded in some. Specifically, LDH levels changed from 282.5 pre-operatively to 289.9 (upper normal limit—460 U/l), AST from 33.1 to 35.2 (limit—55 U/l), ALT, 39.7 to 41.8 (60), total bilirubin 0.65 to 0.56 (1.6), γ-GGT 49.3 to 49.5 (70), Alk Phos 96 to 98.6 (145 U/l), and diastase 52.3 to 53.3 (upper normal limit—200 U/l). Mild post-operative enzymatic increase (above normal limits) was found in 41 (3.9%) patients (one to three enzymes in each). This data reflects insignificant percentage ($P<0.001$, significantly different from the 10% increase limit, according to the *binomial test*, or, alternatively, $P>0.998$, according to *one sample proportion test*). Independently, increased LDH was noted in five patients (0.5%), ALT in 13 (1.3%), AST in 14 (1.4%), bilirubin in five (0.5%), γ-GGT in 11 (1%), Alk Phos in nine (0.9%), and diastase increase in five patients (0.5%). Choledocholithiasis was found in nine (22%) of those patients.

The patients that had increased liver function test did not differ significantly from the remainder of the study group in demographics, age, and associated medical parameters.

The post-operative convalescence was uneventful in all the patients involved in our study.

Discussion

In our study, patients whose liver function tests (LFT) were within normal limits 1 day prior to elective laparoscopic cholecystectomy (a prerequisite selection criterion) demonstrated only mild 24-h post-operative liver enzyme elevation. However, such increase was not associated with any clinical significance as it was far below the upper normal limit associated with each biochemical parameter. Our results are inconsistent with the data published in the medical literature to date, in which significant enzymatic increase has been noted in most patients undergoing laparoscopic cholecystectomy. However, the number of patients involved in those studies was far smaller than in our present study.[16–21] The difference between our results and previous studies is not clear to us. We hypothesize that the mild elevation that was observed in the minority of our patients might be due to reduced visceral perfusion during PP. We, therefore, recommend avoiding prolonged laparoscopic procedures for patients with compromised liver function.

Although the laparoscopic approach is the gold standard for gallbladder removal, the complication rate associated with iatrogenic bile duct injury and its severity, though very low, is still higher in comparison with the open approach.[22–24] This low rate still favors the laparoscopic approach. Eventually, it necessitates excessive awareness during surgical dissection, especially when concerning intense inflammatory processes in the gallbladder proximity.[25–26]

The occurrence of calculi in the bile ducts may approach 10% in patients being operated for symptomatic cholecystolithiasis, and clinical awareness usually becomes apparent when elevated blood levels of liver enzymes together with biliary dilatation are observed prior to surgery. Patients harboring such findings were not included in our study, and for them, post-operative LFT analysis is mandatory. Based on an extensive previous experience, we believe that post-operative bile duct stricture or injury should be manifested clinically together with LFT abnormality. Undetected pre-operative passage of small bile duct stones without laboratory changes usually do not carry clinical significance.

Additional factors that might affect LFT during laparoscopic cholecystectomy are related to the induction of CO_2 positive PP. Increased intra-abdominal pressure usually leads to deranged hemodynamic consequences that manifest with decreased cardiac output and increased systemic vascular resistance, as well as decreased hepatic, renal, and intestinal (visceral) perfusion.[1–12] All the above, together with possible metabolic changes following oxidative stress (due to ischemia/reperfusion mechanism), can initiate the post-operative changes in LFT observed in patients undergoing laparoscopic cholecystectomy.[13–15]

According to our broad sampling, such changes in LFT were not noted post-operatively, and only 3.9% of the study group exhibited mild elevation above upper normal limits. Nine patients included in this sub-group had evidence of bile duct stones, thus making the pure possible influence of PP on deranged post-operative LFT even smaller.

Intra-operative cholangiogram was not performed in the vast majority of our study group as our policy is not to perform this procedure routinely. Intra-operative cholangiogram may also be one of the factors responsible for elevated LFT due to temporarily elevated intra-ductal pressure. Our observation of almost an entire absence of deranged post-operative LFT seems to be more valid considering all the factors that may potentially cause elevated post-operative LFT.

In conclusion, our results confidently enable us to state (in contradiction to previous studies) that elevated intra-abdominal pressure to levels up to 14 mmHg during laparoscopic cholecystectomy (or comparable operations) does not significantly affect hepatic metabolism (as manifested in elevated liver enzymes). Alternatively, LFT are not a sensitive parameter to reflect minor hepatic insult, as may happen following PP, due to ischemia/reperfusion. It can also be presumed that routine examination of LFT has no predictive value concerning the deranged effects of PP or the presence of choledocholithiasis in patients in whom normal LFT are measured in proximity to surgery, on condition that the surgeon does not suspect iatrogenic bile duct injury in cases of uneventful operation.

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Prognosis of Resected Ampullary Adenocarcinoma by Preoperative Serum CA19-9 Levels and Platelet-Lymphocyte Ratio

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Abstract
Background The objective of this study was to evaluate whether preoperative CA19-9 levels and the platelet–lymphocyte ratio (PLR) might reflect prognostic indices for resected ampullary adenocarcinoma.
Materials and Methods Data were collected prospectively over a 10-year period for consecutive patients undergoing pancreatoduodenectomy for malignancy.
Results Both preoperative PLR and CA19-9 results were available in 52 cases of resected ampullary adenocarcinoma. Preoperative CA19-9 levels of ≤150 kU/l (or ≤300 kU/l in the presence of bilirubin levels >35 μmol/l) and a PLR of ≤160 were found to represent the optimal cut-off values to risk stratify patients. If both levels were elevated ($n = 8$), patients had a median overall survival of 10.1 months. If either CA19-9 or PLR were elevated individually ($n = 23$), patients had a median survival of 25.2 months. For cases where both levels were less than the cut-off values ($n = 21$), the median overall survival time was not reached but was greater than 60 months (log rank, $p < 0.001$). This preoperative risk stratification was found to remain a significant independent predictor of survival on multivariate analysis (Cox, $p = 0.001$) alongside resection margin status ($p = 0.002$) and tumor size ($p = 0.051$).
Conclusions Preoperative CA19-9 and PLR both merit further evaluation as prognostic indices in resected ampullary adenocarcinoma.

Keywords Ampullary cancer · Resection · CA19-9 · Prognostic

Introduction

Serum levels of the sialyated Lewis blood group antigen CA19-9 are commonly measured during the initial investigation of patients with suspected pancreatic malignancy[1] and have been demonstrated to have comparable sensitivity for ampullary adenocarcinoma when compared with pancreatic adenocarcinoma.[2] CA19-9 is synthesized by the gallbladder and biliary mucosa and excreted in bile and is therefore commonly elevated in benign conditions associated with cholestasis.[3–5] Approximately 5% of the population have undetectable serum CA19-9 levels due to an inability to synthesize the Lewis antigen glycosyl transferase enzyme.[6]

Preoperative CA19-9 levels have been used as a predictor of resectability for pancreatic malignancy to guide decision making regarding optimum use of staging laparoscopy.[7] Levels of >150 kU/l (or >300 kU/l in the presence of cholestasis) have been shown to identify a patient group with periampullary malignancy at greater risk of unresectability for whom the diagnostic yield from staging laparoscopy is maximized.[8,9] It is well recognized that normalization of serum CA19-9 after resection for

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pancreatic cancer is associated with a more favorable survival outcome[10–12], but only a small number of studies have investigated whether elevated preoperative CA19-9 levels in isolation may also confer significant prognostic information in periampullary cancer.[2,13,14]

Previous studies have suggested that elevated preoperative C-reactive protein (CRP) levels[15], thrombocytosis[16,17] and lymphocytopenia[18,19] may also represent potential prognostic markers in resected pancreatic cancer. We have recently reported that a combination of preoperative hematological indices, the platelet–lymphocyte ratio, represents a significant prognostic index in resected pancreatic cancer.[20] However, no previous studies have investigated whether this marker of systemic inflammation might provide any prognostic information in resected ampullary cancer. Similarly, the prognostic value of preoperative CA19-9 levels adjusted for cholestasis in the same patient group has not previously been investigated. The objective of this study was to determine whether preoperative CA19-9 levels and the platelet–lymphocyte ratio might represent significant prognostic indices in patients undergoing resection for ampullary adenocarcinoma and to determine whether a combination of these parameters might provide superior prognostic information.

Materials and Methods

Prospective data were collected for all patients undergoing pylorus-preserving pancreatoduodenectomy or classical Kausch–Whipple resection between January 1997 and September 2007 at the Royal Liverpool University Hospital. Patient demographics, operative details, and the histological characteristics of the resected specimen were recorded. Processing and reporting of the pathology specimens were undertaken according to the Royal College of Pathologists' Guidelines[21] using the fifth edition of the UICC TNM Classification System.[22] A positive resection margin (R1) was defined as tumor involvement within 1 mm of one or more resection margins on microscopic examination. No R2 resections were identified. Details of preoperative intervention for biliary drainage and adjuvant therapy were also recorded, and survival data were obtained from hospital computer records.

The preoperative full blood count, CA19-9, and concurrent bilirubin levels were recorded where available. The normal diagnostic reference interval for serum CA19-9 used in our laboratory is <35 kU/l. Univariate survival analysis was conducted using a CA19-9 cut-off value of 150 kU/l in non-jaundiced patients and 300 kU/l in patients with CA190-9 levels recorded in the presence of cholestasis (bilirubin >35 μmol/l). These values were selected on the basis of the previously published literature investigating the role of CA19-9 in the assessment of periampullary tumor resectability.[7,8] Using a number of different cut-off points, a platelet–lymphocyte ratio of 160 was found to represent the optimum stratification point at which the survival difference between two groups was maximized.

Statistical Analysis

Continuous data were described using median, interquartile range (IQR) and 95% confidence intervals (CI). χ^2 or Fisher's exact tests were used for comparative analysis of categorical data. Relationships between two continuous variables were analyzed using Spearman's rank correlation. Survival data were analyzed using log rank testing for univariate analysis and Cox proportional hazards with forward-stepwise regression for multivariate analysis ('NR' signifies that the upper limit of the confidence interval for median survival was not reached). Corrected log rank p values were quoted for univariate survival where continuous prognostic data were dichotomized.[23] All patients who died within 30 days of surgery were excluded from survival analysis and a p value of ≤ 0.05 was taken to reflect significance. Statistical analysis was performed using Statview version 5 (ISAS Institute, Cary, NC, USA) and Microsoft Excel (Microsoft Office 2007).

Results

Three hundred fifty-one consecutive patients underwent pancreatoduodenectomy for pancreatic or periampullary tumors during the study period. Seventy-seven cases had histologically confirmed adenocarcinoma arising from the ampulla of Vater. Two patients (2.6%) who died within 30 days of surgery were excluded from subsequent survival analyses. There were 37 censored cases with a median follow-up time of 22.5 months. Table 1 demonstrates the demographics of the study group along with the preoperative CA19-9 and full blood count results.

Median overall survival of the study group was 34.9 (95% CI = 25.2 to 63.8) months. The median survival times according to preoperative CA19-9 levels (using a cut-off value of ≤150 or ≤300 kU/l in jaundiced cases) and platelet–lymphocyte ratio ≤160 together with the median survival recorded for the various histological subgroups are shown in Table 2. The corresponding survival curves when stratifying by preoperative CA19-9 and platelet–lymphocyte ratio are shown in Figs. 1 and 2, respectively. No significant correlation between CA19-9 and platelet–lymphocyte ratio was identified (Spearman, rho = 0.022, $p = 0.875$).

A further stratification index was generated for those patients where both preoperative CA19-9 and platelet–lymphocyte ratio were recorded ($n = 52$). If both levels

Table 1 Demographics and Preoperative CA19-9/Full Blood Count Results for Resected Ampullary Adenocarcinoma Patients

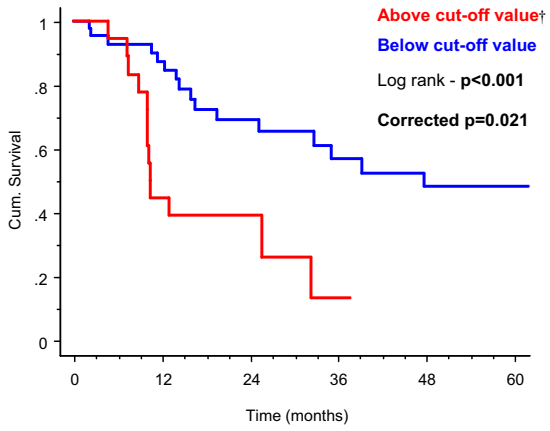
Demographics	Values
No. of patients identified	75
Male/Female	39:36
Median age (IQR)	67 (56–74) years
Overall median survival (95% CI)[a]	34.9 (25.2 to 63.8) months
Preoperative intervention for biliary stenting	
No	15
Yes	60
Adjuvant therapy received[b]	
No	63
Yes	12
Preoperative CA19-9 result available	61
Median preoperative CA19-9 (IQR)	69 (20 to 372) kU/l
Median interval from CA19-9 to operation (IQR)	21 (13 to 34) days
Number of jaundiced cases at time of CA19-9 estimation (bilirubin >35 μmol/l)	
Jaundice	30
No jaundice	30
Not known	1
Preoperative FBC available	65
Median platelet–lymphocyte ratio (IQR)	140.0 (110.6 to 190.5)
Timing of preoperative FBC	
Within 24 h of surgery	52
Within 48 h of surgery	5
>48 h of surgery	8
Neutrophilia present (>7.5×10^6/ml)	8
Lymphocytopaenia present (<1.0×10^6/ml)	2
Thrombocytosis present (>400×10^6/ml)	12

IQR Interquartile range
[a] Median survival and confidence interval calculated from overall Kaplan–Meier analysis
[b] No patients received neoadjuvant therapy.

Table 2 Median Overall Survival Times (log rank) According to Preoperative CA19-9, Platelet–Lymphocyte Ratio and Histological Sub-groups in the Overall Study Group

	Number of patients	Median survival (months)	*p* value
Preoperative CA19-9[a]			
Below cut-off	42	47.8 (25.2 to NR)	<0.001
Above cut-off	19	10.4 (10.1 to 32.4)	(0.021[b])
Preoperative PLR			
≤160	39	78.7 (39.2 to NR)	<0.001
>160	26	16.6 (10.5 to 32.4)	(0.021[b])
Nodal involvement			
Negative	38	47.8 (35.2 to NR)	0.001
Positive	37	14.4 (10.4 to 32.4)	
Size			
≤20 mm	39	44.3 (25.6 to NR)	0.036
>20 mm	34	16.6 (12.4 to 37.6)	
Tumor differentiation			
Well/moderate	53	39.2 (31.4 to 78.7)	0.157
Poor	21	19.6 (10.7 to 25.2)	
Resection margin status			
Negative	49	78.7 (32.7 to NR)	<0.001
Positive	26	11.5 (10.1 to 16.6)	

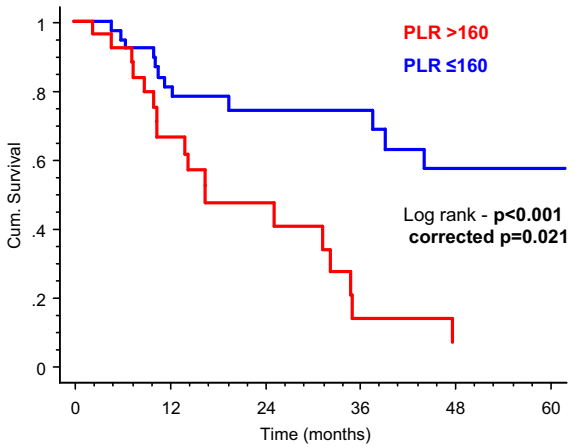
Due to the small number of well differentiated tumors ($n=8$), well and moderately differentiated tumors were grouped together for analysis. 95% confidence intervals in parentheses (*NR* upper value not reached).
[a] A cut-off value of >150 kU/l was used to define the high-risk group for CA19-9 when recorded in the absence of concurrent cholestasis (i.e., bilirubin ≤35 μmol/l), and a cut-off value of >300 kU/l was used in the presence of cholestasis (i.e., bilirubin >35 μmol/l)
[b] Corrected *p* values quoted for log rank analysis



No. at risk						
CA19-9 ≤150kU/l	42	31	19	13	10	9
CA19-9 >150kU/l	19	8	3	1	0	0

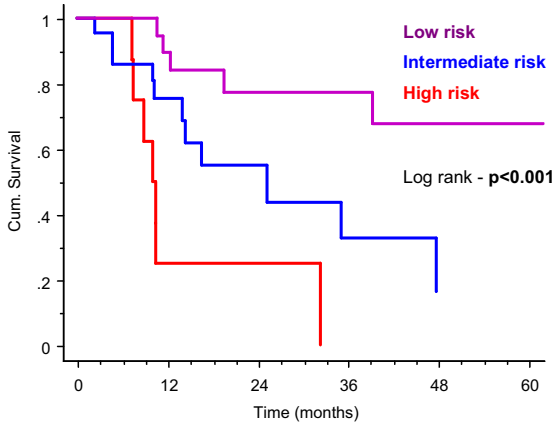
Figure 1 Kaplan–Meier cumulative survival curves for ampullary adenocarcinoma patients stratified by preoperative CA19-9 ≤150 kU/l (or ≤300 kU/l in jaundiced cases). † A cut-off value of >150 kU/l was used to define the high-risk group for CA19-9 when recorded in the absence of concurrent cholestasis (i.e., bilirubin ≤35 μmol/l) and a cut-off value of >300 kU/l was used in the presence of cholestasis (i.e., bilirubin >35 μmol/l).

were above the selected threshold values (n = 8), patients were classified as 'high risk' with an associated median overall survival of 10.1 (95% CI = 7.5 to 32.4) months. If either CA19-9 or PLR were elevated individually (n = 23), patients were classified as 'intermediate risk' with a median survival of 25.2 (95% CI = 14.1 to 47.8) months. For the remaining cases where both levels were less than the cut-off values (n = 21), patients were classified as 'low risk'. The median overall survival time for this group was not reached but was found to be in excess of 60 months on Kaplan–Meier analysis (Fig. 3). When comparing the survival between these groups a log rank p value of less than 0.001 was obtained.



No. at risk						
PLR ≤160	39	29	17	14	10	9
PLR >160	26	14	7	2	2	0

Figure 2 Kaplan–Meier cumulative survival curves for ampullary adenocarcinoma patients stratified by preoperative platelet–lymphocyte ratio (*PLR*) ≤160.



No. at risk						
Low risk	21	17	11	8	7	6
Intermediate risk	23	13	5	3	0	0
High risk	8	2	1	0	0	0

Figure 3 Kaplan–Meier cumulative survival curves for ampullary adenocarcinoma patients according to combined CA19-9 and PLR risk stratification.

Table 3 demonstrates that the only significant difference between the three risk groups in terms of tumor histology was resection margin status with a trend toward an increased likelihood of margin involvement in the intermediate and high-risk groups compared with the low-risk group (Fisher's exact, p = 0.046). There was no significant difference in the proportion of patients undergoing preoperative biliary drainage when comparing the three risk groups (p = 0.674). There was no difference in survival when comparing cases who did (n = 60) or did not require preoperative biliary drainage (n = 15) in the overall patient group [median survival = 34.9 (95% CI = 19.6 to 63.8) months and 32.7 (95% CI = 12.4 to NR) months, respectively—log rank, p = 0.988]. Similarly, there was no significant difference in the proportion of patients who went on to receive adjuvant therapy (p = 0.885).

The results of a multivariate survival analysis using Cox proportional hazards with forward stepwise regression are shown in Table 4. Resection margin status, tumor size, and nodal status were included alongside the preoperative CA19-9/platelet–lymphocyte ratio risk stratification. Nodal status failed to emerge as a significant variable on forward stepwise regression (p = 0.336) and was therefore omitted from the final multivariate model. Although an association between the preoperative risk stratification and likelihood of resection margin involvement was demonstrated (Table 3), the Cox analysis suggests that both were independent prognostic variables in this patient group. Tumor differentiation was not included as an additional covariate in the multivariate model due to the lack of statistical significance on univariate analysis (Table 2, p = 0.157) and the limited number of deaths that were seen (24 out of 52) in the patient group analyzed.[24]

Table 3 Tumor Characteristics According to CA19-9/Platelet–Lymphocyte Ratio Risk Stratification

	Number of cases stratified by CA19–9/PLR risk groups (n=52)			
	High	Intermediate	Low	p value
Size				
≤20 mm	4	8	13	0.193
>20 mm	4	15	8	
Nodal involvement				
Negative	1	10	12	0.104
Positive	7	13	9	
Tumor differentiation				
Well/moderate	5	17	15	0.833
Poor	3	6	5	
Resection margin status				
Negative	2	13	16	0.046
Positive	6	10	5	
Preoperative biliary stenting				
No	1	5	2	0.674
Yes	7	18	19	
Adjuvant therapy received				
No	7	18	18	0.885
Yes	1	5	3	

p values for Fisher's exact (2× 3 contingency tables). Due to the small number of well-differentiated tumors, well and moderately differentiated tumors were grouped together for analysis.

Discussion

CA19–9 is widely used as a diagnostic tool in the preliminary investigation of suspected periampullary malignancy.[25] As a prognostic tool, failure of normalization of CA19-9 levels post-resection is strongly associated with disease recurrence and poorer survival rates.[10–12] Evidence also exists to suggest that pre-resection CA19-9 levels in isolation might act as a significant predictor of survival for periampullary cancer patients,[2,13,14], but the prognostic value of preoperative CA19-9 levels in resected ampullary adenocarcinomas has not previously been independently investigated. Other studies have also suggested that various preoperative inflammatory and hematological parameters such as CRP[15] along with the circulating platelet[16,17] and lymphocyte counts[18,19] confer significant prognostic information in resected pancreatic cancer. However, no studies to date have investigated whether any markers of systemic inflammation might also represent significant independent prognostic factors in resected ampullary cancer.

Chronic inflammation is both an etiological factor and physiological consequence of pancreatic carcinogenesis.[26] The proinflammatory phase associated with systemic inflammation results in release of various immunological mediators such as interleukin (IL)-1, IL-3, and IL-6, which increase circulating platelet counts as a result of megakaryocyte proliferation.[27,28] Preoperative thrombocytosis is commonly associated with a number of different malignancies and has been widely reported as an adverse prognostic marker in several cancers[29,30] including pancreatic adenocarcinoma.[16,17] Release of inhibitory mediators such as IL-10 and transforming growth factor beta 2 as part of the inflammatory response can result in reduced circulating and tumor-infiltrating lymphocyte counts.[31] Pancreatic cancer has been shown to be associated with lower preoperative lymphocyte counts when compared with other gastrointes-

Table 4 Multivariate (Cox Proportional Hazards) Survival Analysis (n=52)

	Coefficient	SE	HR	Chi-square	p value
CA19-9/PLR risk groups:					
Low risk	–	–	–	–	–
Intermediate risk	1.139	0.580	1.963	3.855	0.050
High risk	2.547	0.708	12.765	12.946	<0.001
Resection margin status (R1 vs R0)	1.463	0.468	4.320	9.768	0.002
Tumor size[a]	0.027	0.014	1.028	3.826	0.051

SE Standard error of regression coefficient, *HR* hazard ratio

[a] Modeled as continuous covariate (units=mm) for multivariate analysis—hazard ratios for continuous data reflect increase in relative risk of death with each incremental increase in covariate value of 1 unit. Nodal status failed to emerge as a significant covariate on forward stepwise regression (p=0.336) and was excluded from the final model.

tinal cancers.[32] No previous studies have been published, which have sought to investigate the immunological interaction between tumor and host in the specific context of ampullary cancer.

The results from the present study indicate that an elevated preoperative CA19-9 level and platelet–lymphocyte ratio are both associated with poorer survival in patients undergoing resection for ampullary adenocarcinoma. The lack of any correlation between these two indices suggests that both markers are influenced by different aspects of ampullary tumor biology. The Kaplan–Meier analyses for each parameter (Figs. 1 and 2) demonstrate similar survival curves. However, use of a combined index incorporating both CA19-9 and platelet–lymphocyte ratio enables a low-risk patient group to be identified with a highly favorable survival outcome (>60 months), while a high-risk group can also be predicted for whom a median survival of less than 12 months is observed. This prognostic stratification was found to remain statistically significant when analyzed alongside the important histopathological tumor characteristics. Larger tumors, poor differentiation, nodal metastases, and R1 resections all exhibited the expected trend toward poorer survival on univariate analysis in keeping with previous studies.[33–35]

This finding has potential clinical relevance when considering which patients with resected ampullary cancer might benefit from adjuvant therapy. Although no definitive evidence exists to advocate adjuvant therapy as an optimum treatment strategy in resected ampullary cancer,[36,37] the available evidence suggests that patients with less favorable histological tumor characteristics (e.g., T3/T4 tumors) tend to see more benefit from adjuvant treatment.[38–41] The preoperative risk stratification described in the present study represents a potential prognostic tool that may be of use alongside tumor histology in selecting a patient group at high risk of early recurrence and poorer overall survival for whom adjuvant therapy may be of most benefit.

The majority of patients analyzed (80%) underwent intervention for biliary drainage before resection. Instrumentation of the biliary tree represents a potential source of preoperative sepsis[42] and, as such, may act as a confounding factor in interpreting the survival data. Although previous studies have suggested that biliary drainage in the preoperative setting may increase the risk of early postoperative morbidity such as wound infection,[43] no evidence exists to suggest that preoperative biliary stenting has any influence on operative mortality or subsequent survival after pancreatoduodenectomy. The results from the current study confirmed that the requirement for preoperative biliary drainage had no effect on postoperative survival after resection for ampullary cancer. Furthermore, when stratifying patients into the three risk groups, there was no significant difference in the proportion of cases in each group who underwent preoperative biliary stenting. This suggests that intervention for biliary drainage is unlikely to be a significant confounding factor in explaining the marked survival differences observed when comparing the three risk-stratified groups. Similarly, there was no significant difference in the proportion of patients in each risk group who went on to receive adjuvant treatment. Hence, adjuvant therapy is also unlikely to reflect a significant confounding factor when interpreting the survival data.

Conclusion

The results of the present study suggest that significant prognostic information can be obtained from routine blood results before resection for ampullary cancer. Stratification of ampullary adenocarcinoma patients according to both preoperative CA19-9 levels and platelet–lymphocyte ratio should be considered in the survival analyses of future adjuvant and neoadjuvant trials to validate these findings.

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The Effects of Endothelin Receptor Blockade by Bosentan on the Healing of a Bowel Anastomosis in an Experimental Crohn's Disease Model

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Abstract
Objective It was previously described that endothelins may contribute to the pathogenesis of Crohn's disease. In this study, it was aimed to investigate the effects of endothelin receptor blockade by bosentan on the healing of a bowel anastomosis in an experimental Crohn's disease model.
Material and Methods Twenty-eight Sprague–Dawley rats were divided into four groups. Groups I and II were used as sham-operated and control groups, respectively. Bowel inflammation induced by intrajejunal injection of iodoacetamide in groups III and IV. Rats in group IV were treated with oral preparation of bosentan 60 mg/kg/day. Three days after induction of the inflammation, partial resection of test loop and anastomosis was performed. Re-laparotomy was performed, anastomosis bursting pressures and peritonitis scores were measured, and tissue samples were obtained for the measurements of tissue hydroxylproline level and mucosal damage index 4 days later.
Results The mean mucosal damage index and peritonitis score of group IV were significantly lower, and the mean tissue hydroxyproline level and anastomotic bursting pressure of group IV were significantly higher than those of group III.
Conclusion The blockade of endothelin receptors by bosentan decreases the severity of iodoacetamide induced intestinal inflammation, increases the wound healing in the inflamed intestinal tissue, and decreases the severity of peritonitis.

Keywords Crohn's disease · Endothelin · Bosentan · Wound healing · Intestinal anastomosis

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Introduction

In tissue samples from patients with Crohn's disease, there are vascular and perivascular abnormalities that might lead to ischemic damage.[1] It has also been shown that local endothelin production in inflammatory bowel disease may contribute to intestinal ischemia through vasoconstriction.[2] The most common indication for surgery in Crohn's disease is obstruction and the preferred intervention is resection and anastomosis.[3] It can be hypothetically foreseen that an anastomosis in a patient with Crohn's disease has the risk for dehiscence because of local ischemia owing to vascular abnormalities and high endothelin level.

Three endothelin receptor subtypes have been identified in mammals. Bosentan (Ro 47-0203 or 4-*tert*-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulfonamide) was described by Clozel et al. as the most potent orally active antagonist of endothelin receptors.[4]

In this study, we aimed to investigate the effects of endothelin receptor blockade by bosentan on the healing of a bowel anastomosis in an experimental Crohn's disease model.

Material and Methods

Upon approval by the Ethics Board, this study was conducted in laboratories of Firat University Faculty of Medicine Experimental Research Center, using 28 male Sprague–Dawley rats weighing 220–250 g. Rats were fed standard pellet and tap water and were kept in room temperature and humidity-controlled environment. Only water was provided in the 12 hours preceding the experiments. Rats were randomly allocated to one of the four groups containing seven rats each. Figure 1 shows the experimental design as a timeline figure.

All rats were anesthetized with ketamine hydrochloride (Ketalar, 50 mg/mL, Eczacibasi, Istanbul) and xylazine HCl (Rompun, 23.32 mg/mL, Bayer, Istanbul) before all surgical procedures.

At 48 and 24 h before the first surgical procedure, rats in group IV were treated with the oral preparation of bosentan (60 mg/kg/day), which was obtained courtesy of Dr. Martine Clozel (Actelion Ltd., Allschwil, Switzerland), suspended in 5% gum arabic (as a vehicle). Bosentan was administered by gavage twice before induction of inflammation to ensure that adequate plasma levels of bosentan were present.[4] Thereafter, all rats in group IV were treated once daily for 7 days. Five percent gum arabic was administered to rats in groups II and III in the same manner.

Initial Procedure Laparotomy was performed by midline incision in groups II–IV. The jejunum was identified and a 10-cm loop was closed by gently tying of proximal and distal ends with 00 silk sutures. To induce the inflammation in groups III and IV as described by Rachmilewitz et al., 0.1 mL iodoacetamide (2%) suspended in methyl cellulose (1%) was injected into closed loop.[5] Rats in group II were injected with 0.1 mL methyl cellulose (1%). Thirty minutes later, the loop was opened and returned to the abdominal cavity, and then the abdominal wall was closed.

Secondary Procedure Three days later, laparotomy (or relaparotomy) was performed on rats in all groups including group I. All of the macroscopic abnormalities were noted. The jejunal loop that was closed in the initial procedure was re-identified in groups II–IV and its proximal half was resected. A 5-cm segment of jejunum was resected in group I. The resected segment was placed in formalin for histological examination. The ends were then anastomosed as a one-layer full-thickness anastomosis by using interrupted 6/0 polypropylene.

Rats were allowed water and standard laboratory feeding 24 hours after the operations.

Terminal Procedure Four days later, re-laparotomy was performed. There was peritonitis in some of the rats. The severity of peritonitis was evaluated according to the macroscopic findings as well as the Mannheim peritonitis index (Table 1).[6]

Anastomosis bursting pressures were measured in vivo. Before opening the adhesions, a perfusor was connected 5 cm above the anastomosis line and a manometer was connected 5 cm below it. The inside of the abdomen was filled with water and air was supplied at a fixed rate from the perfusor. The manometer reading of the initial air bubble was recorded as the bursting pressure (mmHg).

A 1-cm segment of the jejunum including the line of anastomosis (0.5 cm pre-anastomotic and 0.5 cm post-anastomotic) was resected, transected longitudinally and rinsed with saline to remove intestinal contents. Then, it was wrapped in aluminum foil and was kept frozen at −80° C for tissue hydroxyproline measuring. The remaining 4.5 cm of test loop was placed in formalin for histological examination.

Histopathological Examination Four- to 4-μm-thick sections were stained with hematoxylin-eosin and examined under light microscope by a pathologist unaware of the experimental protocol. He made a careful examination to see whether or not any epitheloid granuloma with giant Langhans cells. The histological changes were also scored with respect to mucosal damage index (MDI), as described

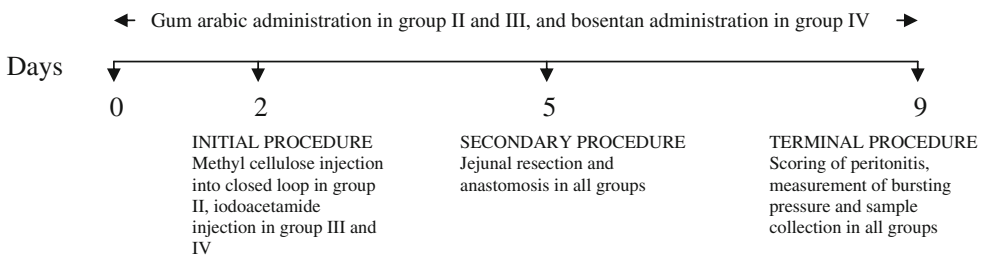

Figure 1 Study design.

Table 1 The Peritonitis Index

Risk Factor	Point
Presence	
None	0
Focal	3
Generalized	6
Exudate	
Clear	0
Cloudy	6
Purulent/Fecal	12
Total Score	

by Mei et al.[7] MDI was as follows: 0: normal mucosa, no damage on mucosal surface; 1: mild hyperemia and edema, no erosion or ulcer on mucosal surface; 2: moderate hyperemia and edema with erosion on mucosal surface; 3: severe hyperemia and edema with necrosis and ulcer on mucosal surface, the major ulcerative area extended less than 1 cm; 4: severe hyperemia and edema with necrosis and ulcer on mucosal surface, the major ulcerative area extended more than 1 cm.

Measurements of Tissue Hydroxyproline Level Hydroxyproline level was determined by modifying the method described by Woessner.[8] After being completely defrosted, the tissue samples were washed with saline and then laid on a dry sheet of paper. They were placed inside glass tubes and further dried in a stove at 100°C temperature. Dried tissue samples were crushed in a mortar until pulverized and the dry weight of the samples were measured. Pulverized tissue samples were placed in glass tubes and added 2 mL of 12 N HCl before closing the lids. Later, they were hydrolyzed by boiling them in a stove at 130°C for 3 hours. The samples that decreased less than 1 mL because of evaporation were added 12 N HCl to maintain levels of at least 1 mL. They were then centrifuged at 3,000 rpm for 15 min. We removed 0.5 ml of the upper layer supernatant, added 0.5 ml isopropanol and centrifuged the samples at 2,500 Gs for 10 min. Then, 0.3 mL of the upper layer was removed and the hydroxyproline level was measured. During the procedure, OH-P standard, chloramin-T, P-dimethyl amino benzaldehyde, perchloric acid, isopropanol, Na-acetate 3 H2O, Na citrate 5,5 H2O, 12 N HCL, 1 mM HCL chemicals were used. Reagents were added onto the sample and blank in order, vortexed, and incubated in water bath at 60°C for 25 min. Optical density was measured at 558 nm and the concentration of hydroxyproline was calculated by comparing it with the blank. Results were expressed as milligram per gram dry tissue.

Statistical Analyses Data obtained from the study groups were expressed as mean ± standard deviation. Differences in parameters among groups were examined using one-way analysis of variance (ANOVA) and post hoc analyses with Scheefe and Student–Newman–Keuls tests. All confidence intervals in this report are calculated at the 95% confidence level.

Results

Data are summarized in Table 2.

Secondary Procedure

One animal in group III died 2 days after the secondary procedure. In autopsy, the dehiscence of anastomosis was observed. There were hemorrhagic, ulcerated, and necrotic areas in inflamed bowel segments of rats in group III (Fig. 2a). On the other hand, there were no macroscopic changes in group IV (Fig. 2b). There was no granuloma-like structure in any of the groups. In the histopathological examination, there were findings of acute inflammation characterized by marked neutrophil infiltration. There were severe congestion, hemorrhage, ulceration, and diffuse necrosis in group III (Fig. 3a). In group IV, there were

Table 2 Means of the Histopathological Scores, Tissue OH-Proline Levels, Bursting Pressures, and Peritonitis Scores of the Experiment Groups (Mean ± Standard Deviation)

	Group I (n=7)	Group II (n=7)	Group III (n=6)	Group IV (n=7)
Histopathological score-1	0.0±0.0	0.14±0.38	7.29±1.11	2.00±1.82[a]
Histopathological score-2	1.29±0.49	1.28±0.29	7.50±1.38	3.29±2.75[b]
Tissue OH-proline level	7.15±0.70	7.01±0.57	3.46±0.65	6.65±0.64[b]
Bursting pressure	263.6±12.2	262.1±11.1	50.8±7.4	190.9±88.6[c]
Peritonitis score	0.43±1.13	0.86±1.46	15.50±3.99	4.71±6.68[b]

[a] Group IV vs groups I and II, $p<0.05$; group IV vs group III, $p<0.001$
[b] Group IV vs groups I and II, $p>0.05$; group IV vs group III, $p\leq0.001$
[c] Group IV vs groups I and II, $p<0.001$; group IV vs group III, $p<0.001$

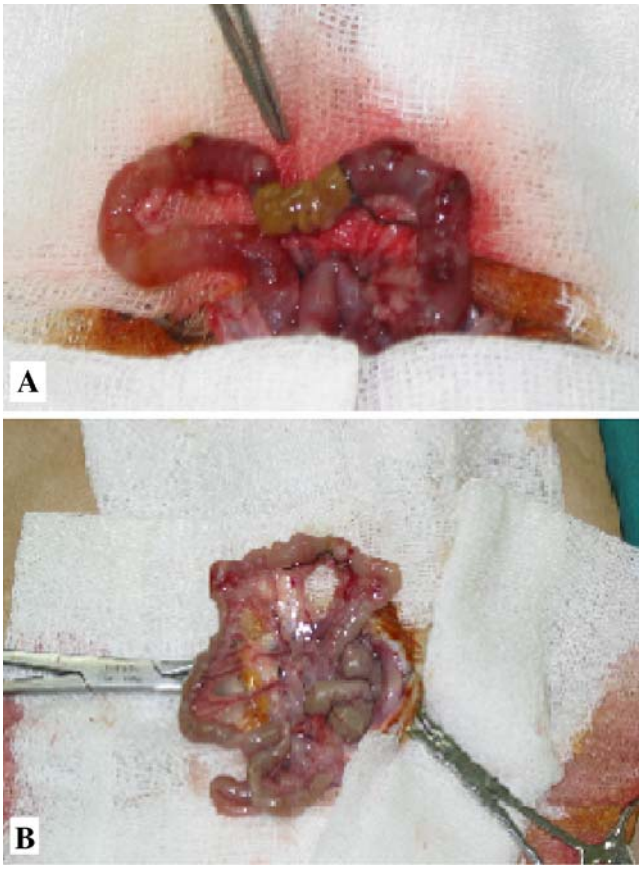

Figure 2 The macroscopic appearances of iodoacetamide-injected jejunal segments. A) The hemorrhagic, ulcerated, and necrotic areas in group III. B) There were no macroscopic changes in a bosentan-pretreated rat in group IV.

mild inflammatory infiltration and edema in the lamina propria (Fig. 3b). The mean histopathological score (histopathological score-1) of group IV was significantly higher than that of groups I and II but significantly lower than that of group III ($p<0.05$ and $p<0.001$, respectively).

Terminal Procedure

There was no granuloma-like structure in any group yet. The mean histopathological score (histopathological score-2) of group III was significantly higher than that of the others ($p<0.001$ for groups I and II, $p=0.001$ for group IV). The mean histopathological score of group IV was not statistically different from that of groups I and II ($p>0.05$). Figure 4a,b demonstrates the histopathologic findings of groups III and group IV, respectively.

The mean tissue hydroxyproline level of group IV was not also different from that of groups I and II ($p>0.5$). The mean tissue hydroxyproline level of group III was significantly lower than that of the others ($p<0.001$).

All of the bursts occurred at the anastomoses. The mean anastomotic bursting pressures of groups III and IV were significantly lower than those of groups I and II ($p<0.001$). However, these were significantly higher in group IV than in group III ($p<0.001$).

Four of six rats in group III had generalized purulent peritonitis; however, the remains had no peri-anastomotic abscess**.** The mean peritonitis score in group III (Fig. 5b) was significantly higher than that of the others ($p<0.001$ for groups I and II, p=0.001 for group IV). However, the mean peritonitis score of group IV (Fig. 5a) was not statistically different from that of groups I and II ($p>0.05$).

Discussion

The etiology of Crohn's disease is still not known. However, current evidence suggests that its pathogenesis involves interactions among genetic susceptibility, immunological factors, and environmental factors.[5] As the etiopathogenesis of the disease is not completely understood, the experimental models are far from reflecting the whole Crohn's disease picture. For instance, granuloma formation, a characteristic of human Crohn's disease, was reported only in trinitrobenzene sulphonic acid (TNBS) or

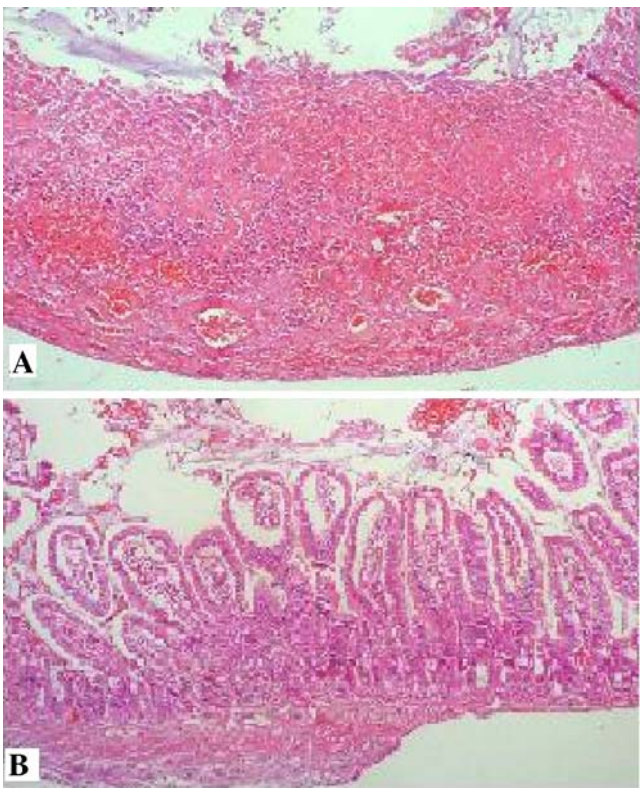

Figure 3 The jejunum cross-sections after iodoacetamide injection (hematoxylin-eosin stain, ×100). **A)** The severe congestion, hemorrhage, ulceration, and diffuse necrosis in group III. B) The mild inflammatory infiltration and edema in lamina propria in group IV.

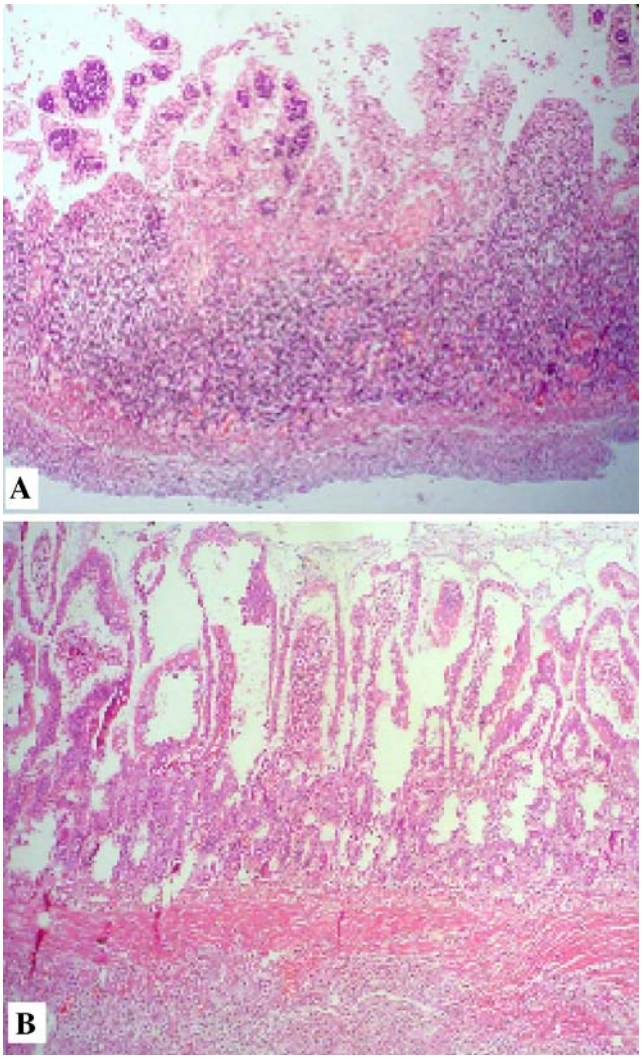

Figure 4 The jejunum cross-sections obtained from terminal procedure (hematoxylin-eosin stain, ×100). A) The severe infiltration of inflammatory cells, marked congestion, ulceration, and disruption of surface epithelium in group III. B) The mild inflammatory infiltration and edema in the lamina propria in group IV.

iodoacetamide-induced inflammation and microvascular ischemia models.[5,9,10]

Rachmilewitz et al. reported that there were many epitheloid cells and granulomas in serosa in 10 of 14 rats 7 days after inflammation induced by iodoacetamide.[5] On the other hand, there was no granuloma formation, which was the evidence of chronic inflammation in the present study. Far from mimicking Crohn's disease, the experiment was an acute inflammation model induced by iodoacetamide.

In this study, it was observed that bosentan, which was given as pretreatment, prevented iodoacetamide-induced tissue damage and significantly reduced acute inflammation, although not being able to block completely. In addition, it was detected that the inflammation in the bosentan treatment group did not show significant difference from the non-inflammation group in the samples obtained 7 days after the establishment of inflammation. Similarly, Hogaboam et al. reported that bosentan prevented tissue injury in a TNBS-induced rat colitis model in a dose-dependent fashion.[11] They suggested that bosentan reduced the tissue injury and granulocyte infiltration in an inflamed colon via few mechanisms: 1) it may directly prevent the granulocyte adherence and infiltration into the vascular endothelium in the inflamed region, 2) it may prevent microcirculatory disruptions which result with distal colon injury, and 3) it may have regulatory effect on endothelin-induced synthesis and secretion of the other causative vasoactive factors of colitis. In fact, polymorphonuclear leukocytes, monocytes, macrophages in the inflamed region lead to endothelin-1 (E1) production.[12–14] E1 can induce inflammatory response that leads to tissue edema. It was shown that intravenously administered E1 gave rise to albumin extravasation in stomach and duodenum and increased the leukocyte rolling in postcapillary venules of the ileal submucosa and the adherence to vessel wall.[15,16] These two effects disappear with blockade of ET_A receptor.

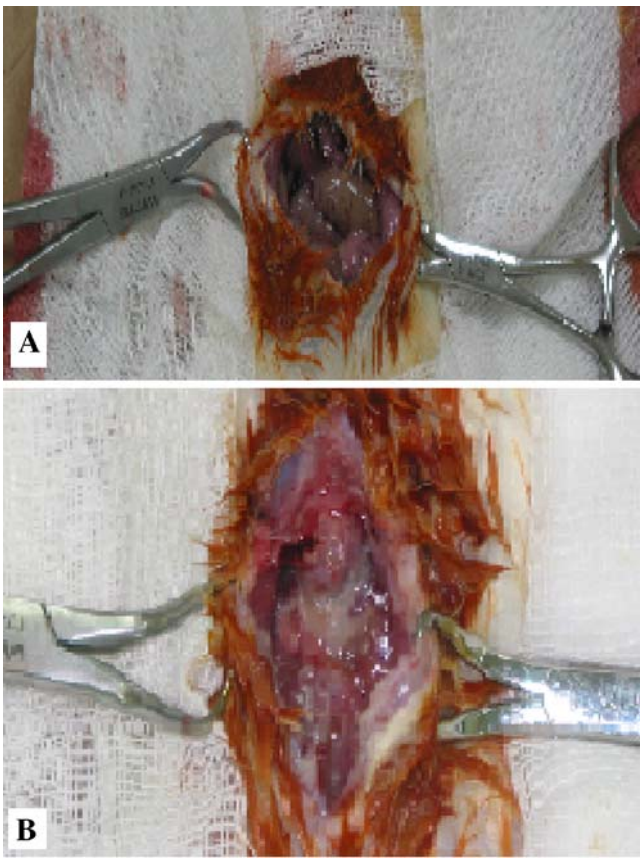

Figure 5 The macroscopic appearances of peritoneal surfaces on terminal procedure. A) There was no change in peritoneal surface of a rat in the bosentan-treated group. B) The figure shows generalized cloudy exudates of peritoneal surfaces in a rat in group III.

In the present and a previous study performed in our clinic, it has been shown that the endothelin receptor blockade with bosentan had positive contributions on the healing of intestinal anastomosis.[17] There are controversial data regarding the effects of endothelin on wound healing in the medical literature. There exist evidences that E1 induces healing with fibrosis in response to tissue injury in lung, heart, and liver. In some of the studies, it was demonstrated that fibrosis could be prevented by ET_A and mixed endothelin receptor blockade.[18–20] Clinical trials support these experimental studies as well. Akimoto et al. reported that tissue endothelin levels were higher during the healing and scarring stages of gastric ulcer in humans.[21] Kanazawa et al. stated that plasma E1 and VEGF levels were high and E1 levels had a positive correlation with the angiogenic mitogen VEGF in active CH as well.[22] In contrast, McCartney et al. reported that E1 and E2 levels were low in human IBD while high in experimental TNBS colitis.[23] However, no information regarding the disease activity was given. The reason for this difference might be the dissimilar disease activities of the patients from whom the tissue samples were obtained in the two studies. On the other hand, King-Van Vlack et al. showed that the flow of the submucosal terminal microvessels in the small intestines of the guinea pigs decreased with intra-arterial E1 administration independently from the difference in the vessel diameter.[24] Although the expected response to tissue hypoxia is an increase in oxygen transport capacity of blood and oxygen uptake of tissues, they observed a decrease in tissue uptake. There is no evidence that can explain how bosentan increased wound healing in this study. In addition, it is obvious that the literature data are inadequate, even controversial to explain the mechanism. New studies are needed to describe this effect.

The most surprising finding of the study was the decreasing in the severity of peritonitis in the bosentan group nearly the same as the control. The cells that contribute to the inflammatory response in the peritoneal cavity are macrophages, neutrophils, adjacent capillary endothelial cells, and mesothelial cells.[25] The first three of these are the source of endothelium.[12] At the same time, E1 increases leukocyte migration.[16] Hyperemia and fluid exudation follow the activation of immune cells (macrophages and probably mast cells) and the change in peritoneal mesothelium. Histamine and the other permeability increasing substances mediate this response. It is known that E1 leads to albumin extravasation in bronchi, spleen, kidney, stomach, and duodenum as well.[15] Endothelin may contribute to the formation of exudate by leading an increase in permeability of the capillary endothelial cells that are adjacent to peritoneum. In fact, it was shown that plasma E1 level increased in sepsis because of fecal peritonitis, and big E1 and active endothelin was higher in peritoneal fluid than in the plasma in fecal peritonitis.[26,27] On the other hand, Metsarinne et al. demonstrated that activated mast cells increase E1 mRNA expression on one hand and cleave endothelium-secreted E1 on the other.[28] In other words, one of the control points of the possible role of endothelium in peritonitis pathogenesis is activated mast cells. In the present study, it can be suggested that nonspecific endothelin receptor blockade showed an extravasation-decreasing effect on the capillary endothelial cells that are adjacent to mesothelium as a decrease in exudate was observed in the bosentan pretreatment group. Measuring the levels of mast cell degranulation products, such as histamine, in addition to endothelin level in plasma and peritoneal fluid in the future studies might more clearly establish the state of mast cells in this pathway.

Conclusion

In conclusion, we could not obtain similar results with Rachmilewitz et al. when we reproduced the iodoacetamide-induced Crohn's disease model. The blockade of endothelin receptors by bosentan decreases the severity of iodoacetamide-induced intestinal inflammation, increases the wound healing in the inflamed intestinal tissue, and decreases the severity of peritonitis. More studies are needed to identify the mechanisms of these effects of bosentan.

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Carcinoid Tumour of the Appendix: An Analysis of 1,485 Consecutive Emergency Appendectomies

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Abstract
Aim The aim of this study is to conduct a retrospective analysis of the incidence and long-term results of carcinoid tumours of the appendix in emergency appendectomies.
Methods A retrospective review of 1,485 appendectomies was performed in two centres from January 2000 until January 2006. Demographic data, clinical presentation, histopathology, operative reports and survival were scored and compared with the literature.
Results In three women and four men, carcinoid tumours were identified (0.47%). The mean age was 32.7 years (range, 20–59 years). The clinical presentation was resembling the symptoms of acute appendicitis in all cases. Laparoscopic appendectomy was the treatment of choice in five patients; in one of these patients, a conversion to laparotomy was necessary. The other two patients underwent primary open appendectomy. Five patients underwent additional surgery after the pathology report became available. Four patients underwent ileocecal resection; one other patient underwent right hemicolectomy. In none of the re-operation specimens was residual carcinoid tumour detected. After a mean follow-up of 65 months (range, 25–92), all patients were alive and disease- and symptom-free.
Conclusion Carcinoid tumours of the appendix most often present as acute appendicitis. It also emphasises the value of histopathological analysis of every removed appendix. The long-term prognosis of incidentally found carcinoids of the appendix is good.

Keywords Acute appendicitis · Carcinoid tumour

Introduction

Carcinoid tumour of the appendix is one of the most common single presentations of this type of tumour and is thereby the most common type of primary malignant lesions of the appendix.[1] The ileum is the second common site of presentation.[1] The carcinoid tumours of the appendix are often asymptomatic and found by chance during appendectomy or other abdominal operations. Carcinoid tumours are found in 0.3–0.9% of patients undergoing appendectomy.[1,2] Tumour characteristics predict the behaviour of the tumour.[2,3] The majority of patients with a incidental carcinoid are cured by appendectomy. The recommendations in literature for adjuvant surgery are irradical resection margins, tumour size greater than 2 cm and goblet-type carcinoid.[4] Most surgeons will encounter this clinical problem only once in a lifetime. For this reason, in our opinion, even a small database will be a useful aid for the management of primary carcinoid tumours of the appendix. In this study, we aim for retrospective analysis of the incidence and long results of carcinoid tumours of the appendix in emergency appendectomies.

Materials and Methods

The study is a retrospective analysis of patients undergoing appendectomies performed between January 2000 till

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January 2006 in the Erasmus University Medical Centre (Erasmus MC) and the Medical Centre Rijnmond Zuid (MCRZ), a Rotterdam community teaching hospital. Data were reviewed on demography, clinical presentation, histopathology, operative reports and long-term outcomes. The used definition of acute appendicitis was granulocyte infiltration through all layers of the appendiceal wall. The follow-up contained medical history, blood samples and octreotide imaging or computed tomography scan. Outpatient clinical controls were at least twice a year.

Results

In the study period, 1,485 patients underwent a laparoscopic or open appendectomy for suspected acute appendicitis. The histopathology of seven patients showed carcinoid tumour. All specimen of those seven patients showed acute appendicitis as well. Of those seven patients, four were men and three were women. They had a mean age of 32.7 years (range, 20–59 years). In five patients, laparoscopic appendectomy was performed; in one of these patients, conversion was necessary because of anatomical reasons. Two patients underwent primary open appendectomy, one because of technical reasons and the other patients because of a ventriculoperitoneal drain. In six patients, the diagnosis was carcinoid; in one patient the histopathology showed a goblet type carcinoid. In four patients, the tumour was located at the tip of the appendix. In two cases, the tumour was located at the base and in one in the body of the appendix. In two patients, tumour diameter was greater than 2 cm, and in one of these, a micro-metastasis was found in the mesentery of the appendix. Two other patients had positive resection margins at the base of the appendix. Four of these patients underwent ileocecal resection, two laparoscopic and two open. One patient underwent laparoscopic right hemicolectomy. In none of the re-operation specimens, residual tumour activity was observed, and no lymph node involvement was seen. Mean lymph node harvest was 11 (range, 5–17). In the other two patients who underwent appendectomy and a tumour less than 1 cm, no re-intervention was performed. All patients have remained tumour-free during a mean follow-up of 65 month (range, 25–92 months).

Discussion

The prevalence of carcinoid tumour in patients undergoing emergency appendectomy is in our database 0.47%. The literature describes percentages between 0.3–0.9% and a little dominant occurrence in female patients.[1,2,5–8] Unusual is the predominance of male patients in our series—four to three—probably due to the small series. The mean age of presentation at 32 years is lower than in large epidemiological studies, suggesting an average diagnostic age between 38 and 49 years, even higher for the goblet-type carcinoid tumour (52 versus 42 years), also possibly related to these small numbers.[7,8] All seven patients presented with acute appendicitis. Probably by the absence of liver metastasis, also in retrospection, no signs of carcinoid tumour could be detected. During appendectomy, in none of the patients, the suspicion of appendicular tumour was raised. In all cases, histopathology reflected an inflammatory response adjacent to the tumour. This pleas for routine pathology of all removed appendices in patients with macroscopically inflamed appendices. In four patients, the tumour was located at the tip of the appendix: in two cases at the base and in one in the body of the appendix. This is in accordance with the literature.[6,9] Carcinoid tumours of the appendix rarely metastasise.[2,3,5] Sporadically extended metastasis disease of a carcinoid tumour of the appendix is described.[3] In a review, Goede et al. describe that acceptable indications for re-intervention represented by all lesions larger than 2 cm in diameter, histological evidence of mesoappendiceal extension, tumours at the base of the appendix with positive margins or involvement of the cecum, high-grade malignant carcinoids and goblet-cell adenocarcinoids.[4] The recommended resection is represented by right hemicolectomy. The consensus that appendiceal carcinoid tumours with a size smaller than 2 cm after radical resection need no further treatment because of minimal metastatic behaviour was followed successfully in this series. In two patients with a tumour at the base of the appendix and a tumour size smaller than 2 cm with positive resection margin, ileocecal resection was performed. No tumour remains in this specimen was found. One patient with a tumour larger than 2 cm with angioinvasive growth underwent right hemicolectomy. This specimen was also without residual tumour on pathology. In the other patient with a tumour larger than 2 cm, ileocecal resection was performed. The patient with the goblet-type tumour underwent also ileocecal resection with no evidence of residual tumour in the specimen. Goblet-cell carcinoids have a worse outcome than the other types of carcinoid tumours and frequently present with metastatic disease.[4,10,11] This patient with a follow-up of 75 months had no recurrence of disease. Lymph from the appendix drains into the retrocecal glands, iloecolic glands, along the iloecolic artery and, finally, to central glands at the base of the superior mestenteric artery. This knowledge gives sense to ileocecectomy for carcionoid of the appendix resecting also the ileocecal artery at its origin from the superior mesenteric artery. At least, it is worth mentioning that patients with carcinoid lesions have a notable risk of developing a synchronous or metachronous colorectal

neoplasm up to 33%.[1,2,4,12] Although none of our patients yet developed a colorectal tumour, follow-up by colonoscopy should be recommended.

From this database, it is concluded that long-term prognosis of incidentally found carcinoids of the appendix is good. It also emphasises the value of histopathological analysis of the removed appendix. Instead of right hemicolectomy, ileocecal resection seems to be the logical operation for tumours larger than 2 cm.

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Serotonin Signaling in Diverticular Disease

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Abstract Diverticulosis is extremely common in Western societies and is associated with complications in up to 15%of cases. Altered motility is an important feature of the pathogenesis of diverticular disease, and serotonin (5-HT) release is a primary trigger of gut motility. This study aims to determine whether colonic 5-HT signaling is altered in patients with diverticulosis or diverticulitis, and whether differences in serotonin signaling may distinguish patients with asymptomatic diverticulosis from those who develop disease specific complications. Sigmoid colon biopsies were obtained from healthy control subjects, individuals with asymptomatic diverticulosis, and those with a history of CT-proven diverticulitis within the preceding 6 months. The key elements of 5-HT signaling including content, release, and 5-HT transporter (SERT) expression were analyzed. A significant decrease in SERT transcript levels was present in the mucosa of patients with a history of diverticulitis when compared with controls, but not in those with asymptomatic diverticulosis. Mucosal 5-HT content, enterochromaffin (EC) cell numbers, and TpH-1 mRNA levels were comparable amongst the groups, as were basal and stimulated 5-HT release. Alterations in 5-HT signaling do not appear to be responsible for the development of diverticula. However, patients with a recent history of acute diverticulitis have a significant attenuation in SERT expression and function, likely secondary to previous inflammation. Our findings may explain the persistent symptoms of pain and altered motility so often observed in patients with diverticulitis long after recovery from the acute inflammatory response.

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Keywords Diverticulosis · Diverticulitis · Serotonin transporter (SERT) · Enterochromaffin cells

Introduction

Diverticular disease is the most common abnormality of colonic structure, with a prevalence in a postmortem surveys of 35%–50%.[1–3] Complications can affect 10%–25% of those with diverticulosis and include bleeding, acute or chronic abdominal pain, intraabdominal abscesses, bowel perforation, fistulae, sepsis, and death.[4,5] Pathogenic factors that have been associated with diverticula include a low-residue diet, elastin and collagen redistribution, compartment segmentation, and disordered motility.[3,6] However, remarkably little is known about the exact pathophysiology of disease, and why specific complications occur in some patients while most remain asymptomatic.

Serotonin (5-HT) is an important chemical mediator involved in bowel function, secretion, and motility independent of the CNS.[7,8] Over 95% of the body's 5-HT stores are located in the intestines, where it is synthesized by a subset of enteroendocrine cells called enterochromaffin (EC) cells. Serotonin is secreted from EC cells into the lamina propria in response to mechanical, chemical, and noxious stimuli, [7] and 5-HT release is a primary trigger for peristalsis, secretion, and visceral sensation.[9] Mucosal 5-HT signaling involves the following sequence of events: (1) luminal stimulation (for example; acidity, mechanical or sheer stress) causes EC cells to release 5-HT into the lamina propria; (2) activation of 5-HT receptors on nearby nerve fibers; (3) removal of 5-HT from the interstitial space via the serotonin-selective reuptake transporter (SERT), which is expressed by all epithelial cells; and (4) intracellular degradation by type A monoamine oxidase.[7,10–12]

It is becoming increasingly clear that key elements of 5-HT signaling are altered in disease states characterized by disordered motility and secretion such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).[10,13,14] Diminished SERT activity appears to be a crucial factor in altered 5-HT signaling.[10] We hypothesized that similar derangements in 5-HT signaling would be detected in patients with diverticulosis. Further, we hypothesized that patients with a history of diverticulitis would greater alterations in 5-HT signaling, possibly explaining a predisposition to disease specific complications.

Materials and Methods

Tissue Acquisition

Human sigmoid colonic mucosal samples were obtained from asymptomatic volunteers who presented for screening colonoscopy after informed consent. Patients were designated as either normal control or asymptomatic diverticulosis based on the presence of diverticula at the time of the procedure. Subjects that had an episode of CT-proven diverticulitis within the prior 6 months, as identified by one of three surgical specialists, were referred for the study if they were undergoing colonoscopic or sigmoidoscopic evaluation. Patients were excluded if the researcher felt that they were unable to provide informed consent (i.e. language or mental status barriers), if they had a history of IBS or IBD, pelvic radiation, history of diverticulitis greater than 1 year prior, bleeding disorders, or if they had not stopped anticoagulation for 7 days before the procedure. Five sigmoid colon mucosal biopsies were obtained in succession from each individual identified as control or diverticulosis using the large capacity biopsy forceps. In patients designated as the diverticulitis group, three biopsies were taken from the area of previous inflammation based on endoscopic, surgical, and radiologic findings, and three were taken from the normal appearing sigmoid colon distal to the area of previous inflammation. We elected to take biopsies in the distal sigmoid as 5-HT signaling varies throughout the colon.[15,16] Each sample was immediately prepared and processed as described below. The study was approved by the Institutional Review Board of the University of Vermont College of Medicine.

Immunohistochemistry

At the time of biopsy, the samples were carefully oriented on a piece of filter paper placed between two mini-sponges and fixed in 4% paraformaldehyde and 0.2% picric acid for 24 hours. The biopsies were then cut into 5-μm sections and mounted in paraffin. Standard deparaffinization techniques were performed and the primary antiserum of mouse antihuman monoclonal antibodies to 5-HT (1:500 Dako Diagnostics, Belgium) were applied followed by the secondary goat antimouse antibodies labeled with 7-amino-4-methyl-coumarin-3 acetic acid (AMCA; 1:250; Jackson Immunoresearch, West Grove, PA). Sections were then incubated with a 1:30,000 dilution of the nucleic acid stain, yo-yo (Molecular Probes, Eugene, OR).

Quantitative Measurement

Tissue sections from each individual were evaluated under the fluorescence microscope and digitized at a single exposure with a 20× objective. Care was taken to count longitudinal sections of bowel wall to include multiple intact crypts. Epithelial cell hyperplasia or hypoplasia was determined by counting the number of stained nuclei in epithelial cells for a given length of colon and quantifying the number of epithelial cells per crypt (three to six crypts). 5-HT-containing epithelial cells (EC cells) were counted as a function of muscularis mucosa length, as a function of 5-HT-positive cells per colonic gland, and as a proportion of the total number of epithelial cells in a region of colon.

Histological Assessment

One portion of the above fixed tissue was stained with hematoxylin and eosin (HE). Each individual was blindly assigned an inflammatory score (0=none, 1=mild, 2=moderate, 3=severe) by an experienced gastrointestinal pathologist. The scoring system was as follows: (0), normal numbers of inflammatory cells in the lamina propria with no active inflammation; (1) normal or minimally increased numbers of inflammatory cells in the lamina propria and/or a rare neutrophil/eosinophil within the crypt epithelium; (2) increased numbers of inflammatory cells in the lamina

propria and/or mild active inflammation; (3) significantly increased numbers of inflammatory cells in the lamina propria and/or moderate/severe active inflammation. Tissue samples with a score of 0 or 1 were considered normal. Any tissue samples scored 2 or 3 were excluded in all groups.

Measurement of 5-HT Content of Colonic Mucosa

One of the biopsy specimens from each individual was homogenized in 0.1 M perchloric acid and buffered in an equivalent amount of 0.5 M borate buffer. The 5-HT content was analyzed using an enzyme immunoassay kit according to the manufacturer's instructions (Beckman Coulter, Fullerton, CA).

Measurement of 5-HT Released from Colonic Mucosa

Two biopsy specimens from each individual were used to assess 5-HT release in the control and diverticulosis groups. 5-HT release was not determined in the diverticulitis group secondary to the number of additional biopsies required. Specimen were processed under two experimental conditions: 1) basal release—biopsies were kept for 5 minutes in HEPES solution without glucose at 37° C; 2) stimulated release—biopsies were kept in HEPES solution without glucose at 37° C for 2 minutes, followed by 3 minutes of mild agitation on a vortex machine at room temperature (VWR vortexer 2 set at level 4). After the 5 minutes of either treatment, 400 μL of each solution were removed and designated separately. For both specimens, the 5-HT release was analyzed using an enzyme immunoassay kit according to the manufacturer's instructions (Beckman Coulter, Fullerton, CA).

Measurement of mRNA in Colonic Mucosa

For RNA extraction, a biopsy sample was placed in 350-μL RNAlater (Qiagen Inc, Valencia, CA). RNA was extracted from the samples using the RNeasy Mini Kits according to manufacturer's instructions (Qiagen Inc, Valencia, CA). Samples of complementary strands of DNA to the mRNA samples were generated using the Superscript II Reverse Transcription Kit according to the manufacturer's instructions (Invitrogen Corp, Carlsbad, CA). A 7500 Fast Real-Time PCR Instrument was used for real-time polymerase chain reaction (RT-PCR; Applied Biosystems, Foster City, CA). SYBR Green Jump Start Taq Ready Mix was used for quantitative PCR (Sigma Aldrich Corp, St. Louis, MI). The expression of ClCN3, SERT, and TpH-1 in each sample was normalized to beta-actin. For SERT, the sense primer was 5′ cgtagttgtggcgggctcat 3′ and the antisense primer was 5′ gccgtggtcatcacctgctt 3′. For ClCN3 the sense primer was 5′ tgctttagtggctgcatttg 3′ and the antisense primer was 5′ ccagaacgggatactttcca 3′. For TpH-1 the sense primer was 5′ tgcggacttggctatgaac 3′ and the antisense primer was 5′ cacaggacggatggaaaac 3′. For beta-actin the sense primer was 5′ catgtttgagaccttcaacac 3′, and the antisense primer was 5′ ccaggaaggaaggctggaa 3′. No differences in beta-actin message levels were detected amongst the sample groups. The data are reported as fold change in calculated relative expression normalized to beta-actin from the average of the controls.

Statistical Analyses

Statistical analyses were performed using Prism software (v. 4.00; Graphpad software, San Diego, Ca.). Comparisons between the four groups (control, diverticulosis, diverticulitis-affected, diverticulitis-adjacent normal) involved a one-way ANOVA followed by the Newman–Keuls multiple comparisons test. Comparisons between control and diverticulosis were performed using an unpaired *t* test. Differences were considered statistically significant if the *p* value was ≤ 0.05.

Results

A total of 51 subjects, identified from December 2005 through May 2006, met inclusion criteria and were recruited for the study. Thirteen of the subjects had experienced a CT scan proven case of acute diverticulitis 1 to 6 months before the time of the colon/sigmoidoscopy. In all 13 cases, a defined area of edema, induration and/or rigidity was identified in the segment of colon that had been radiographically involved with diverticulitis. In a previous

Table 1 Characteristics of 51 Patients Undergoing Biopsies during Colon/sigmoidoscopy

Group	Control	Diverticulosis	Diverticulitis/Normal Adjacent
N	22	16	13
Sex: M	M=10	M=9	M=4
F	F=12	F=7	F=9
Age Range (Median)	36–69 (52)	37–80 (59)	37–73 (55)
# on SSRIs # on	2	2	4
Benzodiazepines # with Grade 2 or 3	2	1	0
Inflammation	1	1	1/2

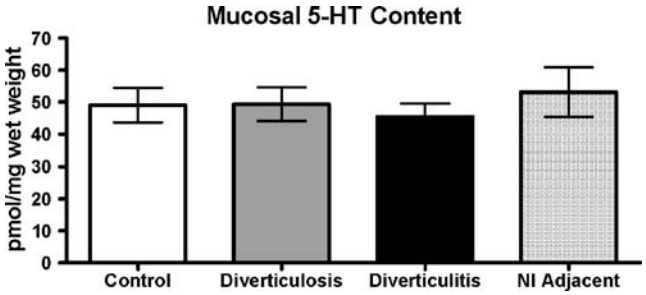

Figure 1 Mucosal 5-HT content measured enzyme immunoassay demonstrating that no difference in 5-HT content was detected amongst groups. Groups are: control, diverticulosis, diverticulitis = areas of indurated sigmoid, and normal (nl) adjacent = normal sigmoid distal to active inflammation.

study involving 38 healthy controls (18F/16 M), no correlation were detected between age, gender or SSRI use and the various elements of serotonin signaling that were investigated in the current study.[10] Mucosal inflammatory scores were determined and were not significantly different between groups (Table 1).

5-HT Content, EC Cell Numbers and TpH-1 mRNA

No differences were detected in the mean mucosal 5-HT content between controls, subjects with asymptomatic diverticulosis, or individuals with diverticulitis in either the actively inflamed or adjacent region (Fig. 1). No differences in EC cell numbers were detected among the four groups with respect to the numbers of epithelial cells per gland, numbers of EC cells per gland, percent EC cells, or the number of EC cells per millimeter muscularis mucosa. (Fig. 2A–D).

Type 1 tryptophan hydroxylase (TpH-1) is the rate-limiting enzyme for 5-HT biosynthesis in the intestinal mucosa.[17] To evaluate the 5-HT synthesis capacity in mucosa of healthy controls vs diverticulosis and diverticulitis samples, we measured TpH-1 transcript levels in mucosal biopsy specimens. Consistent with the lack of differences in 5-HT content and EC cell numbers, no differences in TpH-1 mRNA levels were detected amongst the populations that were studied (Fig. 3A–B).

5-HT Release

An *ex vivo* model of 5-HT release has been developed in an attempt to evaluate basal and stimulated release of 5-HT from EC cells in human tissue specimens. Mechanical stimulation resulted in a significant increase in 5-HT relative to basal release in both the control and the diverticulosis groups ($p<0.0005$) However, no differences in basal or stimulated 5-HT release were detected between the control and the diverticulosis specimens.

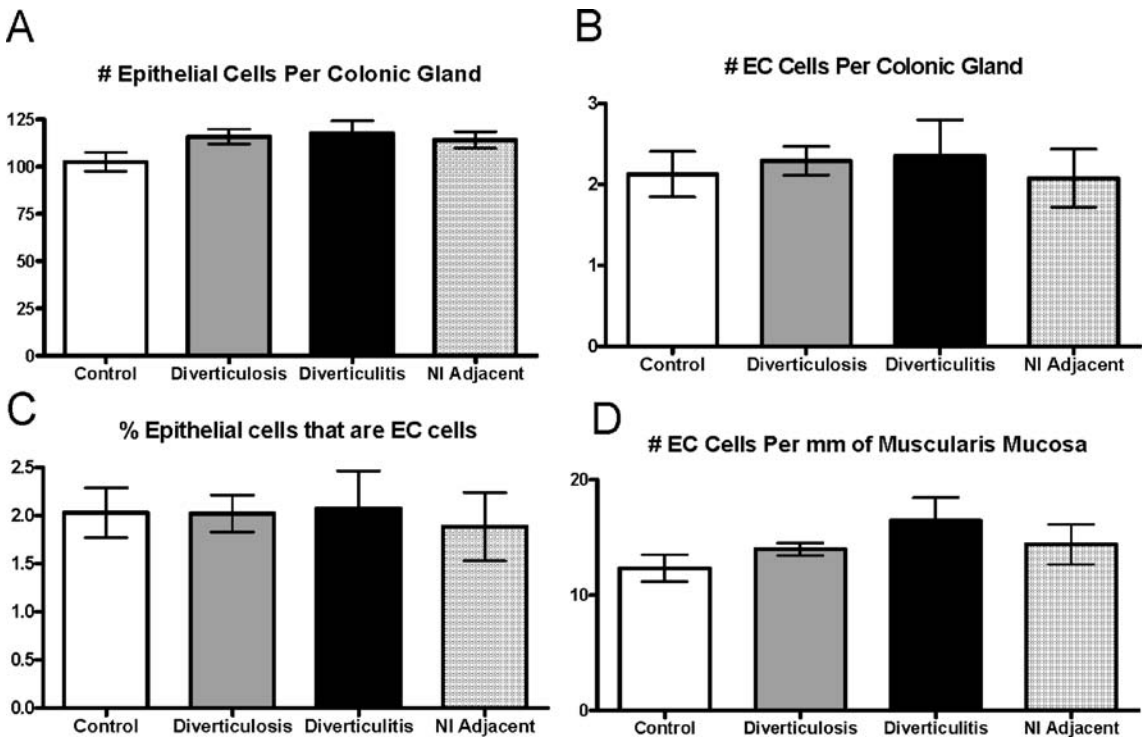

Figure 2 (**A**–**D**) There were no differences found among the groups either in numbers of EC cells or epithelial cells per gland to suggest an increase in 5-HT content.

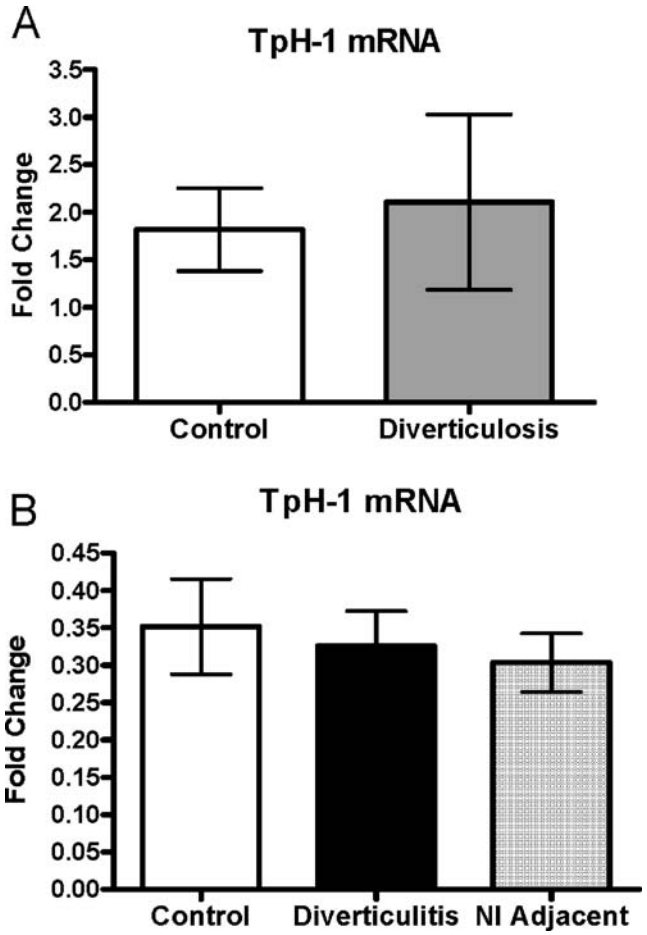

Figure 3 (**A**–**B**) The rate limiting enzyme in 5-HT production, TpH-1 mRNA relative expression was measured using quantitative RT-PCR. There was no difference in TpH-1 mRNA levels between any of the groups.

SERT mRNA

The 5-HT transporter, SERT, is expressed by all epithelial cells and terminates 5-HT signaling in the interstitial space.[18] In the current study, a highly significant decrease in SERT transcript levels was detected within the affected area of diverticulitis specimens (<0.01), and a trend toward decreased expression was detected in the normal adjacent mucosa, when compared to control (Fig. 4A). On an important note, no difference in SERT transcript levels were detected between patients with asymptomatic diverticulosis and control samples (Fig. 4B).

Discussion

Despite the high prevalence of diverticular disease, little is known about its pathophysiology, and there is currently no way to predict who will develop complications. Nonetheless, altered motility appears to be a key feature associated with diverticulosis. 5-HT signaling alterations are thought to be a cause of the disordered motility, secretion, and sensation found in IBD and IBS.[10,19] Our data suggest that similar abnormalities exist in patients who have had an episode of acute diverticulitis but not in those with asymptomatic diverticula.

Disordered serotonergic signaling may result from changes in availability, release, or reuptake of 5-HT. Serotonin availability is measured directly by 5-HT content and indirectly by counting the numbers of EC cells, as well as measuring TpH-1 transcript levels. 5-HT release is simulated *ex vivo* in our laboratory, as it is currently not feasible to measure 5-HT concentrations in the lamina propria in vivo, and 5-HT released in solution containing mucosa at body temperature is measured. 5-HT reuptake is approximated indirectly by measurement of the expression of SERT mRNA. 5-HT acts on numerous receptors which mediate its signals to the intrinsic neural circuitry of the gut as well as to the central nervous system through spinal nerves and the vagus nerve. This study does not attempt to quantify the effects of increased or decreased 5-HT levels on these receptors.

Our data demonstrate that SERT transcript levels were significantly lower in areas of active inflammation in

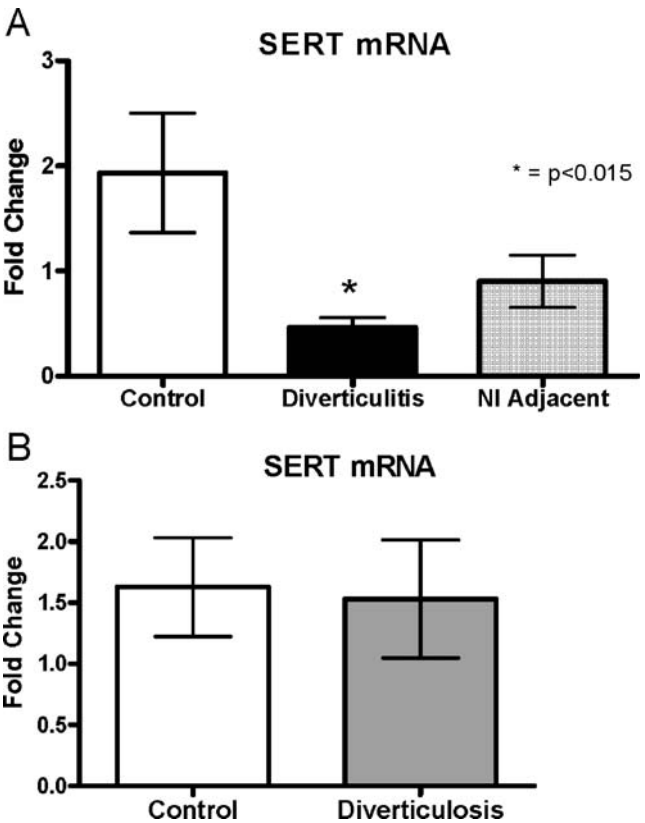

Figure 4 (**A**–**B**) SERT mRNA relative expression was measured using quantitative RT-PCR and normalized to beta-actin. There was no difference between control and diverticulosis, but there was a statistically significant decrease in the mRNA expression in subjects with diverticulitis while the adjacent bowel was decreased but not significantly. These data are demonstrated on separate graphs as the groups were run separately.

diverticulitis, and that there was a trend toward decreased expression of SERT mRNA in the adjacent normal bowel of patients with diverticulitis when compared to control. There were no differences detected between those with diverticulitis and control with regard to measures of 5-HT content or release. This study showed no differences detected in any of the studied aspects of 5-HT signaling between diverticulosis and control subjects.

Our results are consistent with data from other studies of mucosal inflammation in animals, as well as in human studies of IBD and IBS.[10,13,14,20] Furthermore, we have recently reported that SERT expression and function are decreased by TNF-alpha and IFN-gamma in the Caco-2 human epithelial cell line.[21] Interestingly, the extramucosal inflammation, which is a hallmark of diverticulitis, appears to cause a similar effect on SERT expression in the mucosa. It is not possible to discern with certainty from this study whether decreased SERT expression predisposes patients with diverticulosis to develop diverticulitis, or if the inflammation associated with diverticulitis causes altered SERT expression. The latter is the more likely hypothesis because it is clear that inflammation down-regulates SERT expression and function.[13,14,20,21]

Changes in 5-HT reuptake are likely to lead to altered 5-HT availability upon release, and to alterations in 5-HT-activated reflexes. Recently, Bian and colleagues demonstrated that delayed expression of SERT in the mucosa of neonatal guinea pigs is associated with elevated levels of free mucosal 5-HT.[22] Furthermore, Chen et al. showed that transgenic mice that lack SERT exhibit diarrhea,[23] and the use of serotonin selective reuptake inhibitors is similarly associated with an increased incidence of diarrhea.[24] In addition, acute administration of the selective serotonin reuptake inhibitor, citalopram leads to increased motility, including high amplitude propagated contractions that are associated with cramping, as well as compliance, in the human colon.[25] Our results may explain why many patients continue to suffer from discomfort and altered gut motility long after an acute flare of diverticulitis despite normalization of the white blood cell count and physical findings. This clinical scenario may mimic postinfectious-IBS, a well documented disorder related to disordered 5-HT signaling after mucosal inflammation from prior *campylobacter* infection.[26]

In conclusion, disordered 5-HT signaling does not appear to contribute to the development of colonic diverticula. However, decreased expression of SERT was noted in patients who have had CT proven acute diverticulitis up to 6 months after resolution of their clinical episode. These findings provide a plausible explanation for the persistent symptoms of altered motility often observed in patients who appear to have recovered from the acute inflammatory phase of diverticulitis.

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Recurrence and Quality of Life Following Perineal Proctectomy for Rectal Prolapse

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Abstract

Background Surgical outcome and quality of life (QOL) following perineal proctectomy for rectal prolapse remain poorly documented.

Methods From 1994 to 2004, patients with full-thickness rectal prolapse were treated exclusively with perineal proctectomy independent of age or comorbidities. Subjective patient assessments and recurrences were determined retrospectively from hospital and clinic records. Consenting patients completed the gastrointestinal quality of life index (GIQLI).

Results Perineal proctectomy was performed in 103 consecutive patients with a median age of 75 years (range 30–94). Most patients underwent concurrent levatorplasty (anterior 85.8%, posterior 67.9%). Durable results were obtained in all patients; the recurrence rate was 8.5% over a mean follow-up of 36 months. Preoperatively, 75.5% of patients reported fecal incontinence, and 32.1% had obstructed defecation. Incontinence significantly improved post-proctectomy (41.5%, $p<0.001$), as did constipation (10.4%, $p<0.001$). GIQLI respondents reported satisfaction following proctectomy with 63% scoring within one standard deviation of healthy controls. Patients with recurrent prolapse reported a lower QOL. Risk factors for recurrence included duration of prolapse, need for posterior levatorplasty, and prior anorectal surgery.

Conclusions Perineal proctectomy provides significant relief from fecal incontinence and obstructive symptoms caused by rectal prolapse, with an acceptable recurrence rate and low morbidity.

Keywords Procidentia · Rectal prolapse · Proctectomy · Quality of life · Incontinence

Introduction

Although perineal proctectomy (rectosigmoidectomy) for the treatment of full thickness rectal prolapse was described first by Mickulicz in 1889, it was more than 80 years later when this approach gained significant popularity in the United States following the report by Altemeier and colleagues in 1971.[1] Since this time, most surgeons have reserved the perineal proctectomy, (along with other perineal-only approaches) for elderly, infirm, or institutionalized patients who were deemed too high-risk for transabdominal procedures.[2] This trend has continued in the era of various laparoscopic rectopexy repairs and resections.[3,4] Despite the myriad of treatment options for rectal prolapse and due largely to the lack of adequate randomized studies, a recent Cochrane review was unable to either support or refute the perceived superiority of transabdominal repairs for this condition.[5] Across the United States, perineal procedures continue to comprise between 50 and 60% of the procedures performed for rectal prolapse.[6]

For the last 12 years, all patients presenting to Washington University with isolated rectal prolapse had undergone perineal proctectomy regardless of their ability to tolerate a transabdominal procedure. We report the results of following this non-selective policy for the

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surgical treatment of rectal prolapse in more than 100 consecutive patients, along with an analysis of risks for recurrent prolapse. In addition, since very little is known about the quality of life (QOL) following prolapse surgery, we administered a standardized, validated instrument to study participants. To our knowledge, this is the first attempt at determining factors that may influence both the perceived QOL among prolapse patients and assist in selecting appropriate surgical therapy.

Material and Methods

Since 1994, all patients presenting to the Section of Colon & Rectal Surgery at Washington University have been treated non-selectively with perineal proctectomy, independent of age, concomitant comorbidities, or prior abdominal or anal surgery. Participants in the current study were identified using a diagnosis- and procedure-coded, prospectively maintained database. All patients treated surgically for full thickness rectal prolapse were included; exclusion criteria included those patients with internal prolapse only (rectal intussusception), inflammatory bowel disease, or solitary rectal ulcer syndrome. Patients with rectocele in addition to prolapse also were excluded. Outpatient clinic charts and hospital records were reviewed retrospectively for all patients undergoing perineal proctectomy over the 10-year period from January 1995 to December 2004. Standard clinic evaluations consisted of the documentation of presenting symptoms, patient self-assessment of fecal incontinence, and complete medical, surgical, and obstetric history. Fecal incontinence (graded using an established scale from 0 [fully continent] to III [frequent incontinence to liquid and solid stool]) and subjective complaints regarding defecation were reassessed at all subsequent follow-up visits.[7–9]

The surgical technique of perineal proctectomy for prolapse has been well-described.10–12 In brief, patients were positioned in prone-jackknife, with surgery performed under either general or regional (spinal) anesthesia. The degree of prolapse determined length of rectal resection, and all anastomoses were hand-sewn in a single layer. Whether an anterior, posterior, or combined levatorplasty was performed was left to the discretion of the operating surgeon upon evaluation of the pelvic floor. When employed, levatorplasty consisted of plication of the levator ani musculature using interrupted absorbable sutures, as previously described by Prasad et al.[12] Perineal rectopexy was not performed in any patient. Antibiotics were continued for 24 hours, with oral intake resuming on postoperative day 1. Bowel function (incontinence, constipation, need for laxatives, prolapse recurrence) was reassessed in the clinic at 1, 3, and 12 months postoperatively, and then annually on an as-needed basis. Recurrence was defined as rectal prolapse demonstrable during evaluation in the outpatient clinic.

A modified gastrointestinal quality of life index (GIQLI) was administered via mail and telephone interviews to all patients with a minimum of 1 year follow-up. The GIQLI is an established tool for assessing the health-related QOL in patients with a variety of gastrointestinal disorders.13–16 This 36-item questionnaire asks the patient for a self-assessment of the preceding 2 weeks with regard to gastrointestinal symptoms (19 questions), overall physical function (seven questions), emotional status (five questions), social interactions (four questions), and perceived treatment effectiveness (one question). Items are scored from 0 (least desirable) to 4 (most desirable), for a total maximum score of 144. Prospective evaluation of the GIQLI in healthy control populations yielded a mean score of 125.8 points (95% CI [121.5, 127.5]).[13] Modification of this tool consisted of supplemental questions addressing subjective prolapse recurrence, fecal continence, and the need for additional anal surgery. Patient survival was determined from medical records and the Social Security Death Index database.

Fisher's exact test was used to analyze categorical variables. Wilcoxon or *t*-test comparisons were employed to compare continuous variables between two groups, based on underlying distributions of the variable. Cox regression analysis was planned from the outset of the study to determine risk factors for recurrent prolapse. However, with fewer than 10 events, multivariate modeling was deemed inappropriate. Data are expressed as mean ± standard deviation, and p values less than 0.05 were considered significant. The study was approved by the Washington University Human Studies Committee.

Results

Over the 10-year-period of the study, 106 perineal proctectomies were performed on 103 patients with full-thickness rectal prolapse. The median age at the time of surgery was 75 years (range 30–94). Women comprised 94.2% of the cohort, with a mean parity of 2.2 (range 0–9). Twenty-seven patients (26%) reported duration of rectal prolapse of greater than 1 year. Eighty patients (78%) reported routine incontinence to either liquid or fecal stool (e.g., Gr II or III incontinence), with 34 (33%) complaining of obstructed defecation. Patients reported an average of 2.4±2.1 bowel movements per day, with 10% requiring daily laxatives. The cohort exhibited a spectrum of comorbid disease (Fig. 1).

All patients underwent perineal proctectomy with resection of redundant rectum. General anesthesia was used in

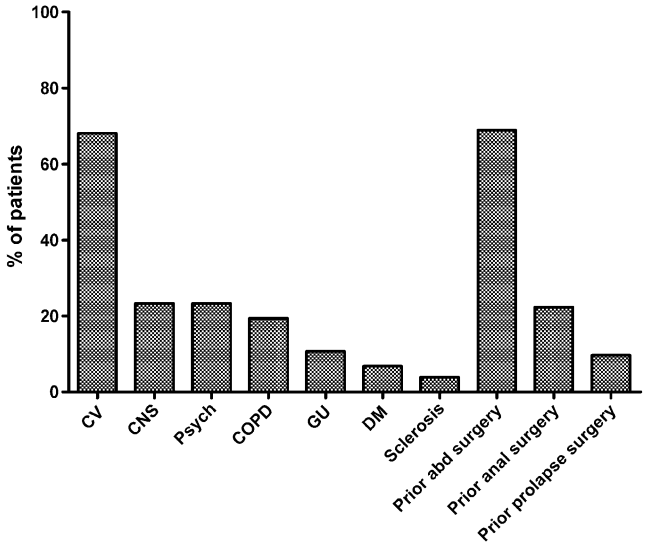

Figure 1 Patient comorbidities. *CV* cardiovascular disease, *CNS* central nervous system disease, *psych* major psychiatric disease, *COPD* chronic obstructive pulmonary disease, *GU* urologic disease, *DM* diabetes mellitus, sclerosis, any collagen-vascular or other sclerosing disorder.

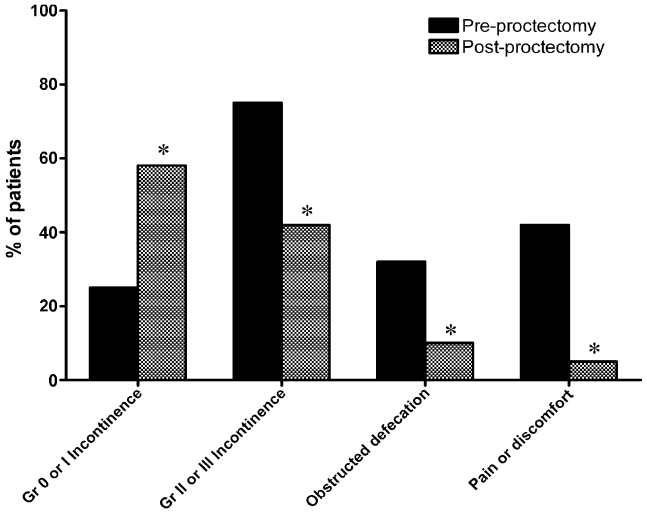

Figure 2 Pre- versus postoperative patient symptoms. Asterisk denotes $p<0.001$ versus pre-proctectomy symptoms.

37% of patients, with the remainder receiving a spinal anesthetic. The median length of resected rectosigmoid was 8 cm (mean 11.3, range 4–50 cm). There were no significant differences in the lengths resected between patients with primary versus recurrent prolapse (11.7 vs. 7.9 cm, $p=0.09$), or those with short versus long (greater than 12 months) duration prolapse (10.6 vs. 12.8 cm, $p=0.39$). Anterior levatorplasty was deemed necessary in 86% of patients, and posterior plication of the levator musculature required in 68%. There was a single 30-day postoperative death due to myocardial infarction (mortality rate 0.9%). Four patients developed anastomotic leaks or fistulas, and another three patients had clinically apparent abscesses requiring drainage. The overall morbidity rate was 8.5%, and there were no correlations between operating surgeon and complication or recurrence rates (data not shown).

The median length of clinical follow-up was 21 months (mean 36, range 1–120 months). As depicted in Fig. 2, only 44 patients (43%) reported persistent Gr II or III fecal incontinence postoperatively ($p<0.001$ vs. preoperative incontinence). Likewise, symptoms related to obstructed defecation were significantly improved ($n=11$ (10%); $p<0.001$ vs. preoperative function). The postoperative mean 0.9±0.9 bowel movements per day was significantly lower than experienced preoperatively ($p<0.0001$). For the entire cohort, the recurrence rate for rectal prolapse was 8.5%, whereas the recurrence rate for patients under the age of 50 years was 6.7%. In those who developed recurrence, median time to recurrent rectal prolapse was 13 months (range 2–60 months). Results of the univariate analysis of risk factors for recurrent prolapse are listed in Table 1. Surgery before 1999 (e.g., in the first half of the series), duration of rectal prolapse for greater than 1 year, a history of prior anal surgery, and the performance of a posterior levatorplasty were all associated with recurrence. Of these, the duration of prolapse before surgery held the strongest

Table 1 Risk Factors for Recurrent Prolapse Following Perineal Proctectomy

	Univariate Analysis (p values)
Gender	0.57
Age greater than 50 years	0.11
Parity	0.21
Duration over 12 months	**0.0002**
Incontinence grade	0.72
Prior anal surgery	**0.007**
Prior rectal prolapse surgery	0.17
Prior colon surgery	0.59
Prior abdominal surgery	0.53
Cardiovascular disease	0.15
CNS disease	0.97
COPD	0.25
Urologic disorders	0.94
Psychiatric disease	0.97
Diabetes	0.40
Collagen-vascular or other sclerosing diseases	0.22
Anesthetic type	0.31
Surgery prior to 1999	**0.005**
Length of resected rectosigmoid	0.08
Anterior levatorplasty performed	0.78
Posterior levatorplasty performed	**0.03**

CNS central nervous system, *COPD* chronic obstructive lung disease, *OR* odds ratio for recurrence, *95% C.I.* 95% confidence interval

association with recurrent prolapse, and the recurrence rate for patients with prolapse greater than 1 year was 35%.

Of 79 patients alive at the present time, 38 patients were contacted and completed the entire GIQLI, yielding a response rate of 48%. The respondents had a median follow-up of 53 months (mean 56 months), with six patients (16%) developing recurrent prolapse during this time. Two of the six patients underwent repeat perineal proctectomy with satisfactory results and no evidence of recurrence; the remainder declined further surgery. Thirteen of 38 patients (34%) reported Gr II or III fecal incontinence, with the rest reporting good control.

Among all respondents, the mean GIQLI score was 105.2±22.7. Thirty patients (63%) scored within one standard deviation of historical normal controls with regards to their gastrointestinal-specific QOL. Patients developing recurrent rectal prolapse reported significantly lower overall QOL than those without recurrence (Fig. 3A). The mean total GIQLI score for those with recurrence was 88.8±18.3 versus 109±22.3 for those with intact repairs (p=0.05). In a similar way, those with recurrence scored lower on questions addressing bowel function-specific aspects of QOL such as urge, frequency and control (10.6± 3.2 vs. 17.5±4.7, p=0.005). However, impaired fecal continence did not significantly influence either the overall GIQLI score or response to bowel-specific questions (Fig. 3B). There was no correlation between recurrent rectal prolapse and the self-reported fecal incontinence grade (data not shown).

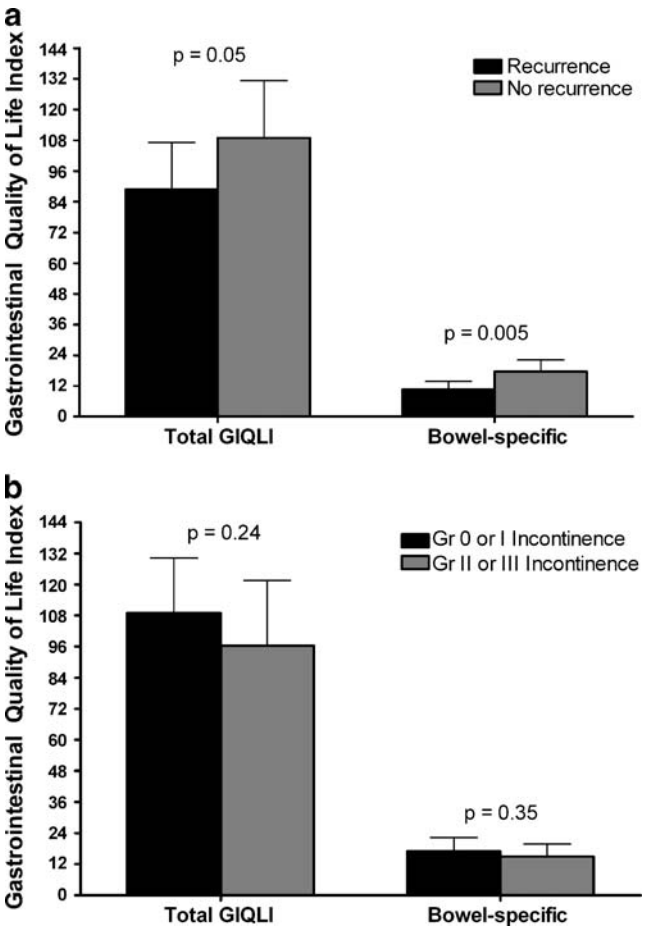

Figure 3 A and B. The influence of (A) recurrent rectal prolapse and (B) persistent fecal incontinence on total GIQLI and bowel-specific scores following perineal proctectomy.

Discussion

There are more than 100 described surgical procedures for the treatment of rectal prolapse.[17] The fact that so many surgical options exist suggests that no single operation is superior for correcting this disorder in all patients. Further complicating this issue is the lack of long-term follow-up in most series due to the advanced age or institutionalized status of many patients with rectal prolapse. Surgical dogma holds that perineal approaches should be reserved only for infirm patients due to a perceived higher rate of recurrent prolapse relative to transabdominal procedures.[2] However, for the last decade we have non-selectively utilized perineal proctectomy, typically with posterior levatorplasty, in all patients with rectal prolapse regardless of other mitigating factors. In line with prior reports, the current study confirms that perineal proctectomy can be performed with very low surgical morbidity and mortality.[2,10,18,19] In addition, we report a low overall recurrence rate of 8.5% after a 3-year average follow-up. Finally, patients generally experience a good QOL following perineal proctectomy, with the majority not varying significantly from normal controls in their responses on a standardized gastrointestinal-specific assessment tool.

The GIQLI is an established instrument for addressing a wide spectrum of gastrointestinal disorders that has been cross-validated with other generic QOL indices and reviewed by gastroenterology specialists.[13] The mean GIQLI score in a normal control population is 126, although several authors have reported lower scores in patients with colorectal disorders.[14,15] The mean GIQLI score for patients following perineal proctectomy was 105.2, and almost two thirds of patients fell within one standard deviation of normal controls, reflecting overall satisfaction with their postsurgical outcome. Perineal proctectomy provided relief from both the severe fecal incontinence (preoperative 77%, postoperative 42%) and the obstructed defecatory symptoms (preoperative 33%, postoperative 10%) that commonly occur in conjunction with rectal prolapse. Furthermore, those patients with incontinence did not perceive a significantly worse QOL than those with normal continence. The finding of poor correlation between fecal incontinence, bowel-movement frequency and QOL was reported previously and likely

relates to personal behavior modifications allowing near-normal function.[15,20] Conversely, patients who developed recurrent rectal prolapse reported a significantly lower QOL (GIQLI 88.8 vs. 109 for non-recurrent prolapse, $p=0.05$). Responses to the bowel function-specific questions mirrored the findings on the GIQLI, suggesting that the reduction in QOL stems directly from dissatisfaction with bowel habits (as opposed to pain, self-image, or overall physical function). Thus, it stands to reason that the avoidance of recurrent prolapse should remain a high priority in selecting appropriate surgical therapy.

The recurrence rate of 8.5% at 3 years in the current study compares favorably with previous reports on perineal proctectomy in which prolapse recurred in 6.4 to 16% of patients at significantly shorter follow-up times.[2,10,18] However, among patients choosing to respond to the modified GIQLI questionnaire, the recurrence rate was 16% at an average follow-up of more than 4.5 years. This apparent increase in recurrence rate may result from a selection bias (e.g., those with recurrences were more likely to respond to the questionnaire and thus are overrepresented), or it may represent a true finding evident upon longer clinical follow-up. However, the short median time to recurrent prolapse following proctectomy (13 months) suggests that most treatment failures occur within the first several years postoperatively. Recurrent prolapse greatly influenced perceived QOL, with those suffering a recurrence scoring significantly lower on both the GIQLI and on the subset of bowel function-specific questions.

Long-standing rectal prolapse, proctectomy before 1999, prior anal surgery for other conditions, and the performance of a posterior levatorplasty were identified on univariate analysis as risk factors for recurrent rectal prolapse following perineal proctectomy. There may be a learning curve to performing a technically sound perineal proctectomy. The remaining factors are likely surrogate markers for an impaired sphincter mechanism. Levatorplasty was performed at the discretion of the operating surgeon, typically when the sphincters were judged to be lax. Although it's conceivable the levatorplasty itself contributes to recurrent prolapse, more probable is that the impaired sphincter mechanism from longstanding prolapse predisposes to recurrence independent of whether levatorplasty is performed. Likewise, prolonged duration of rectal prolapse correlates with diminished anal manometric measurements and altered sphincter morphology.[9,21,22] We previously reported that preoperative manometric squeeze pressure greater than 60 mmHg has a positive predictive value of 90% for improved continence, but no impact on recurrence rates.[9] Overall, only 6.7% of tested prolapse patients had "normal" preoperative anal manometry, suggesting a high prevalence of sphincter impairment in this population, although the etiology of sphincter dysfunction in relation to rectal prolapse remains controversial (e.g., cause vs. effect).[7,8,23] Interestingly, the length of resected rectosigmoid colon approached significance on univariate analysis as a risk factor for recurrence. Due to the low number of primary events (e.g., recurrences), multivariate analysis of these risk factors could not be performed. Although conjecture, it may be that prolonged rectal prolapse irreparably damages the anal sphincters, thereby making perineal proctectomy less likely to provide a durable repair. Unlike prior reports, we found no relation between patient age at proctectomy and recurrent prolapse.[18]

Conclusion

Perineal proctectomy for rectal prolapse produced acceptable intermediate-term results in a non-selected, consecutive series of more than 100 patients. The recurrence rate of 8.5% was obtained with low operative morbidity and a single mortality. Perineal proctectomy significantly improved both obstructive symptoms and fecal continence. Overall, post-proctectomy patients reported a near-normal gastrointestinal-specific QOL independent of persistent fecal incontinence, although recurrent prolapse negatively influenced their perception. The presence of rectal prolapse for greater than 1 year represents a risk factor for treatment failure following perineal proctectomy; consideration should be given to other surgical approaches in this subset of patients. Since duration of prolapse adversely affects recurrence, every effort should be made to perform timely repair as soon as rectal prolapse is diagnosed. Perineal proctectomy seems to be a reasonable alternative to abdominal repairs, even in patients with no medical contraindications to abdominal surgery.

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Targeted Suppression of β-Catenin Blocks Intestinal Adenoma Formation in APC Min Mice

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Abstract
Introduction Mutations involving the adenomatous polyposis coli (*APC*) tumor suppressor gene leading to activation of β-catenin have been identified in the majority of sporadic colonic adenocarcinomas and in essentially all colonic tumors from patients with Familial Adenomatous Polyposis. The C57BL/6J-APC^{min} (Min) mouse, which carries a germ line mutation in the murine homolog of the *APC* gene is a useful model for intestinal adenoma formation linked to loss of APC activity. One of the critical downstream molecules regulated by APC is β-catenin; molecular targeting of β-catenin is, thus, an attractive chemopreventative strategy in colon cancer. Antisense oligodeoxynucleotides (AODNs) capable of downregulating murine β-catenin have been identified.
Analysis of β-catenin Protein Expression in Liver Tissue and Intestinal Adenomas Adenomas harvested from mice treated for 7 days with β-catenin AODNs demonstrated clear downregulation of β-catenin expression, which was accompanied by a significant reduction in proliferation. There was no effect on proliferation in normal intestinal epithelium. Min mice treated systemically with β-catenin AODNs over a 6-week period had a statistically significant reduction in the number of intestinal adenomas. These studies provide direct evidence that targeted suppression of β-catenin inhibits the formation of intestinal adenomas in APC-mutant mice. Furthermore, these studies suggest that molecular targeting of β-catenin holds significant promise as a chemopreventative strategy in colon cancer.

Keywords Beta-catenin · Adenoma · Colon cancer · Chemoprevention

Introduction

Approximately 150,000 people will develop colorectal cancer in the United States in 2007, and it is estimated that over 50,000 people will die from it.[1] While surgery and chemotherapy are useful modalities for treating patients with colon cancer, additional strategies in the treatment of such patients are needed. It has become apparent that the accumulation of genetic mutations in a clonal cell population results in the transition of normal colonic epithelium to carcinoma.[2] Defining the molecular abnormalities involved in the development of neoplasia offers hope for specific molecular targeting in the treatment of established tumors as well as for chemopreventative interventions.

The wnt or β-catenin signaling pathway is important in developmental processes and carcinogenesis.[3] In normal cells, β-catenin is tightly regulated by a multiprotein destruction complex that includes GSK-3β, APC, axin, and protein phosphatase 2A.[4] In the presence of wnt glycoproteins, β-catenin is stabilized by the inhibition of GSK-3β. Free cytoplasmic β-catenin translocates into the

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nucleus where it binds to T-cell factor proteins and serves as a coactivator to stimulate transcription of multiple target genes that regulate cellular proliferation, survival, and migration including c-myc, cyclin D1, AF-17, MMP-7, and PPARδ among others.[5–11]

The adenomatous polyposis coli (*APC*) gene product is a critical regulatory component of the wnt signaling pathway and is involved in downregulating β-catenin within the cell. Dysregulation of this pathway, leading to the aberrant accumulation of β-catenin and subsequent stimulation of target gene transcription, has been described as a critical step in carcinogenesis.[12,13] Mutations involving APC or β-catenin have been described in the vast majority of sporadic colonic adenocarcinomas.[14,15] Additionally, inherited germ line mutations in the *APC* gene result in Familial Adenomatous Polyposis, an autosomal dominant disorder.[16,17] Affected individuals develop numerous colonic adenomas at an early age with malignant degeneration occurring in most by age 50.[18] C57BL/6J-APC^{min} (Min) mice carry a germ line mutation in the murine homolog of the *APC* gene.[19] These mice develop multiple intestinal adenomas which make them an excellent model for studying chemopreventative agents for neoplasia related to inactivation of the *APC* gene.

Targeted therapeutics directed at downregulating β-catenin expression represent an attractive approach to modifying aberrant downstream signaling in colon cancer. Studies utilizing antisense oligodeoxynucleotides (AODNs) and small interfering RNA to selectively inhibit β-catenin expression have demonstrated efficacy in the inhibition of APC-mutant colon cancer cell growth both in vitro and in vivo.[20–22] These studies provide a direct link between the disruption of abnormal β-catenin or Tcf signaling and growth inhibition of established tumors. Numerous chemoprevention studies using a variety of agents have demonstrated efficacy in the suppression of adenoma formation in Min mice.[23] Many of these agents have been shown to downregulate β-catenin among multiple other targets, but specific targeting of β-catenin signaling in intestinal adenoma formation has not been described.[24–31]

To directly examine the role of β-catenin in the development of intestinal adenomas and the role of targeted suppression of β-catenin in the prevention of intestinal adenomas, our laboratory employed a strategy utilizing AODNs in Min mice. A novel 20-base phosphorothioate AODN sequence capable of specifically and potently suppressing the expression of murine β-catenin in a dose-dependant manner was utilized. The studies presented here demonstrate a role for aberrant β-catenin signaling in the development of intestinal adenomas and supports chemopreventative strategies aimed at the targeted suppression of β-catenin.

Materials and Methods

Experimental Animals

Female C57BL/6J-APC^{min} (Min) mice, at 7 weeks of age, were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Balb/c nude mice, 4- to 8-week-old female, were purchased form the National Cancer Institute (NCI, Frederick, MD, USA). Animals were housed in the University of Pennsylvania, School of Medicine Animal Research Facility with a 12-h light–dark cycle under constant temperature and humidity. Animals were given a standard mouse chow diet and drinking water ad libitum. Guidelines established by the University of Pennsylvania regarding the humane care and use of laboratory animals were followed.

ODNs and Antisense Treatment Protocol

Phosphorothioate AODNs with the following sequences were obtained from Trilink Biotechnology (San Diego, CA, USA):

Murine β-catenin Antisense 20mer: 5′-GCT TTT CTG TCC GGC TCC AT-3′
Murine β-catenin Scrambled 20mer: 5′-TGC CTG CCT ACT GTT CTG TC-3′

It should be noted that the scrambled control ODN contains the same nucleotides but differs from the β-catenin AODN at 16 of 20 bases. Lyophilized AODNs were reconstituted in sterile phosphate-buffered saline (PBS) to 20 mg/ml and stored in aliquots at −20°C. Stock β-catenin and scrambled AODNs were diluted in sterile Hanks Balanced Salt Solution (Mediatech, Herndon, VA, USA) to concentrations of 10 and 20 mg/kg in a volume of 0.5 ml per mouse. Following treatment the mice were sacrificed by CO_2 asphyxiation. The intestinal tracts were removed from each animal from distal esophagus to rectum, opened longitudinally, flushed with saline, and flattened for tumor counting. Tumors were counted under a 10× dissecting microscope by an observer blinded to the treatment group and genotype of the animal. In some experiments, mice were pulsed with bromodeoxyuridine prior to sacrifice, and the tissue was processed for immunohistochemistry.

Analysis of β-catenin Protein Expression in Liver Tissue and Intestinal Adenomas

Following the 7-day intraperitoneal (i.p.) injection course consisting of either ODN or control, the Balb/c nude mice were sacrificed, and liver specimens were surgically removed. Following sacrifice of the Min mice, representative adenomas

were surgically excised under the dissecting microscope after counting was completed. Tissue was homogenized in 10 volumes (*w*/*v*) homogenization buffer containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.2), 50 mM NaCl, 1 mM ethylene diamine tetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, and 1% aprotinin. Tissue debris was removed by centrifugation at 2,000×*g* for 10 min. The supernatant fraction was transferred to a fresh tube and lysed by the addition of Triton X-100 to a final concentration of 1%. The sample was centrifuged at 30,000×*g* for an additional 30 min to remove insoluble material. Protein concentration was measured by Bradford colorimetric assay (Bio-Rad).

Immunohistochemical Analysis

Balb/c nude mice liver tissue was fixed with Bouin's solution, embedded in paraffin, and sectioned at 3 μm. Tissue sections were deparaffinized and stained as described below. Min mice were given an intraperitoneal injection of 5-bromo-2-deoxyuridine (120 mg/kg) and 5-fluoro-2′-deoxyuridine (12 mg/kg; Sigma Chemical Co., St. Louis, MO, USA) 90 min prior to sacrifice. Following sacrifice, the intestinal tract was removed, flushed with cold PBS, fixed in Bouin's solution for 6–12 h, and washed with 70% ethanol. The intestinal tract was opened with an incision along its cephalocaudal axis on the antimesenteric border and, then, rolled from its proximal to distal end. Each of these resulting "swiss rolls" was, then, cut in half parallel to the duodenal ileal axis and placed on a tissue cassette with the cut edge of one half facing down and the cut half of the other half facing up. The tissue was embedded in paraffin, and serial sections were prepared. Sections were stained with hematoxylin and eosin to inspect mucosal histology.

Tissue was deparaffinized in xylene and isopropanol and subjected to an antigen unmasking procedure consisting of a 15-min incubation at 37°C in type II bovine pancreas chymotrypsin (Sigma Chemical Co., St. Louis, MO, USA). Endogenous peroxidases were inactivated by immersing the slides in 2% H_2O_2 and 100% methanol for 5 min at room temperature. The treated sections were, then, washed in PBS, placed in blocking buffer (1% BSA, 0.3% Triton X-100, and 0.2% powdered milk in PBS), and stained overnight at 4°C with either a monoclonal antibody specific for β-catenin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or a monoclonal antibody specific for BrdU (Calbiochem, San Diego, CA, USA). Antigen-antibody complexes were detected by using a peroxidase-labeled secondary antibody. BrdU-positive cells were quantified, and the proliferative index was determined by calculating the percentage of BrdU-positive cells to the total number of columnar epithelial cells in a given field. Statistical analysis was performed using the Student's *t*-test.

Northern Blotting

Representative adenomas from each of the treatment groups were surgically excised and snap frozen in liquid nitrogen. Tissue was homogenized in 1 mL of TRIzol® (Invitrogen, Carlsbad, CA, USA), and total RNA was isolated according to the supplier's instructions. Total RNA was, then, subjected to the Oligotex® mRNA spin column (Qiagen, Valencia, CA, USA) to yield purified mRNA. Equal amounts of mRNA was, then, electrophoresed on a formaldehyde containing 1% agarose gel, transferred onto a nylon membrane (Boehringer Mannheim, Indianapolis, IN, USA), and hybridized with a digoxigenin-labeled murine β-catenin cDNA probe. A digoxigenin-labeled Glyceraldehyde 3-phosphate dehydrogenase cDNA probe (CLONTECH, Palo Alto, CA, USA) was used as a loading and transfer control.

Western Blotting

Equal amounts of cell lysate from tissue specimens in each treatment group were subjected to 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroblotted onto a polyvinylidene fluoride membrane (Immobilon-P, Milipore, Bedford, MA, USA). β-Catenin-specific antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). An actin-specific monoclonal antibody was obtained from Chemi-Con (Temecula, CA, USA) and was used to control for equal protein loading. All antibodies were used according to the supplier's recommendations.

Results

Inhibition of β-Catenin Expression in Intestinal Adenomas using Antisense ODNs

A 20-base pair ODN capable of potent suppression of human β-catenin expression has been previously described.[20,21] The murine and human β-catenin genes are highly homologous; therefore, the design of the murine β-catenin antisense ODN involved targeting a similar region of the murine β-catenin mRNA sequence as in the human β-catenin antisense sequence. A novel 20-base phosphorothioate antisense oligodeoxynucleotide sequence capable of specifically and potently suppressing the expression of murine β-catenin in a dose-dependant manner was identified.

Dose-response experiments were carried out in Balb/c nude mice and consisted of daily intraperitoneal injections with either murine β-catenin AODN, murine β-catenin scrambled ODN, or Hank's balanced salt solution (HBSS). Systemic treatment of Balb/c mice with daily intraperitoneal injections of AODNs targeting β-catenin significantly reduced liver β-catenin protein levels as detected by Western blot (Fig. 1a) and immunohistochemistry (Fig. 1b,c,d). Reduction of β-catenin protein levels was most potent at a dose of 20 mg/kg. There was no effect on β-catenin expression with either dose of scrambled AODN or HBSS alone. Similar downregulation of both β-catenin mRNA and protein in intestinal adenomas was demonstrated in Min mice treated with a dose of 20 mg/kg (Fig. 2). There was no apparent toxicity of ODN treatment as all animals survived the duration of the study in apparent good health.

β-Catenin Antisense Inhibits Cellular Proliferation in Intestinal Adenomas

β-Catenin is known to stimulate the transcription of a number of target genes, many of which are involved in cellular proliferation.[20–26] Previous studies have demonstrated the ability of β-catenin antisense ODNs to decrease TCF transcriptional activity as well as downregulate known genetic targets of β-catenin such as c-myc and cyclin D1.[10,22] Systemic treatment of Min mice with β-catenin antisense ODNs resulted in a significant decrease in the number of adenoma cells in S-phase as detected by BrdU incorporation (Fig. 3a,b). Intestinal crypt cells demonstrated similar uptake of BrdU among groups treated with the β-catenin antisense ODN and the scrambled sequence when compared to the untreated controls. The calculated proliferative index for the β-catenin antisense ODN groups were significantly less than both the scrambled ODN and HBS-treated groups (Fig. 3c). While the proliferative index was reduced after 1 week of antisense treatment, polyp number was not reduced (data not shown).

β-Catenin Antisense Inhibits Intestinal Adenoma Formation in Min Mice

In order to evaluate effects of longer term β-catenin downregulation on adenoma development, Min mice were randomized and subjected to daily intraperitoneal injections of either β-catenin AODN, a scrambled sequence ODN or HBS alone for 6 weeks. As shown in Fig. 4, Min mice injected with the β-catenin AODN had significantly less intestinal adenoma formation when compared to both the scrambled ODN (Average 13.4 vs 33.9; $p<0.00003$) and HBS-treated groups (Average 13.4 vs 31.5; $p<0.00001$). There was no significant difference between the scrambled ODN and HBS-treated groups.

Discussion

There have been numerous published studies in Min mice using a variety of agents which demonstrate inhibition of

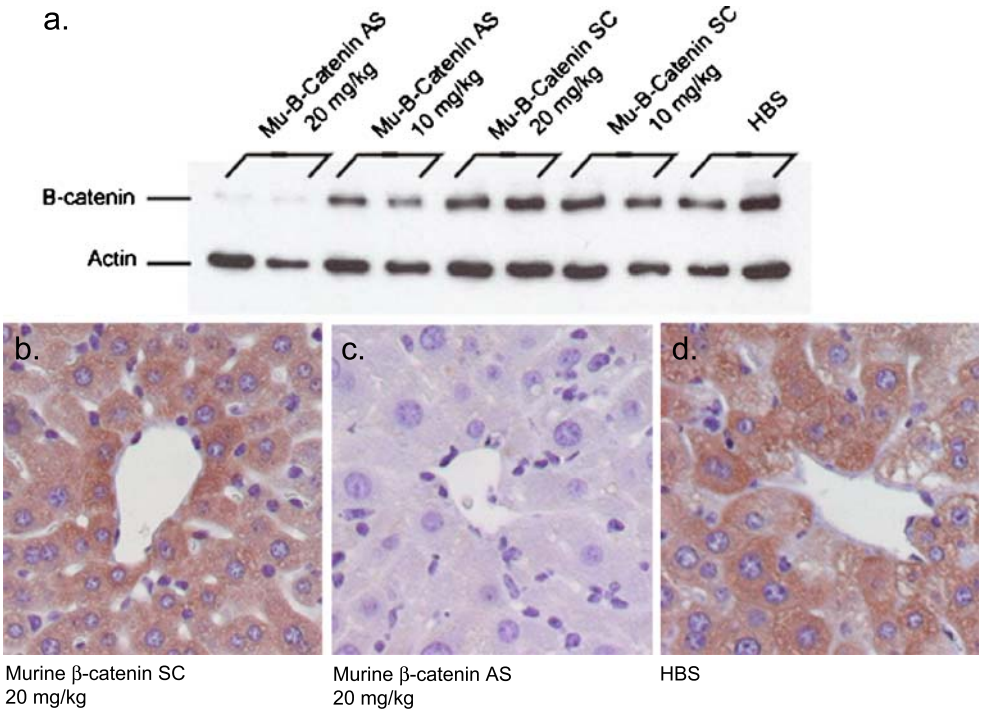

Figure 1 Effects of systemic administration of AODNs on β-catenin expression in Balb/c nude mice liver tissue by Western blot and immunohistochemistry. **a.** A novel 20-base pair murine β-catenin AODN administered at a dose of 20 mg/kg resulted in significant downregulation of β-catenin. Injection of either the scrambled ODN or HBS resulted in no change in β-catenin protein levels. **b**. 7-day treatment with a scrambled sequence ODN demonstrated no downregulation of β-catenin expression when compared to the HBS-treated group (**d**.). **c**. Treatment with murine β-catenin AODN resulted in significant downregulation of β-catenin expression.

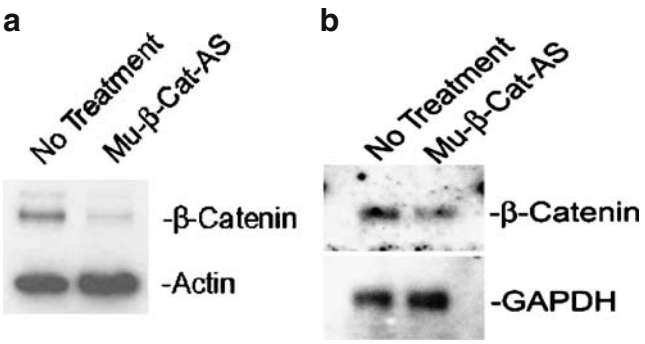

Figure 2 Downregulation of β-catenin expression in intestinal adenomas in Min mice following systemic administration of murine β-catenin AODN. Min mice were treated with daily i.p. injections of murine β-catenin AODN (20 mg/kg) or scrambled antisense ODN (20 mg/kg) for 6 weeks. **a**. β-Catenin protein levels were significantly reduced in the murine β-catenin AODN-treated group compared with the scrambled AODN-treated group. **b**. β-Catenin mRNA was significantly reduced in the murine β-catenin AODN-treated group compared with the scrambled AODN-treated group.

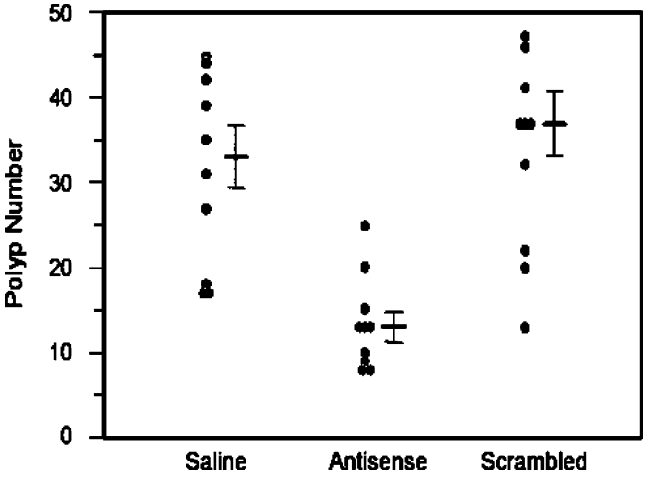

Figure 4 Min mice treated with murine β-catenin AODN over a 6-week period had significantly lower numbers of intestinal adenomas when compared to both scrambled ODN ($p<0.00003$) and HBS-treated groups ($p<0.00001$).

intestinal adenomas.[23] A number of these studies examined effects on β-catenin signaling, particularly with the use of nonsteroidal anti-inflammatory drugs (NSAIDs). However, NSAIDs have multiple targets including cox-2, NF-κB, PPARγ, PDK-1/Akt, and RSK-2/MAPKs, thereby making specific conclusions regarding the contribution of aberrant β-catenin expression to adenoma formation difficult.[32] None of the previously published chemoprevention studies have utilized direct targeting of β-catenin to evaluate its contribution to adenoma formation. The studies presented above have used AODNs to specifically downregulate β-catenin expression in APC-mutant mice and have shown that systemic administration of β-catenin AODNs results in specific and potent suppression of both β-catenin mRNA and protein levels in intestinal adenomas. Downregulation of β-catenin expression was accompanied by a significant decrease in cellular proliferation in intestinal adenomas as well as a significant reduction in the total number of intestinal adenomas. Collectively, these experiments provide evidence for the critical role of altered β-catenin signaling in the development of intestinal adenomas.

Previous studies utilizing β-catenin AODNs to inhibit the growth of APC-mutant colon cancer cells demonstrated these molecules to be sequence- and target-specific, providing evidence that the mechanism for growth inhibition in these cell lines is directly related to suppression of β-catenin signaling.[20,21] Further direct evidence of the importance of β-catenin in the growth and survival of APC

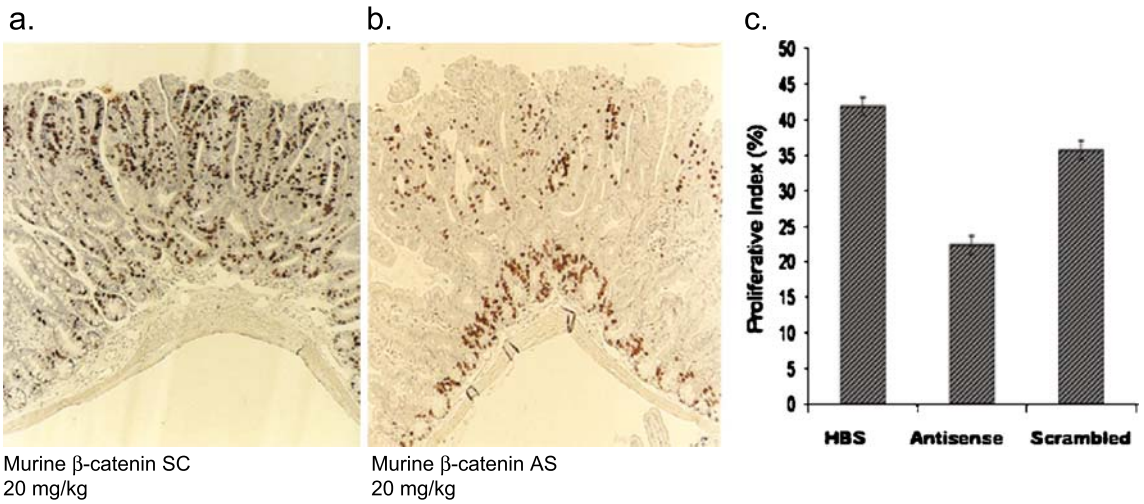

Figure 3 BrdU staining of intestinal adenomas in control and β-catenin AODN-treated mice. **a**. Increased uptake of BrdU is demonstrated in both the intestinal crypts and villi in mice treated with the scrambled AODN. **b**. The β-catenin AODN-treated group demonstrates similar uptake in the intestinal crypts with decreased uptake in the villi consistent with a decreased rate of proliferation in these epithelial cells. **c**. The calculated proliferative index for the BrdU-stained adenomas was significantly decreased in the β-catenin antisense ODN treatment group.

and β-catenin-mutant colon cancer cells was provided through studies demonstrating growth inhibition of colon cancer cells utilizing siRNA directed against β-catenin.[22] Together, these studies have provided a direct link between aberrant β-catenin expression and neoplastic growth.

The experiments presented above have demonstrated that target-specific suppression of β-catenin using AODNs results in significant inhibition of adenoma formation. It is likely that chemoprevention studies demonstrating greater inhibition of adenoma formation are, in part, due to a particular agent's ability to affect a diverse range of targets involved in cell growth and proliferation. It is also possible that AODNs may reflect less potency in downregulating β-catenin than other agents, particularly NSAIDs. Agents utilized clinically as chemopreventative agents may have toxicities linked to effects on targets other than β-catenin. Our data would support further development of potent, specific agents that target β-catenin for the chemoprevention of colon cancer.

It has become clear that altered β-catenin expression plays a significant role in the enhanced growth and survival of neoplastic cells. The results above indicate that agents designed to specifically target and potently antagonize activation of β-catenin signaling have efficacy in chemoprevention of intestinal adenoma formation. The diversity of genetic disruption involved in the initiation and progression of neoplasia suggests that the most efficacious therapies will be those that target multiple defective pathways. Further study of agents which have the ability to inhibit β-catenin signaling is warranted to determine both the molecular mechanism and advance the design of targeted therapeutics.

Conclusion

Targeted suppression of β-catenin inhibits intestinal adenoma formation in APC-mutant mice. Molecular targeting of β-catenin holds significant promise as a chemopreventative strategy in colon cancer.

Acknowledgements This work was supported by National Institutes of Health grant RO1 CA100189 and a Clinician Scientist Award in Translational Research from the Burroughs Wellcome Fund (to JAD).

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Laparoscopic Repair of a Giant Hiatus Hernia—How I Do It

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Abstract The laparoscopic approach is now the technique of choice for the repair of large hiatus hernia. It is associated with a low risk of complications. However, controversy exists as to the optimal technique for laparoscopic repair. In this paper, we describe our approach. This entails full dissection of the hernia sac from the mediastinum, hiatal repair with posteriorly placed sutures, and then construction of an appropriate fundoplication. Whether the use of mesh for hiatal repair will reduce the risk of subsequent reintervention and not add any new risks is, however, unclear. For this reason, we believe that the mesh should only be used in appropriately designed clinical trials, and for now, the standard approach to laparoscopic repair of a large hiatus hernia is sutured repair.

Keywords Laparoscopic surgery · Hiatus hernia · Paraesophageal hernia · Esophagus · Fundoplication

Introduction

Soon after introduction of the laparoscopic Nissen fundoplication, surgeons began to report experience with laparoscopic repair of large hiatus hernias, and this approach is now considered to be the technique of choice for the repair of large hiatus hernia.[1] This has led to low morbidity and mortality rates, even in the older patients. However, there is still a significant risk of recurrent, although frequently asymptomatic, herniation at longer-term follow-up. Hence, controversy exists regarding what is the optimal technique for laparoscopic repair of a large hiatus hernia. In this paper, we describe our approach to the laparoscopic repair of large hiatus hernias and discuss some alternative techniques and new developments.

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Surgical Procedure

Preoperative Workup

A careful history of the patients' symptoms is taken, with emphasis on reflux, regurgitation, dysphagia, and (postprandial) chest pain. In the elective setting, we routinely perform a barium-swallow X-ray and upper gastrointestinal endoscopy to delineate the relevant anatomy, the degree, if present, of gastroesophageal reflux, and other pathology. Further assessment with esophageal manometry or pH monitoring is not routine as we usually add an anterior 90° partial fundoplication to the repair (discussed later). However, if a Nissen fundoplication is to be added to repair of a large hiatus hernia, esophageal manometry and pH monitoring should be undertaken to confirm reflux and adequate esophageal body peristalsis.

Theater Setup

A nasogastric tube is not routinely passed, although it can be placed temporarily intraoperatively, as needed, to decompress the stomach if it is found to be full of gas at laparoscopy. The patient is positioned in the lithotomy position with the legs extended in stirrups (French position) and 20 to 30° head up (reverse Trendelenburg). The video monitor is placed at the patient's eye level and in line with

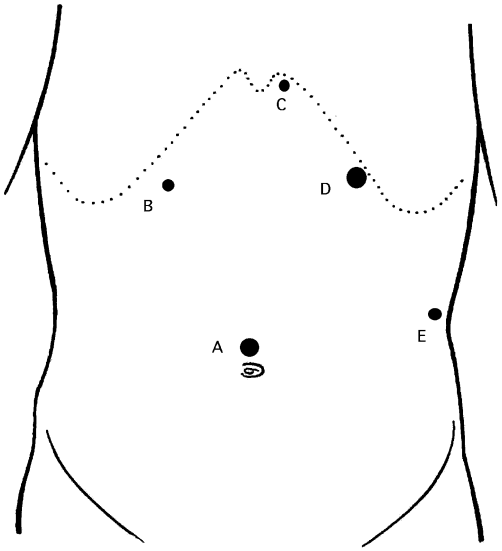

Figure 1 Port placement: *A*, camera port; *B*, *D*, surgeon's operating ports; *C*, liver retractor insertion site; *E*, port for assistant's retractor.

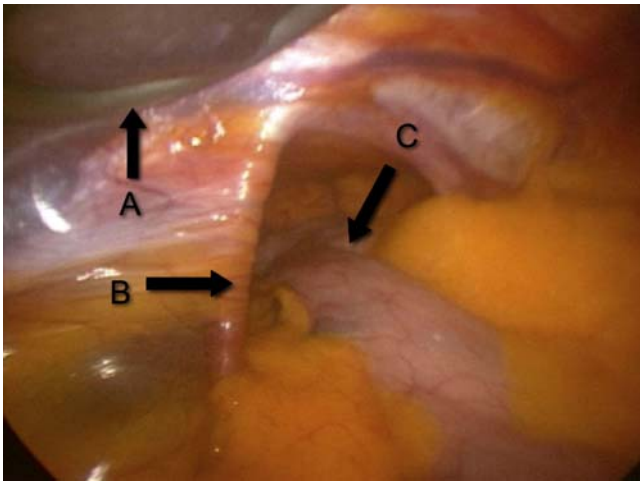

Figure 3 The initial laparoscopic view—a large paraesophageal hernia with more than 50% of the stomach in the chest via a large hiatal defect is seen. The apex of the liver retractor (*A*) is seen lifting the liver anteriorly. *B*, The hiatal rim; *C*, intrathoracic component of the stomach.

the operating surgeon who stands between the legs of the patient. The surgeon's assistant stands at the patient's left side.

The instrumentation used is fairly simple. We use two 11-mm and two 5-mm trocars (port placement is shown in Fig. 1). Instrumentation consists of two atraumatic grasping instruments, a diathermy hook, and a needle holder for the operating surgeon. A toothed grasping instrument is used initially by the assistant surgeon and an atraumatic grasping instrument later in the procedure. The assistant also controls a 30° laparoscope, and a pair of scissors is used for cutting sutures. The most important instrument is the Nathanson liver retractor (Figs. 2 and 3—Cook Medical Technology, Eight Mile Plains, Queensland, Australia). This provides a safe, stable elevation of the liver. We do not use a harmonic scalpel or similar technology.

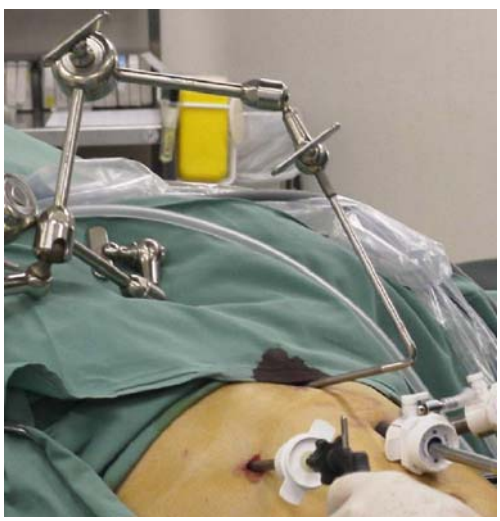

Figure 2 External view of liver retractor held in position by an "iron intern."

Operative Technique

An 11-mm port is introduced immediately supraumbilically using an open insertion technique (Fig. 1), and pneumoperitoneum is established. The liver retractor is introduced via a 5-mm stab wound, which is placed as high as possible in the angle between the xiphoid and the apex of the left costal margin, to the left side of the falciform ligament. With this device, the left lobe of the liver is retracted upward and slightly to the right, to expose the hiatal defect. Good exposure is usually obtained even in patients with a fatty liver. Three further ports are placed next: a 5-mm port immediately subcostal in the right midclavicular line, an 11-mm trocar immediately subcostal in the left midclavicular line, and a 5-mm port in the anterior left axillary line approximately 3 to 4 cm below the costal margin.

The size of the hernia and the contents of the hernia sac are first inspected (Fig. 3). However, no attempt is made to reduce the contents of the hernia, as this is usually not feasible in very large hernias until the hernia sac has been fully dissected. This is because the upper part of the stomach is incorporated into the posterior wall of the sac. Hence, the first part of the operation should be to fully dissect the hernia sac from the mediastinum. As this is undertaken, the contents (stomach and sometimes bowel) progressively reduce into the abdomen, and a sufficient intra-abdominal length of the esophagus is usually evident. We have only rarely encountered a short esophagus (two patients in nearly 400 laparoscopic procedures), and we

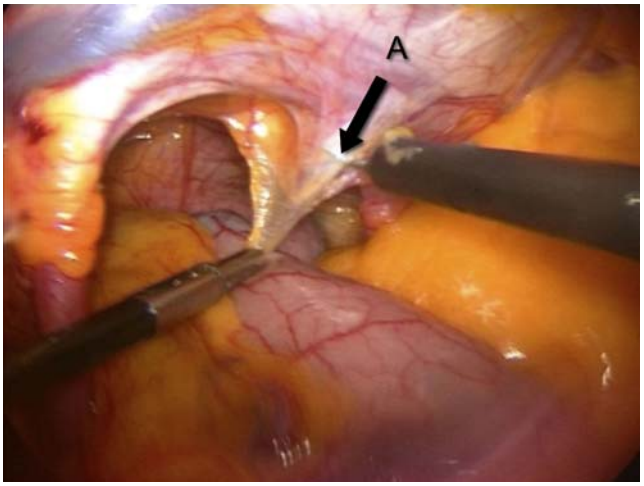

Figure 4 Division of the first layer of the hernia sac (peritoneum), commencing anterolaterally on the left side, close to and just inside the hiatal rim (*A*)

would not undertake an esophageal lengthening procedure (Collis gastroplasty) at an initial operation.

The first step is to divide the lesser omentum to expose the right hiatal pillar within the lesser sac. During this maneuver, the hepatic branch of the vagus nerve is usually divided, although occasionally, this nerve can be spared. If an aberrant left hepatic artery is encountered, this can be divided between clips or spared as necessary. Next, we commence dissection of the sac at its neck by dividing the layers of the hernia sac, close to but 0.5–1 cm inside the hiatal rim. Our preference is to commence this dissection anterolaterally on the left side of the hiatus (Fig. 4). Two layers need to be divided to enter the correct plane. The first of these is the peritoneum, and beneath this is a fascial layer composed of attenuated phreno-esophageal ligament. Once the correct plane is entered, the assistant uses a toothed grasping instrument to pull the cut edge of the hernia sac into the abdomen.

The plane of dissection is then extended across the front of the hiatus toward the right pillar and posteriorly along the left pillar. It is important for the assistant to pull firmly down on the sac as this maneuver gradually reduces the sac contents. The retraction is toward the right lower abdomen when dissecting the left pillar and to the left lower side when dissecting the right pillar. When dividing the sac from the hiatal rim, the dissection must be maintained 0.5 to 1 cm inside the hiatal rim to avoid excising the fascial coverings, which protect the muscle fibers at the hiatal rim (Fig. 5).

Dissection alternates between the left and right sides of the hiatus, and the sac is gradually reduced into the abdomen (Fig. 6). At this time, the hernia sac is dissected in the mediastinum using predominantly blunt dissection, although the diathermy hook is used if small blood vessels are encountered. If dissection is in the correct plane, separation of the sac from the mediastinum is usually very easy and bloodless. It is also mandatory to dissect any posterior sac (which is often present) from the mediastinum and to fully expose behind the esophagus. Next, an atraumatic grasping instrument held in the surgeon's left hand is passed from right to left behind the esophagus. We then pass a long linen tape through the 11-mm left upper abdominal port to this instrument, pull the tape behind the esophagus, pass it back to the instrument passing through the left upper abdominal port, and remove both ends of the tape through the left upper abdominal port. This port is removed over the tape and then resited so that the two ends of the tape pass through the wound, adjacent but not through the left upper abdominal port. The ends of the tape are secured at the level of the skin with a clamp. Traction

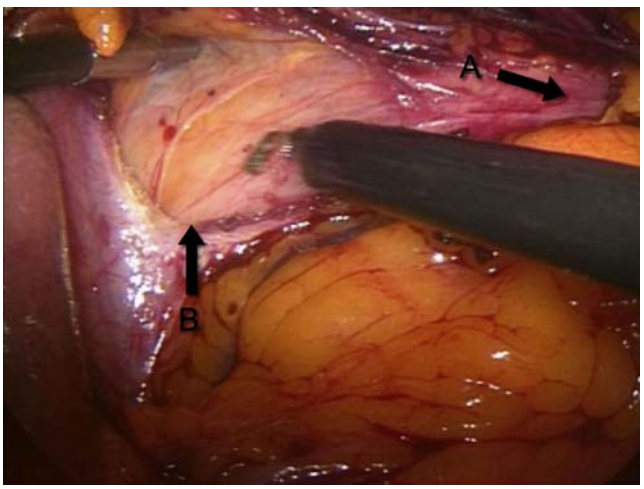

Figure 5 Once the correct plane is entered, the assistant pulls the edge of the sac (*A*) into the abdomen, and the plane of dissection is extended across the front of the hiatus toward the right pillar (*B*).

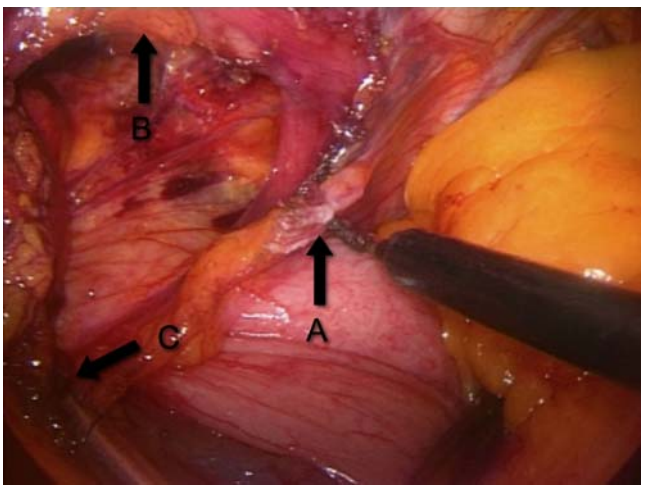

Figure 6 Dissection continues down the left pillar close to the stomach (*A*) as shown (*B*, apex of esophageal hiatus; *C*, edge of sac retracted to right side).

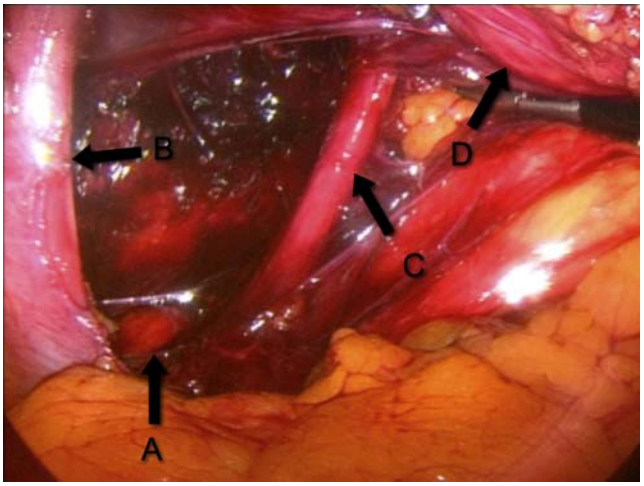

Figure 7 Dissection of the right and left pillar behind the esophagus is continued posteriorly to the decussation (*A*), and a "window" is developed behind the esophagus (*B*, right pillar; *C*, left pillar; *D*, posterior wall of the esophagus).

can then be applied to the tape, either from outside the abdomen or using a grasping instrument inside the abdomen. The esophagus can then be elevated anteriorly and retracted from the mediastinum, and this provides a better view of the hiatus, facilitating further dissection of the posterior aspects of the hernia sac to the base of the right and left pillars and behind the esophagus.

Any posterior component of the hernia sac must now be fully mobilized and brought intra-abdominally. This can be very large in some patients. A window is then created behind the esophagus exposing the left hiatal pillar from the right side (Fig. 7). It is important to undertake sufficient dissection to enable the posterior confluence of the hiatal pillars to be fully identified and for no hernia sac to lie anterior to this. In the process of posterior dissection, we routinely dissect the posterior vagal nerve away from the esophagus, so that it lies close to the confluence of the pillars.

The hiatus is next repaired with sutures. The left and right pillars are approximated with three or more interrupted figures of eight stitches (nonresorbable; monofilament sutures) commencing posteriorly and working anteriorly in 5-mm steps, until the hiatus is reduced to a diameter of approximately 30 mm (Fig. 8). If the repair appears to be under excessive tension, additional sutures can be placed in the anterior hiatus. We have always been able to obtain a satisfactory repair, although we are careful to preserve the fascial coverings over the left and right pillars. An intraesophageal bougie is not usually used to calibrate the hiatal repair, and we only use a bougie if a Nissen fundoplication is added to the procedure.

Once the hiatus is adequately narrowed, we add either an anterior 90° partial fundoplication, an anterior 180° fundoplication, or a Nissen fundoplication to the procedure. If reflux symptoms are the main indication for surgery, we perform an anterior 180° or a Nissen fundoplication, with the final choice based on preoperative esophageal motility testing and patient preference. If mechanical symptoms such as dysphagia or chest pain predominate, then we usually add an anterior 90° partial fundoplication, as this provides additional stability to the hernia repair and adds an antireflux effect but with minimal side effects. The details of the partial fundoplication procedures have been described in detail elsewhere.[2,3]

The anterior 90° partial fundoplication commences with an esophagopexy suture between the right posterolateral aspect of the distal esophagus 2 cm above the esophagogastric junction and the right hiatal pillar near the most anterior hiatal repair suture. Next, the gastric fundus is

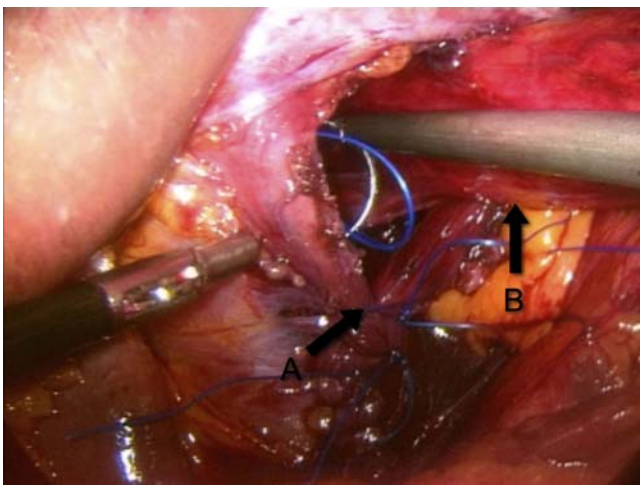

Figure 8 The hiatus is repaired with a series of interrupted sutures (*A*, hiatal repair sutures, *B*, esophagus).

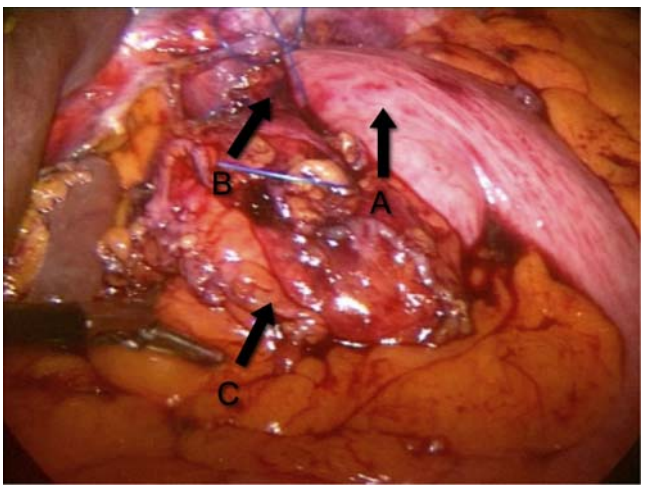

Figure 9 Completed anterior 90° partial fundoplication. *A*, Fundoplication covering left anterolateral esophagus; *B*, right anterolateral esophageal wall; *C*, empty hernia sac, reduced from mediastinum.

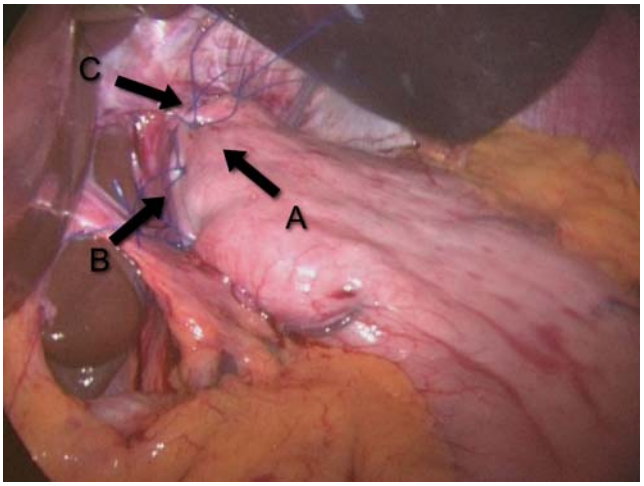

Figure 10 Completed anterior 180° partial fundoplication (*A*, fundoplication covering anterior esophagus; *B*, fundus sutured to right hiatal pillar; *C*, fundus sutured to apex of hiatus).

approximated to the left side of the esophagus using two sutures to accentuate the angle of His. The uppermost of these sutures also incorporates the left hiatal pillar.

The gastric fundus is then sutured loosely over the front of the esophagus, and this suture incorporates the apex of the hiatus and the anterior esophagus. Finally, the inferior edge of the fundal fold lying in front of the abdominal esophagus is sutured to the anterior esophagus to produce a loose fundoplication, which covers the left antero-lateral side of the esophagus (Fig. 9). It also provides three points of fixation between the esophagus and the diaphragm.

For a laparoscopic anterior 180° partial fundoplication,[3] the anterior wall of the gastric fundus is sutured to the right lateral wall of the distal esophagus and to the right hiatal pillar (with three interrupted stitches). The fundus is also sutured to the apex of the esophageal hiatus with two additional interrupted stitches (Fig. 10). If a Nissen fundoplication is added, we construct a loose 360° fundoplication using the anterior wall of the fundus of the stomach.[4] The wrap is calibrated using a 52-Fr bougie, and the short gastric vessels are not divided. The fundoplication is not sutured to the diaphragm.

Our standard approach to hiatal repair does not include reinforcement with mesh. Repair with a range of different techniques and mesh types have been described. However, there is little standardization of practice in this area. Hence, our current practice is to reinforce the hiatal repair with mesh only within the context of a randomized trial, which compares hiatal repair with sutures, with sutures reinforced with an on-lay resorbable or nonresorbable mesh. If mesh is added, we first repair the hiatus with sutures as described above. We then place a 3×5-cm piece of mesh across the hiatus repair, away from the esophageal wall, and secure this in place using either sutures or a hernia tacker (Fig. 11). The mesh is placed loosely to reinforce the posterior sutures, and the mesh is not placed around the anterior or lateral aspects of the hiatal rim.

Intraoperative Complications

Bleeding from the liver can occur because of local trauma from the liver retractor or direct surgical trauma. Bleeding from the liver is usually minor and has almost never required direct intervention. The Nathanson liver retractor also minimizes this problem. Bleeding from the short gastric vessels can occur with division of the vessels when creating a Nissen fundoplication. We no longer divide these vessels.[5]

The inability to reduce a large hiatus hernia, dense adhesions, and iatrogenic perforations of the esophagus or stomach can be other reasons for open surgery. Perforation of the esophagus or stomach has been described in several series.[6,7] This can occur with direct damage to the wall of the esophagus during dissection. In general, we avoid using ultrasonic shears or electrocautery when dissecting the esophageal wall and prefer a blunt dissection approach to minimize this risk. Another cause of esophageal perforation is injury when passing a bougie. We usually avoid using a bougie when undertaking repair of a large hiatus hernia, as it is not necessary when adding an anterior partial fundoplication. We do, however, use a bougie when constructing a Nissen fundoplication, and great care should be taken when passing the bougie in these patients.

Intraoperative pneumothoraces are documented in approximately 3–5% of patients. This is usually due to direct injury to the pleura when dissecting the hiatal sac from the mediastinum, and injury of the left pleural membrane is more likely than injury to the other side. Pneumothorax usually causes a few intraoperative problems, and the CO_2

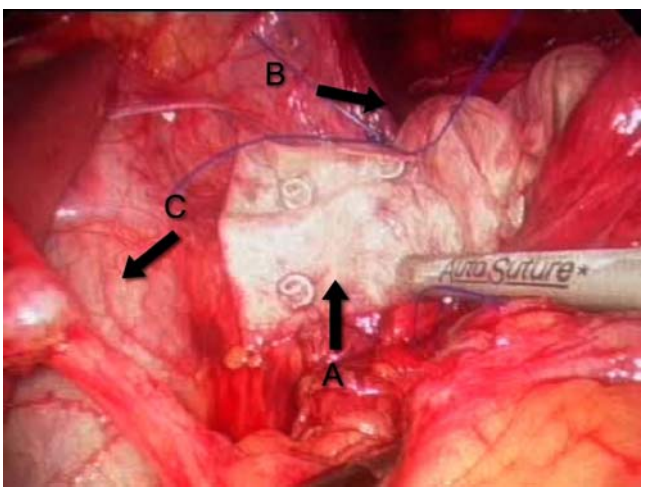

Figure 11 Posterior placement of absorbable mesh (*A*). The mesh is fixed to the hiatal repair with a hernia tacker (*B*, most anterior hiatal repair suture; *C*, inferior vena cava).

gas in the pleural cavity resolves spontaneously without the need for further intervention in most patients.[8]

Postoperative Care

A nasogastric tube is avoided. Thromboembolism prophylaxis is routinely used. Antiemetics are routinely given intraoperatively to minimize the risk of early postoperative retching and vomiting. Long-acting local anesthetic is injected at all wound sites, and opioid analgesics are avoided if possible. We routinely arrange a barium-swallow X-ray on the first day after surgery. If any early problems are identified (such as acute reherniation), early identification facilitates early repair in the first few postoperative days. The X-ray also provides a baseline for comparison should any future problems arise.

Patients are allowed to drink liquids immediately after the operation, and a vitamized/pureed diet is usually commenced on the first postoperative day. Discharge is usually on the first or second postoperative day. The pureed diet is continued for 4 weeks until the first outpatient visit, and after this, a normal diet is gradually resumed as tolerated. Mild symptoms of dysphagia are treated conservatively, and these usually resolve by 6 to 8 weeks.

Outcome

There are now several published studies that report the outcome of laparoscopic repair of large paraesophageal hernias. Anatomical recurrence rates, as judged by radiologic examination, range from 5 to 40%.[6] Clinically significant recurrence, i.e., anatomical recurrence with symptoms requiring reintervention, is much less common, as many patients with an anatomical recurrence are asymptomatic, and the presence of symptoms does not predict the presence of anatomical recurrence.[6,8] Overall satisfaction with laparoscopic repair of large hiatus hernia is high with 80–90% of patients reporting the outcome of their operation to be good or excellent.[6,9] Quality of life is also improved significantly after operation.[10]

Whether the use of mesh to reinforce the hiatal repair or to obtain a tension-free repair will reduce the recurrence rate and symptomatic outcome is still unclear. Three randomized controlled trials have compared the results of procedures with mesh and with no mesh.[11–13] Although the results of these trials appear to be promising, follow-up remains short term, and the primary outcome measure in all studies was the radiological appearance, not the clinical outcome.

It remains to be seen as to whether reinforcement of hiatus hernia repair with mesh will reduce the risk of later reintervention and at the same time not add any new risks or complications. Until this is clear, we believe that mesh should only be used in appropriately designed clinical trials. For now, the standard approach to laparoscopic repair of very large hiatus hernia is sutured repair, and the overall clinical outcome for this approach is very good in most patients.

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Renal Cell Carcinoma Metastatic to the Duodenum: Treatment by Classic Pancreaticoduodenectomy and Review of the Literature

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Abstract Renal cell cancer (RCC) most commonly metastasizes to the lungs, bones, liver, renal fossa, and brain, although metastases can occur elsewhere. RCC metastatic to the duodenum is especially rare, with only a small number of cases reported in the literature. Herein, we describe a case of an 86-year-old woman with a history of RCC treated by radical nephrectomy 13 years previously. The patient presented with duodenal obstruction and anemia from a solitary duodenal mass invading into the pancreas and was treated via classic pancreaticoduodenectomy. Preoperative imaging and intraoperative assessment showed no evidence of other disease. Pathology confirmed metastatic RCC without lymph node involvement. Our case report and review of the English language literature underscore the rarity of this entity and support aggressive surgical treatment in such patients.

Keywords Renal cell carcinoma · Pancreaticoduodenectomy · Metastases · Duodenum

Introduction

Renal cell carcinoma (RCC) commonly metastasizes to sites such as lung, lymph nodes, liver, pancreas, bone, brain, contralateral kidney, and adrenal glands.[1] Autopsy studies have shown that metastatic disease of any type to the small intestine is unusual and accounts for only 1–2% of all metastases. Approximately 7% of small bowel metastatic lesions is of renal cell origin.[2] RCC metastatic to the pancreas has been frequently reported.[3–5] Solitary metastasis from RCC to the duodenum is exceedingly rare. Commonly, renal cell metastases present many years after initial treatment, with recurrences reported up to 17.5 years after initial surgery.[6] Most cases of duodenal metastasis from RCC present with upper gastrointestinal bleeding or obstructive symptoms, and sequelae may include anemia, melena, fatigue, and early satiety. Several treatments of solitary RCC metastasis have been reported. These include a variety of surgical and interventional therapy options that have been shown to provide effective survival benefits. Herein, we report a case of an 86 year-old woman with metastatic RCC confined to the duodenum, with successful complete resection by classic pancreaticoduodenectomy.

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Clinical Material

An 86-year-old woman with a past medical history notable for hypertension and RCC, 13 years status, post-right radical nephrectomy, presented to her primary care physician with fatigue. When found to be anemic, she was treated with iron supplementation. Several months later, she continued to have fatigue, anorexia, early satiety, and weight loss of approximately 6 lb and was found to have heme-positive stools. Jaundice and pancreatitis were absent. The patient underwent a capsule endoscopy; however, the capsule never left the stomach. The patient then had an esophagogastroduodeno-

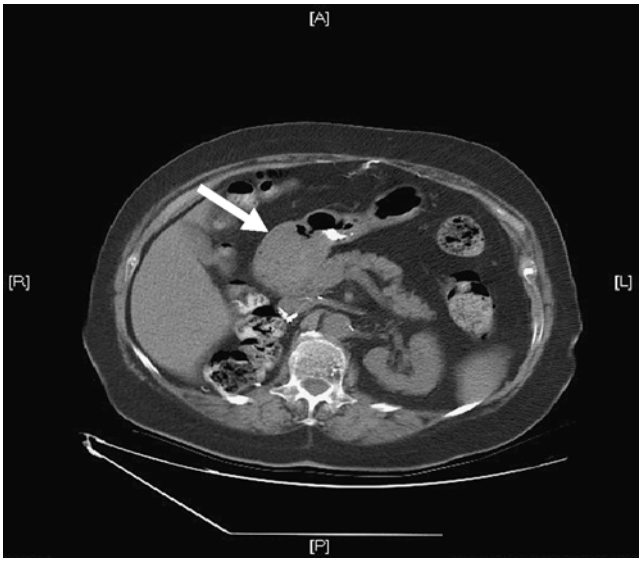

Figure 1 Non-intravenous contrast CT image indicating (*white arrow*) large soft tissue structure at level of the gastric antrum and duodenal bulb (note prior right nephrectomy with absence of the right kidney).

scopy, which showed an ulcerated mass involving the distal antrum and proximal duodenum. Biopsy of the ulcerated mass was consistent with metastatic RCC. An abdominal computed tomography (CT) scan (Fig. 1) showed a large soft tissue mass at the level of the gastric antrum and duodenal bulb, no evidence of liver metastasis, and her visceral vessels appeared normal. She was scheduled for surgical resection and received perioperative prophylactic antibiotic, subcutaneous heparin, and sequential compression devices.

At the time of operation, exploration of the duodenum and head of the pancreas revealed a mass clearly located in the proximal duodenum with involvement of the head of the pancreas and distal antrum. There was no evidence of malignant ascites, carcinomatosis, omental implants, or involvement of the liver. Attempts were made to dissect the

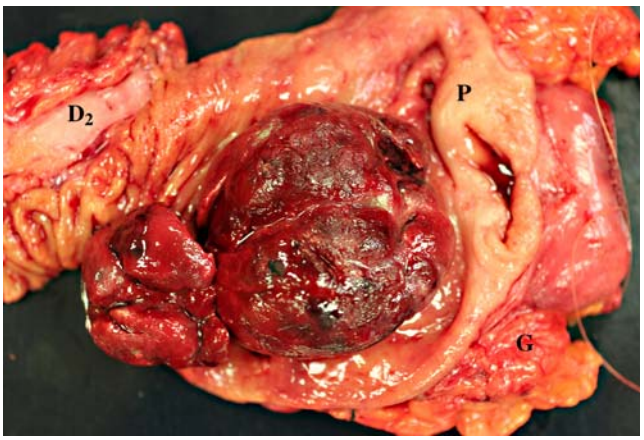

Figure 2 Gross specimen revealing a 9×5.5×3-cm tan-red polypoid, pedunculated tumor protruding into the duodenum. The distal gastric specimen (*G*) is to the right, the second portion of the duodenum is to the left (D_2), and the pylorus (*P*) is noted.

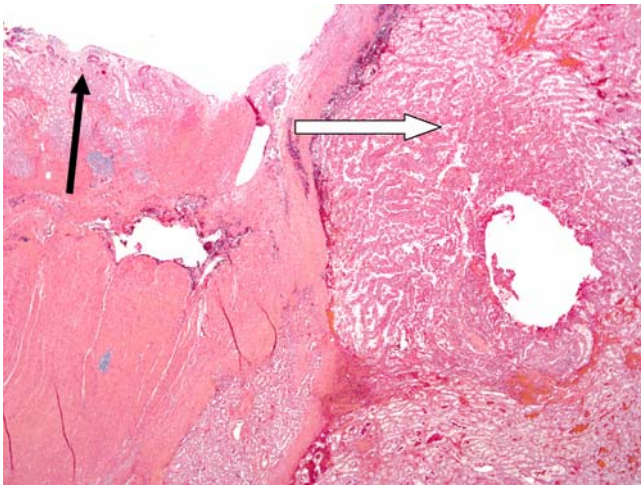

Figure 3 Duodenal wall with Brunner's glands (*black arrow*) and invading tumor (*white arrow*; hematoxylin and eosin, ×100 magnification).

tumor free of the head of the pancreas; however, the duodenal mass was adherent to and invading into the pancreas. A classic Whipple procedure was performed, and the resultant specimen was sent for pathologic evaluation. Postoperatively, the patient's hospital course was complicated by hypoxemia and bilobar pulmonary emboli treated with appropriate anticoagulation. Ultimately, the patient was discharged to a rehabilitation facility on postoperative day 16 in stable condition, doing well. In follow-up, the patient is leading an active life and continues to do well, now 7 months after resection, without evidence of further metastatic disease.

Pathology

Gross examination of the classic pancreaticoduodenectomy specimen revealed a 9×5.5×3-cm tan-red polypoid, pedunculated tumor protruding into the duodenum (Fig. 2). The

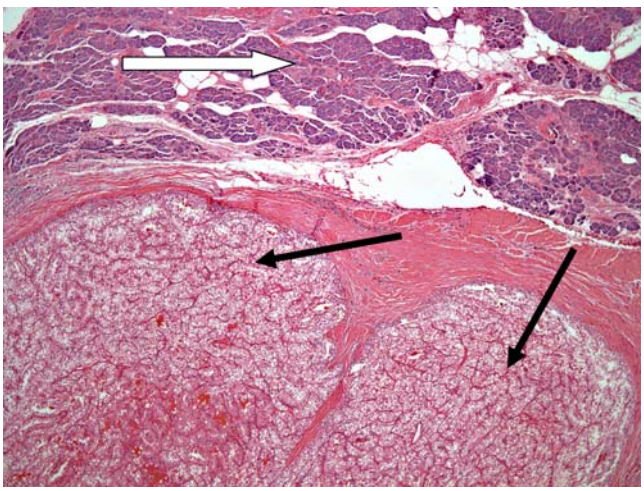

Figure 4 Metastatic renal cell carcinoma (*black arrows*) within pancreas (*white arrow*) with reactive fibrosis (hematoxylin and eosin, ×100 magnification).

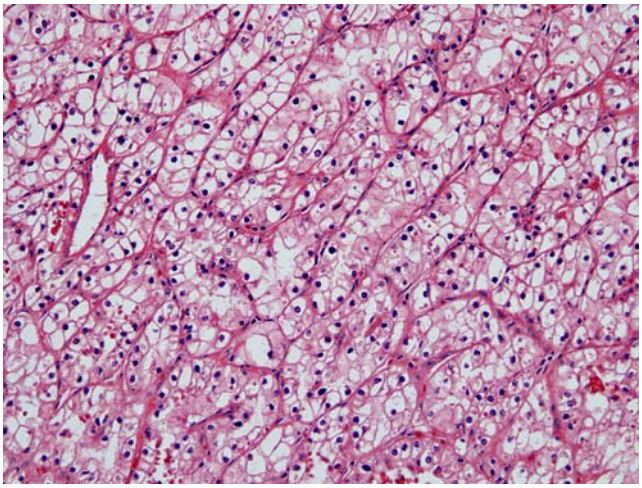

Figure 5 The tumor was composed of clear cells arranged in trabecular and alveolar patterns. The tumor cells had centrally located small nuclei with inconspicuous nucleoli, and were surrounded by a prominent vascular network (hematoxylin and eosin, ×400 magnification).

tumor involved the full thickness of duodenal wall and invaded into the pancreatic head (Figs. 3 and 4). Microscopically, the tumor was composed of clear cells arranged in a trabecular and alveolar pattern. The tumor cells had centrally located small nuclei with inconspicuous nucleoli and were surrounded by a prominent vascular network (Fig. 5). Eight lymph nodes were identified in the resection specimen, and all were negative for metastatic tumor.

Discussion

Metastatic RCC to the duodenum is extremely rare. For the purposes of this report, we have included patients with the bulk of the gross tumor in the duodenal or ampullary regions, distinct from pancreatic involvement. Our review of the English language literature (using keywords including: RCC, solitary, metastasis, duodenum and ampulla) yielded 16 reports of solitary duodenal/ampullary renal cell metastases (Table 1). The mean time period from nephrectomy to

Table 1 Published Case Reports of Solitary Renal Cell Carcinoma Metastatic to Duodenum/Ampulla

Reference	Year	Age	Sex	Years post-nephrectomy	Presentation	Location of Metastasis	Operation/ procedure	Lymph node Status	Survival (months)
Adamo et al.	(this article)	86	F	13	Anemia, early satiety	Duodenum	Classic Whipple	0/8	7
Chang et al.[7]	2004	63	F	9	GI bleeding	Duodenum	Radical subtotal gastrectomy	0/6	10
Loualidi et al.[8]	2004	76	M	5	GI bleeding	Duodenum	Palliative radiotherapy	N/A	N/A
Nabi et al.[9]	2001	40	M	4	Epigastric pain, obstruction with bilious vomiting	Duodenum	Proximal gastrojejunal bypass	N/A	Died 7 days post-op of sepsis
Sohn et al.[4]	2001	N/A	N/A	6	N/A	Periampullary	Classic Whipple	0	22
Le Borgne et al.[3]	2000	48	M	13	GI bleeding	Duodenum	Classic Whipple / SMV resection	0	53
"	2000	72	F	7	GI bleeding	Duodenum	Classic Whipple	0	18
Janzen et al.[6]	1998	75	M	17.5	GI bleeding	Ampulla	Total pancreatectomy, duodenectomy	N/A	N/A
Leslie et al.[14]	1996	78	F	10	Abdominal discomfort, pruritis, GI bleeding, weight loss	Ampulla	PPPD	0	30
Leslie et al.[14]	1996	53	M	8	GI bleeding, weight loss	Ampulla	PPPD	0/5	78
Toh et al.[11]	1996	59	F	10	Abdominal pain, anorexia	Duodenum	Duodenotomy, excision of mass	N/A	N/A
Freedman et al.[10]	1992	65	M	12	GI bleeding, fatigue	Duodenum	Classic Whipple	N/A	66
Venu et al.[15]	1991	64	M	11	GI bleeding, fatigue	Ampulla	N/A	N/A	Died massive PE
Lynch-Nyhan et al.[12]	1987	16	M	1	GI bleeding	Duodenum	Embolization	N/A	6
Lynch-Nyhan et al.[12]	1987	61	M	6	Jaundice	Duodenum	Embolization	N/A	N/A
Lawson et al.[13]	1966	69	F	0	GI bleeding	Duodenum	Classic Whipple	N/A	N/A

GI Gastrointestinal; *SMV* superior mesenteric vein; *PPPD* pylorus preserving pancreaticoduodenectomy; *N/A* not available, *PE* pulmonary embolism
[a] All survivals reported at time of respective report publishing

diagnosis of these solitary metastases was 8 years, with our patient having a 13-year interval between initial resection and recurrence. The most common presentation of these patients was gastrointestinal bleeding (69%), whereas a minority presented with pain, jaundice, or early satiety.

RCC can spread via lymphatic or hematogenous spread, as well as by direct invasion into adjacent anatomic structures.[7] The pancreas is an unusual site of metastatic disease for other tumors, but it is common in RCC. The duodenum is a particularly rare site of metastasis in RCC, which is perhaps counterintuitive given its retroperitoneal proximity to the right kidney.

The natural history of RCC is unpredictable. Disease eradication and cure are possible after nephrectomy; however, there is also the possibility of a long period of disease latency, followed by recurrence of metastatic disease at unsuspected anatomic locations.[1] As such, it is imperative to remain vigilant in post-nephrectomy patients upon presentation of new clinical symptoms. When metastatic RCC involves the duodenum, it most commonly is located in the periampullary region or the duodenal bulb.[8] The presenting symptoms in these rare cases typically include gastrointestinal bleeding, anemia, fatigue, and obstruction with early satiety or jaundice. Our patient initially presented with fatigue and was found to be anemic with heme-positive stools. Any patient with gastrointestinal symptoms and a history of RCC should undergo full diagnostic work-up with both radiologic and endoscopic evaluation.[9] Endoscopy allows for biopsy of suspicious lesions and is mandatory for this subset of patients.

Surgical treatment of solitary RCC metastasis has been shown to improve survival.[3–5,10,11] Therapeutic goals include complete metastatectomy whenever surgically feasible. Several case series have been reported of successful surgical treatment of solitary pancreatic RCC metastases[3,4]; however, few isolated reports of excision of duodenal or ampullary metastases exist in the literature. Procedures ranging from classic pancreaticoduodenectomy (Whipple procedure) to interventional embolization have been reported (Table 1). In patients with massive gastrointestinal bleeding secondary to metastatic duodenal RCC, arteriography with embolization of the gastroduodenal artery has been described.[12] Our patient had isolated proximal duodenal involvement with adherence to the head of the pancreas making it impossible to separate the duodenal/pancreatic border. Thus, given the involvement of the mass with the proximal duodenum, a classic pancreaticoduodenectomy was performed, with successful excision of all involved structures with clear margins and with no lymph node involvement. In fact, all resection specimens from all reviewed case reports have revealed no lymph node involvement (Table 1).

In summary, solitary metastatic RCC to the duodenum is extremely rare. Appropriate awareness and aggressive work-up of gastrointestinal symptoms in patients post-nephrectomy for RCC are of paramount importance. In this subgroup of patients with symptoms of gastrointestinal bleeding, fatigue, anemia, and early satiety or obstruction, all should undergo complete endoscopic evaluation, as well as radiologic investigation to evaluate for the presence of and the extent of metastatic disease. Any patient with solitary metastatic RCC to the duodenum should be considered a candidate for complete surgical excision if medically and technically feasible, both for palliation of symptoms and because it provides the opportunity for meaningful disease free survival.

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Small Bowel Volvulus Diagnosed by the CT "Whirl Sign"

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Abstract A 24-year-old man presented to the emergency department with acute onset, colicky, abdominal pain. A CT scan showed the "whirl sign" diagnostic of small bowel volvulus. Diagnosis of a small bowel volvulus can be challenging, and CT scan is the imaging modality of choice.

Keywords Whirl sign · Small bowel volvulus · Acute abdomen

A 24-year-old man with no previous medical history presented to the emergency department with acute onset, colicky, abdominal pain. On physical examination, he had normal vital signs and no raise in temperature. He suffered from extreme pain with marked peri-umbilical tenderness, but there was no rebound tenderness or guarding. Laboratory findings were normal. Plain abdominal X-rays showed some dilated loops of small bowel. A computed tomography (CT) scan was performed showing a "whirl sign" diagnostic of small bowel volvulus (Fig. 1). Laparotomy was performed confirming the diagnosis of a volvulus of the entire small bowel around the superior mesenteric artery (Fig. 2). No malrotation or adhesions were found. There was a mobile cecum which was in the normal right lower-quadrant after derotation of the bowel. The bowel appeared viable.

Small bowel volvulus is a rare condition where a loop of small bowel is twisted around the axis of its own mesentery causing small bowel obstruction. This can cause torsion and occlusion of the vasculature leading to ischemia and necrosis.[1]

Primary or idiopathic volvulus occurs when there is no predisposing anatomical abnormality as was the case in our patient. Although rare in North America and Western Europe, the incidence of primary small bowel volvulus is five to ten times higher in Africa, Asia, the Middle East, and India. This is believed to be caused by the ingestion of large volumes of fiber-rich food after long periods of fasting. Secondary volvulus is caused by anatomical anomalies such as postoperative adhesions or malrotation. In children, malrotation is much more common than in adults, but in adults with known malrotation, small bowel volvulus is the leading cause of small bowel obstruction.[2,3]

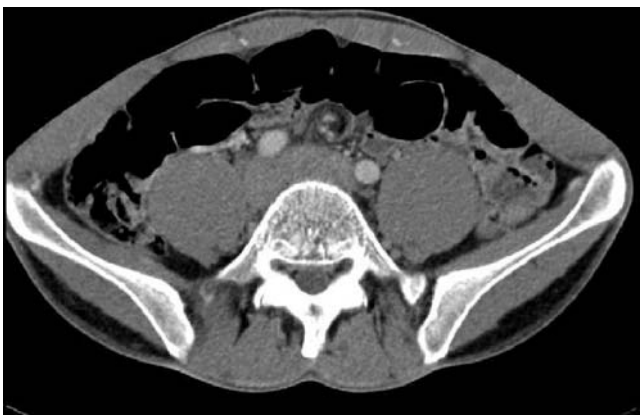

Figure 1 CT scan showing the "whirl sign" consisting of collapsed small bowel wrapped around a twisted superior mesenteric artery.

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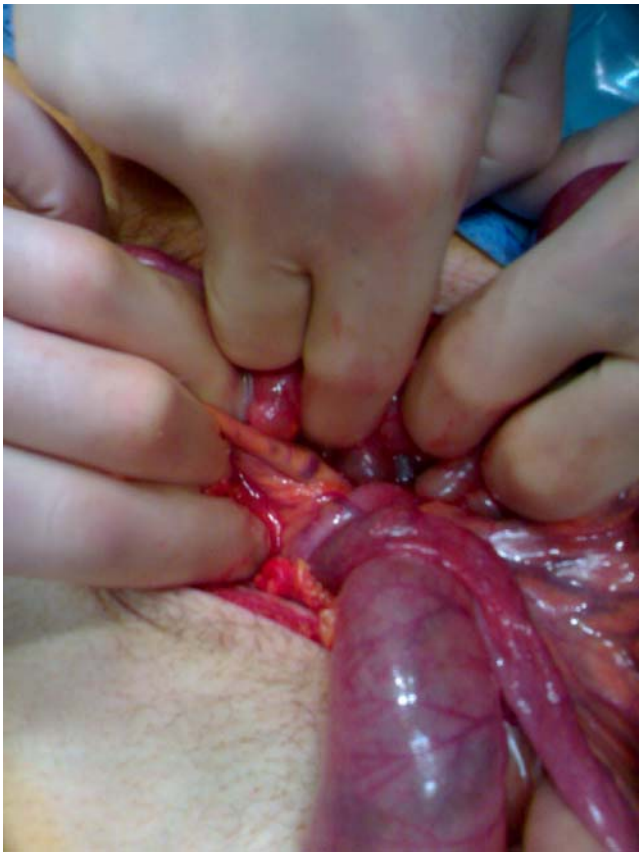

Figure 2 Laparotomy showing the twisted mesentery with collapsed loops of small bowel wrapped around it.

Diagnosis can be difficult because symptoms are non-specific. On physical examination, peritoneal irritation is often absent.[3] Laboratory findings are often unremarkable and plain abdominal radiographs may or may not show signs of abdominal obstruction. When small bowel volvulus is suspected, CT scanning is the imaging modality of choice. There are several specific appearances on CT that are highly suggestive of volvulus. The whirl sign is one of them consisting of collapsed loops of small bowel wrapped around a twisted superior mesenteric artery creating a whirl-like appearance (Fig. 1).[4,5]

Small bowel volvulus is a rare but potentially fatal surgical emergency that should be considered in every patient presenting with abdominal pain.

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Intrapancreatic Accessory Spleen Mimicking Endocrine Tumor of the Pancreas: Case Report and Review of the Literature

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Abstract Accessory spleen is an anomaly that is observed in about 10% of individuals by the autopsy study, and most accessory spleens are located close to the splenic hilum. Although accessory spleen is a frequently encountered entity, intrapancreatic accessory spleen (IPAS) is rarely recognized radiologically and is sometimes mistaken for another type of pancreatic neoplasm. Only 10 IPAS cases surgically resected as solid pancreatic mass have been reported in the English literature. We herein report a case of IPAS mimicking an endocrine tumor of the pancreas and review of the literature.

Keywords Intrapancreatic accessory spleen · Pancreas · Endocrine tumor

Case Report

An 81-year-old woman with a history of sudden glucose intolerance was referred to our hospital for further examination of a solitary tumor in the tail of the pancreas. Laboratory data showed no abnormalities. Among various tumor markers, only carbohydrate antigen 19-9 was slightly elevated to 112.4 U/ml (normal, <37 U/ml). Serum levels of the hormones, glucagon, insulin, gastrin, and somatostatin were all within normal range.

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Ultrasonography revealed the presence of a hypoechoic tumor, about 1.5 cm in diameter, in the tail of the pancreas. Plain computed tomography (CT) showed an isodensity tumor compared with the adjacent pancreas, and enhanced CT showed a well-defined and homogeneously enhanced tumor, about 1 cm in size, in the tail of the pancreas (Fig. 1a). T1-weighted magnetic resonance imaging (MRI) revealed the presence of isointensity tumor (Fig. 1b), and T2-weighted imaging of iso to high intensity tumor (Fig. 1c) relative to the surrounding pancreatic tissue. Although celiac angiography revealed a vague tumor stain, CT angiography showed strong enhancement (Fig. 1d). On the basis of these findings, nonfunctioning endocrine tumor was suspected, and distal pancreatectomy with splenectomy was performed because of the possibility of malignancy.

The surgical specimen contained a well-demarcated dark-red nodule, 1.1×0.9 cm in size, surrounded by pancreatic tissue (Fig. 2a). The nodular lesion was elastic soft, and the gross appearance of the nodule was quite different from that of endocrine tumors. Microscopically, the nodule was composed of lymphoid follicles and splenic pulp (Fig. 2b). The surrounding pancreatic parenchyma showed focal pancreatitis, with infiltration of adipose tissue, parenchymal atrophy, and periductal lymphocyte infiltration. There was no apparent neoplastic component. Thus, the nodule was diagnosed as an intrapancreatic accessory spleen.

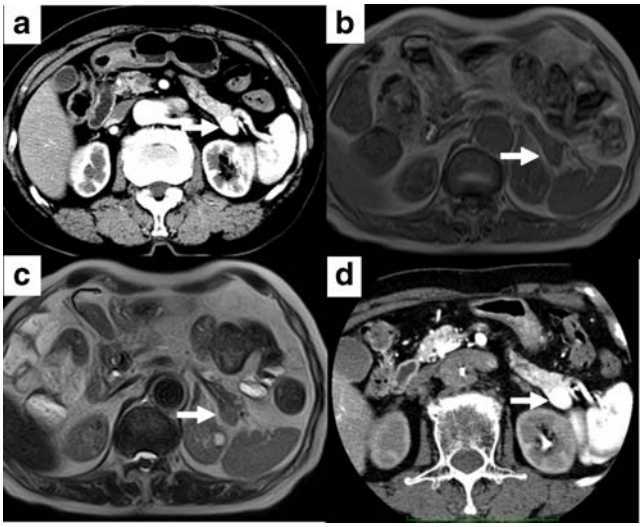

Figure 1 **a** Enhanced CT showed a well-defined, homogeneously enhanced tumor, about 1 cm in size, in the tail of the pancreas (*arrow*). **b** MRI revealed isointensity on T1-weighted images (*arrow*). **c** MRI showed iso to high intensity on T2-weighted images (*arrow*). **d** CT angiography showed strong enhancement (*arrow*).

Discussion

Accessory spleen is a congenital abnormality consisting of normal splenic tissue in ectopic sites, anywhere along the splenic vessels, in the wall of the jejunum, in the mesentery, or even in the pelvis.[1,2] In the autopsy study, 311 out of 3,000 patients (about 10%) had an accessory spleen, and the accessory spleen was solitary in 272 out of the 311 patients (87%).[1] The similar result in the additional 2,700 patients was reported from the same institute.[2] It has been reported that 80% of accessory spleens are located at or near the splenic hilum, and nearly one of every five accessory spleens is located close to the tail of the pancreas.[1,2]

Although accessory spleen is sometimes observed, reports of intrapancreatic accessory spleen (IPAS), however, are limited, and IPAS is rarely recognized radiologically.[3] An IPAS appears as a solid enhancing neoplasm on CT scan, and differential diagnosis includes solid pseudopapillary tumor,

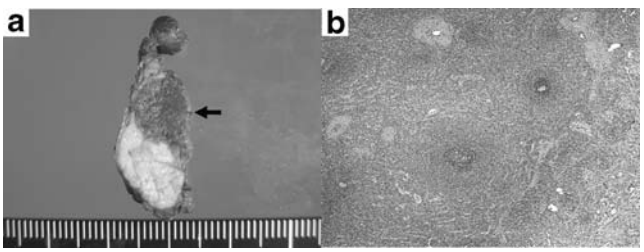

Figure 2 **a** The surgical specimen contained a well-demarcated dark-red nodule (*arrow*) surrounded by pancreatic tissue. **b** Microscopic findings. The nodule was composed of lymphoid follicles and splenic pulp (hematoxylin and eosin stain, original magnification, ×40).

Table 1 Summary of Surgically Resected IPAS

Case	Author	Year	Sex/age	Size (cm)	Preoperative diagnosis	Radiologic findings
1	Takayama et al.[4]	1994	F/62	1.2	Asymptomatic endocrine tumor	Enhanced CT and angiography: well-defined, hypervascular mass, MRI: not shown
2	Harris et al.[5]	1994	F/50	1.5	Islet cell tumor	CT: not detected, MRI: low signal on T1, increased signal on contrast-enhanced T1, bright signal on T2
3	Churei et al.[6]	1998	M/38	2.0	Islet cell tumor or nonfunctioning adenoma	Enhanced CT: homogeneously enhanced mass, MRI: low signal on T1, homogeneous enhancement on enhanced T1, high signal on T2
4	Lauffer et al.[7]	1999	M/71	1.5	Pancreatic neoplasm	Enhanced CT: hypervascular, homogeneous enhancement, MRI: not shown
5	Sica and Reed[8]	2000	F/40	2.5	Solid enhancing mass	CT: focal, mass-like enlargement on plain CT, MRI: iso to mildly hyperintense on T2, low on T1, moderate homogeneous enhancement on enhanced T1
6	Brasca et al.[9]	2004	F/52	1.0	Nonfunctional neuroendocrine tumor	CT: not shown, MRI: mildly hyperintense on T2, hypointense on T1, conspicuous enhancement on enhanced T1
7	Hamada et al.[10]	2004	M/61	1.5	Nonfunctioning endocrine tumor	CT: well-defined isodense mass on plain CT, homogeneously enhanced mass on enhanced CT, MRI: not shown
8	Davidoff et al.[11]	2006	M/66	1.6	Nonfunctioning neuroendocrine tumor	Enhanced CT: hypervascular tumor, MRI: not shown
9	Kim et al.[12]	2006	M/45	1.2	Islet cell tumor	CT: slightly higher attenuation than the spleen on the portal venous phase, MRI: slightly higher intensity on T2
10	Kim et al.[12]	2006	F/30	2.5	ITP because of IPAS	CT: lobulated and high attenuating nodule in the pancreatic tail and two accessory spleens, MRI: low intensity on T1, high intensity on T2
11	Our case	2007	F/81	1.1	Nonfunctioning endocrine tumor	CT: isodensity on plain CT, homogeneously enhanced tumor on enhanced CT, MRI: isointensity on T1, iso to high intensity on T2

ITP: idiopathic thrombocytopenic purpura

islet cell tumor, pancreatic adenocarcinoma, endocrine tumor, and metastasis. Indeed, if the accessory spleen is located in the pancreas, it may mimic a well-defined primary pancreatic tumor and thus be resected surgically.[4–12] Imaging characteristics and preoperative diagnosis of IPAS surgically resected and pathologically confirmed are summarized in Table 1. Only 1 among 11 cases was diagnosed as IPAS preoperatively because repeated surgical operation for idiopathic thrombocytopenic purpura led to the conclusive diagnosis of accessory spleen. The mean size was 1.6 cm, and most cases were mistaken for nonfunctioning endocrine tumors or islet cell tumors. Epidermoid cyst in an IPAS is also reported in some cases showing cystic appearance and is sometimes mistaken for cystic tumors.[13]

Endocrine tumors of the pancreas without any clinical symptoms and hormone overexpression are widely defined as nonfunctioning endocrine tumors and account for 15% to 41% of the endocrine pancreatic tumors.[14] The prognosis of nonfunctioning endocrine tumors varies according to the type of tumor, and the rate of malignancy is reported to be 62% to 92%.[15] According to multivariate analysis of predictive factors for survival after surgical treatment of 22 cases of malignant nonfunctioning endocrine tumors of the pancreas, definitive surgical resection of the primary tumor was a predictor of long-term survival. No difference in the overall disease-specific survival was observed with respect to age, sex, tumor location, tumor size, histological differentiation, or status of lymph node involvement.[16]

Radiologic features of endocrine pancreatic tumors and IPAS have been reported. Semelka et al. reported that fat-suppressed and dynamic gadolinium-enhanced MRI may be superior to dynamic contrast-enhanced CT for the detection of metastatic as well as primary endocrine pancreatic tumors, which show ring-like or uniform enhancement.[17] Radiologic features of IPAS are as follows: By contrast-enhanced sonography, IPAS showed inhomogeneous enhancement in the early vascular phase, enhancement similar to that of the spleen during the postvascular phase, and prolonged enhancement in the hepatosplenic parenchymal phase.[18] Most IPAS showed an attenuation on dynamic CT scans and signal intensity on MR images similar to that of normal spleen. They also showed a characteristic heterogeneous enhancement pattern in early CT phases. Moreover, superparamagnetic iron oxide-enhanced MRI in which IPAS has a signal drop similar to that of the spleen is suggested to be an excellent alternative to technetium-99m heat-damaged red blood cell scintigraphy for confirming the diagnosis of IPAS.[12]

Although there was no apparent malignancy in our case, nonfunctioning endocrine tumor was not completely ruled out, and surgical treatment was applied. A definitive preoperative diagnosis is difficult on the basis of nonspecific radiologic findings unless IPAS is strongly suspected.

For cases in which CT reveals an enhancing mass in the pancreas, IPAS should be added to the differential diagnosis, and additional sensitive radiologic studies should be performed.

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Errata

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In Volume 12, Number 3, of *Journal of Gastrointestinal Surgery*, the introduction for the SSAT/AGA/ASGE State of the Art Conference on Cystic Neoplasms of the Pancreas was inadvertently omitted. That article, SSAT/AGA/ASGE State of the Art Conference on Cystic Neoplasms of the Pancreas by Diane M. Simeone (DOI 10.1007/s11605-007-0351-4), follows on the proceeding pages.

The symposium included the following articles:

Cystic Neoplasia of the Pancreas: Pathology and Biology
N. Volkan Adsay
DOI 10.1007/s11605-007-0348-z

Management of Cystic Lesions of the Pancreas
James M. Scheiman
DOI 10.1007/s11605-007-0350-5

Management of Serous Cystadenoma of the Pancreas
Jennifer F. Tseng
DOI 10.1007/s11605-007-0360-3

Mucinous Cystic Neoplasms
Carlos Fernández-del Castillo
DOI 10.1007/s11605-007-0347-0

Surgical Management of Intraductal Papillary Mucinous Neoplasm of the Pancreas
Michael B. Farnell
DOI 10.1007/s11605-007-0349-y

J Gastrointest Surg (2008) 12:1475–1477
DOI 10.1007/s11605-007-0351-4

SSAT/AGA/ASGE State of the Art Conference on Cystic Neoplasms of the Pancreas

Diane M. Simeone

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Abstract Cystic tumors of the pancreas are an increasingly recognized clinical entity, and the management of these lesions continues to evolve. The Society for Surgery of the Alimentary Tract, American Gastroenterological Association, and American Society for Gastrointestinal Endoscopy recently held a "state-of-the-art" conference to discuss the current recommendations for diagnostic evaluation and clinical management of pancreatic cystic tumors. In this article, a brief review of the conference and important teaching points presented at the conference are highlighted.

Keywords Cystic pancreatic neoplasm · Endoscopic ultrasound · Mucinous · Serous · Surgical resection

Cystic tumors of the pancreas are an increasing identified clinical entity, and the management of these lesions continues to rapidly evolve. Because of increased utilization of cross-sectional imaging for unrelated abdominal complaints, the high rate of incidental detection of cystic tumors of the pancreas makes management challenging. Surgical resection of all cystic tumors of the pancreas has been surgical dogma for some time, however, recent studies suggest that a more selective approach to surgical resection may be considered in some patients. In these review articles written as part of the Society for Surgery of the Alimentary Tract "State of the Art" Conference, the current recommendations for diagnostic evaluation and clinical management of pancreatic cystic tumors of varying types are outlined.

In the first article, Dr. N. Volkan Adsay from Emory University reviews the pathology and biology of different types of cystic lesions of the pancreas. Dr. Adsay breaks down cystic lesions of the pancreas into three broad categories: no lining (pseudocyst), true lining (mucinous and serous), and degenerative/necrotic change in a solid neoplasm (solid-pseudopapillary neoplasm, cystic degeneration of ductal adenocarcinoma or neuroendocrine tumor). Pseudocysts represent approximately 30% of all cystic lesions of the pancreas, whereas mucinous and serous cystic tumors represent 50–60% of all cystic lesions and cystic degeneration of a solid neoplasm represent about 10% of all cystic lesions. Of the cystic lesions with true lining, mucinous lesions have malignant potential. These mucinous lesions fall into two groups: intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN). IPMNs are characterized by cystic dilatation of the pancreatic ducts in which papillary projections of neoplastic mucin-producing cells develop. In some cases, the main pancreatic duct is involved, whereas in others, the branch ducts are involved, producing a more discrete cystic mass. Mucinous cystic neoplasms, like IPMNs, produce mucin, but are distinct in that they form cystic tumors that are surrounded by a highly characteristic ovarian type of stroma. Serous cystadenomas are benign neoplasms without significant malignant potential; however,

The articles discussed in this paper were originally presented as part of the SSAT/AGA/ASGE State-of-the-Art Conference on Management of Cystic Lesions of the Pancreas at the SSAT 48th Annual Meeting, May 2007, in Washington, DC, and include Adsay NV, Cystic Neoplasia of the Pancreas: Pathology and Biology; Scheiman JM, Management of Cystic Lesions of the Pancreas: Diagnosis: Radiographic Imaging, EUS and Fluid Analysis; Tseng JF, Management of Serous Cystadenoma of the Pancreas; Fernández-del Castillo C, Mucinous Cystic Neoplasms; and Farnell MB, Surgical Management of Intraductal Papillary Mucinous Neoplasm (IPMN) of the Pancreas.

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these lesions may occasionally grow to a large size and warrant surgical removal based on patient symptoms.

The diagnostic evaluation of cystic lesions of the pancreas is described in the second article by Dr. James Scheiman at the University of Michigan. As Dr. Scheiman points out, the initial approach to the patient should include a detailed history looking for symptoms related to the lesion itself or a related condition, such as pancreatitis. When a cyst arises in a patient with known chronic pancreatitis, the clinical concern of a neoplasm is quite low. However, when patients present with unexplained pancreatitis for the first time with a cyst or if there is no history of pancreatitis, the suspicion of a cystic tumor should be high. In this case, the development of pancreatitis is presumably from the compression of the pancreatic duct. MRI, CT scanning, and endoscopic ultrasound (EUS) are all useful imaging modalities to provide detailed information about cyst location and morphology, although all of these tests are imperfect in their ability to distinguish among the different types of cystic lesions of the pancreas. A particular feature of cyst imaging that is associated with malignancy is the presence of a solid component within the cyst. Aspiration and characterization of cyst fluid, best done under EUS guidance, may be useful in decision making in some patients. The most useful marker in cyst fluid is CEA, which when elevated can help distinguish mucinous from nonmucinous lesions with 79% sensitivity. At the end of his article, Dr. Scheiman provides a very useful algorithm to the approach of patients with pancreatic cystic tumors.

In the third article, Dr. Jennifer Tseng of the University of Massachusetts describes the treatment of serous cystadenoma of the pancreas. Helical CT scans, the most commonly utilized radiographic test to image the pancreas, may be helpful in the differentiation between serous and mucinous neoplasms, in particular if the serous tumor has the classic findings of a central scar with a "honeycomb" appearance of microcysts. Oligocystic or macrocystic variants of serous lesions are more difficult to differentiate. When the decision to operate or not to operate hinges upon differentiating a serous from a mucinous lesion, cyst fluid analysis via EUS guidance may be useful. In general, operative resection for serous cystadenomas is carried out for symptoms, large size (≥4 cm), or the inability to distinguish a serous cystic neoplasm from a mucinous neoplasm, which has malignant potential. The size cutoff of 4 cm in size for resection is based on a large study of patients with serous cystadenomas of the pancreas where it was determined that tumors ≥4 cm in size had much accelerated growth rates (1.98 cm/year) compared to smaller tumors (0.12 cm/year), and thus were at increased risk to cause future patient symptoms.[1]

The fourth article, written by Dr Carlos Fernandez-del Castillo from the Massachusetts General Hospital, addresses the management of patients with mucinous cystic neoplasms. Mucinous cystic neoplasms (MCNs) typically present as a septated, mucin-filled cyst, occurring almost exclusively in the pancreatic body and tail of middle-aged women. No communication with the pancreatic ductal system should be evident. A recent consensus conference held in Sendai, Japan by the International Association of Pancreatology (IAP) put forth guidelines requiring the presence of ovarian stroma to establish the diagnosis of MCN.[2] In a recent large series of 163 patients who underwent surgical resection for MCN, the overall incidence of malignancy was 17.5%.[3] All MCNs in this series that had invasive cancer were ≥4 cm in size or had a solid component. Patients with invasive cancer tended to be older, suggesting a progression from adenoma to carcinoma. Dr. Fernandez-del Castillo advocates surgical excision for all MCNs in patients who are suitable surgical candidates as (1) extensive histological sampling (and thus the likelihood of malignancy) cannot be achieved until the tumor is excised; (2) the mean age of presentation of patients is 45 years, therefore, an expectant approach with frequent surveillance with CT, MRI, or EUS would be required for a long period of time; and (3) most patients would require a distal pancreatectomy, which has a lower operative morbidity and mortality rate than a pancreatic head resection, and in some cases, can be performed laparoscopically.

In the fifth and last article, Dr. Michael Farnell of the Mayo Clinic addresses the management of IPMN of the pancreas. As Dr. Farnell points out, the Sendai consensus conference convened by the IAP in 2006 reviewed collected series of patients with IPMN involving the main pancreatic duct and found that the majority of patients either had carcinoma in situ or invasive cancer, whereas for collected series of patients with side-branch disease, the risk of malignancy was much lower.[2] These data suggest that the two IMPN subtypes may be managed differently. Based on current thinking, patients with main duct IPMN have a risk of malignancy of approximately 70%, and fit surgical candidates should be offered surgical resection regardless of symptoms. Main duct disease may present as diffuse main duct ectasia, which may be because of duct obstruction secondary to mucus production by tumor cells in the head of the gland or a neoplasm diffusely involving the entire main pancreatic duct. The initial approach to treat this type of tumor would be to perform a pancreaticoduodenectomy and obtain a frozen section analysis of the pancreatic margin intraoperatively. If carcinoma is present at the margin, resection of additional pancreas or even total pancreatectomy may be indicated. Segmental main duct ectasia usually involves the distal pancreas, and for these patients, a distal pancreatectomy is warranted. Resection of additional pancreatic tissue should be performed if the frozen section of the pancreatic margin reveals carcinoma.

The risk of malignancy with side branch IPMN is much lower, and in two recent series, factors that correlated with malignancy in this group of patients included the presence of symptoms, solid nodules, or cyst size ≥3 cm.[4,5] In the absence of these criteria, observation may well be indicated.

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