Barrett's Esophagus

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Norman Barrett described the condition that bears his name in 1950.[1] He believed that he was observing a congenitally short esophagus and an intrathoracic stomach.[2] It was Philip Allison, in 1953, with the careful examination of seven esophagectomy specimens, who showed conclusively that it was indeed the tubular esophagus lined with columnar epithelium.[3] Now, more than 50 years later, virtually every aspect of the disease we refer to as Barrett's esophagus is controversial and frequently debated[4] including its definition,[5] pathophysiology,[6] prevalence,[7] significance,[8] the benefit of screening and surveillance,[9] and treatment.[10] Its importance is underscored by its increasing frequency in all too often young men and women,[11] many of whom have few symptoms of gastroesophageal reflux, develop difficulty swallowing, and are found to have esophageal adenocarcinoma.

EPIDEMIOLOGY

Before World War II, the finding of columnar epithelia in the tubular esophagus was an uncommon event in clinical practice. Its recognition has changed dramatically over the past 50 years, to the point that it is now a significant public health problem. At present, long-segment Barrett's esophagus is found in roughly 4% to 6% of patients with symptoms of reflux, 1% of all patients undergoing upper endoscopy, and 0.3% of the United States population. Short-segment Barrett's esophagus (<3cm) is probably even more prevalent, accounting for one-half to two-thirds of all cases of Barrett's esophagus identified in most recent studies[13,14] (Fig. 1). The prevalence of Barrett's esophagus has steadily increased from 1 per 1000 endoscopies in the early 1980s, to 10 per 1000 in the late 1980s, to 55 to 60 in the late 1990s.[12]

Barrett's esophagus is more common in men than in women by a ratio of 3:1. Recent studies have shown that patients who have symptoms of gastroesophageal reflux disease (GERD) for more than 10 years[15] and large hiatal hernias[16] are at increased risk. Similarly, patients with severe bipositional reflux[17] and those with a component of duodenogastroesophageal reflux[18] have been shown to have a higher prevalence of Barrett's metaplastic changes. Case-control epidemiologic studies have shown that patients with Barrett's esophagus develop reflux symptoms at an earlier age and have more severe symptoms than age- and sex-matched GERD or upper endoscopy control patients.[19] Complications of reflux, including esophagitis, stricture, and ulceration, also occur more frequently in patients with Barrett's esophagus. Physiologic studies reveal markedly abnormal esophageal acid exposure, an incompetent lower esophageal sphincter, and poor esophageal body clearance in more than 90% of patients.[20] Both the frequency and duration of reflux episodes are increased in comparison to patients with no columnar metaplasia. Contractility of the esophageal body is often impaired in patients with Barrett's esophagus and may be profoundly reduced. The impaired esophageal body motility results in poor clearance of the refluxed material and prolonged contact times between the refluxing material and the esophageal mucosa with the potential for severe mucosal damage. The clinical and physiologic abnormalities in patients with short-segment Barrett's esophagus are generally intermediate between those found in patients with long-segment Barrett's esophagus and erosive esophagitis[21] (Table 1). Given these facts, irrespective of any concern for the development of dysplasia and cancer, the presence of Barrett's esophagus, particularly long-segment Barrett's esophagus, portends severe GERD and, as such, is an indication that surgical treatment should be considered.

DIAGNOSIS AND DETECTION

The definition of Barrett's esophagus has also evolved considerably over the past decade[22] (Fig. 2). Historically, Barrett's esophagus was identified by the presence of any columnar mucosa extending at least 3 cm into the esophagus with any one of three

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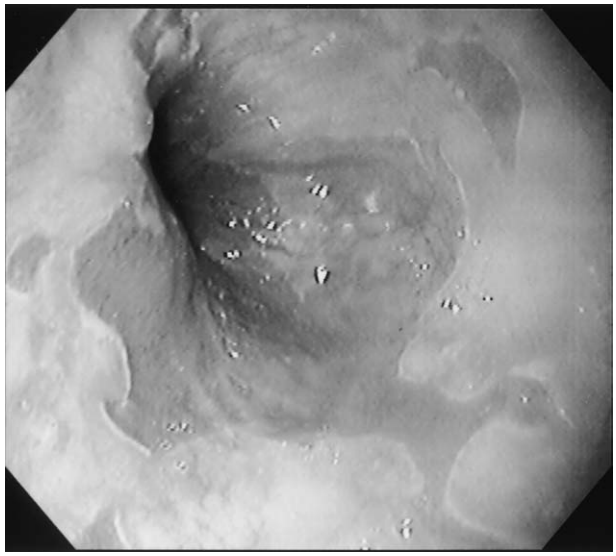

Fig. 1. Endoscopic appearance of short-segment Barrett's esophagus.

histologic types on biopsy (fundic, transitional, or specialized intestinal).[23] It is now believed (although not well proved) that specialized intestinal-type epithelium is the primary tissue predisposed to malignant degeneration. The hallmark of intestinal metaplasia is the presence of goblet cells[24] (Fig. 3). This, coupled with the finding of a similar risk of malignancy in segments of intestinal metaplasia less than 3 cm long,[25,26] has resulted in the current practice of diagnosing Barrett's esophagus given any length of endoscopically visible tissue that demonstrates intestinal metaplasia on histologic examination. Whether to call long segments of columnar mucosa without intestinal metaplasia Barrett's esophagus is unclear, although this circumstance is rare.

Further complicating the definition of Barrett's esophagus, clinical investigation beginning in the early 1990s identified a high prevalence of biopsy-proved intestinal metaplasia at the cardia, in the absence of endoscopic evidence of a columnar-lined esophagus.[27] The significance and natural history of this finding, referred to as cardia intestinal metaplasia or intestinal metaplasia of the gastroesophageal junction, remains unknown, although it may be the earliest stage of transformation to what we know as Barrett's esophagus. The distinction between intestinal metaplasia of the gastroesophageal junction and short-segment Barrett's esophagus is the recognition of an endoscopic segment of columnar lining in the esophagus. This fact adds subjectivity and the experience of the endoscopist into the diagnostic mix, and significantly complicates clinical investigation of early Barrett's changes. Most investigators presently use the term Barrett's esophagus to mean an endoscopically visible segment of columnar-lined esophagus of any length that shows intestinal metaplasia on biopsy.

PATHOPHYSIOLOGY OF INTESTINAL METAPLASIA AND THE METAPLASIA-DYSPLASIA-ADENOCARCINOMA SEQUENCE

Significant evidence suggests that the development of Barrett's esophagus is a two-step process. The metaplastic process at the gastroesophageal junction likely begins by conversion of distal esophageal squamous mucosa to cardiac-type epithelium, which

Table 1. Clinical and anatomic characteristics of varying degrees of intestinal metaplasia of the esophagus and gastroesophageal junction

Characteristics	Total population (N)	GEJ-SIM (N)	SSBE (N)	LSBE (N)	*P* value
Sex (M/F)	394/344	25/22	45/19	35/5	0.0001
White race	485 (66%)	31 (66%)	55 (86%)	40 (100%)	0.0011
Hiatal hernia	252 (34%)	19 (40%)	39 (61%)	32 (80%)	0.0001
Hernia size (cm)	2 (range 1–9)	2 (range 1–8)	3 (range 1–8)	4 (range 2–7)	0.0001
Heartburn	343/550 (62%)	20/34 (59%)	33/40 (83%)	10/16 (63%)	0.077
Duration of heartburn (yr)	2 (range 0.2–45)	3.5 (range 0.25–30)	3.5 (range 0.1–35)	20 (range 0.16–54)	0.009
Esophagitis	110/549 (20%)	7/34 (21%)	10/40 (45%)	3/16 (19%)	0.003
Dysplasia	0/720	2/47 (4.3%)	4/50 (8%)	2/13 (15%)	
Cancer	0	1/47 (2.1%)	1/50 (2%)	2/13 (15.4%)	
Dysplasia + cancer	0	3/47 (6.4%)	5/50 (10%)	4/13 (31%)	0.043

N = number of patients; GEJ-SIM = gastroesophageal junction specialized intestinal metaplasia; SSBE = short-segment Barrett's esophagus; LSBE = long-segment Barrett's esophagus.

Adapted from Hirota WK, Loughney TM, Lazas DJ, et al. Specialized intestinal metaplasia, dysplasia and cancer of the esophagus and esophagogastric junction: Prevalence and clinical data. Gastroenterology 1999;116:277–285.

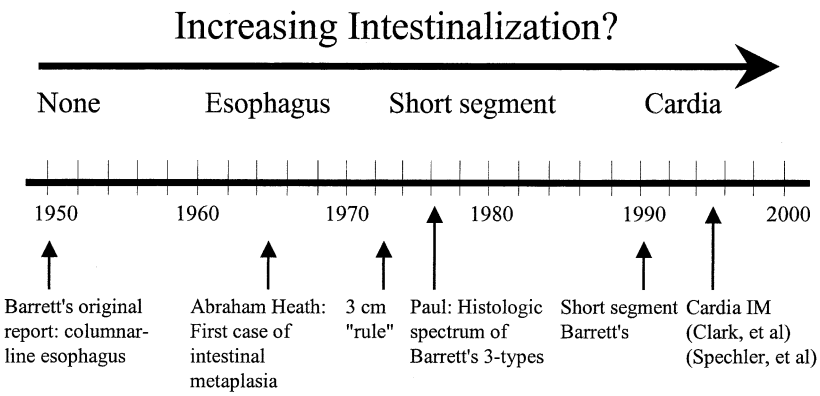

Fig. 2. Time line of historical landmarks in the understanding of Barrett's esophagus. Note the possibility that the development of intestinalized metaplasia (*IM*) has increased over the years.

has historically been considered a normal finding at the gastroesophageal junction[27,28] (Fig. 4). This is likely due to exposure of the lower esophageal squamous mucosa to an acid environment, particularly after meals, a phenomenon that has recently been shown to occur in elegant studies from Glasgow.[29]

Several rather disparate observations support a two-step hypothesis. First, and perhaps most important, cardiac-type epithelium is not present in as many as 25% of adults undergoing upper endoscopy where the transition is directly fundic to squamous, the length of cardiac epithelium at the gastroesophageal junction increases with age, and when present, cardiac epithelium is almost always inflamed.[27] Second, as the severity of GERD progresses, the length of cardiac epithelium above the anatomic gastroesophageal junction increases.[28] Third, cardiac epithelium has been well documented to develop above the fundic to squamous anastomosis created in patients after esophagogastrectomy and gastric interposition to the neck (as has Barrett's epithelium). Finally, squamous-lined ducts, known to be present beneath the squamous mucosa of the esophagus but not beneath normal gastric mucosa, can be observed beneath cardiac mucosa lining the gastroesophageal junction. Taken together, these observations strongly support the fact the primary metaplastic process is not the development of Barrett's mucosa but is a precursor epithelium—heretofore thought to be normal, that is, cardiac-type mucosa. Thus the presence and extent of columnar epithelium lining the distal esophageal sphincter results from a metaplastic process associated with a loss of sphincter function and increased esophageal acid exposure. Intestinal metaplasia within the sphincter may result, similar to the pathophysiology that leads to Barrett's metaplasia, within the esophageal body.

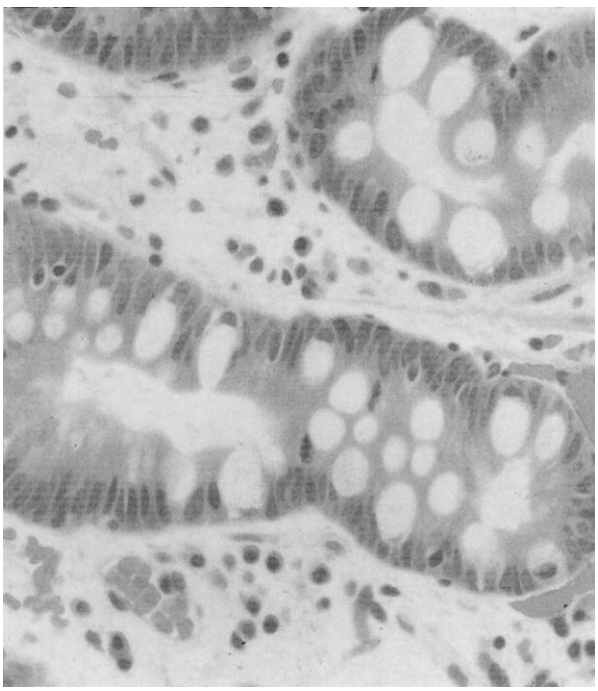

Fig. 3. Photomicrograph of intestinal metaplasia with numerous goblet cells.

The second step then becomes the "intestinalization" of this underlying cardiac epithelium. Factors predisposing to the development of intestinal metaplasia in a columnar-lined segment are slowly emerging and include (1) time spent with reflux disease and (2) the presence of duodenal content in the refluxed material, whereas the degree of disruption of the anatomic gastroesophageal barrier likely determines the length or extent of the intestinalized segment.

The cell of origin of Barrett's epithelium is unknown, although it seems reasonable to assume that

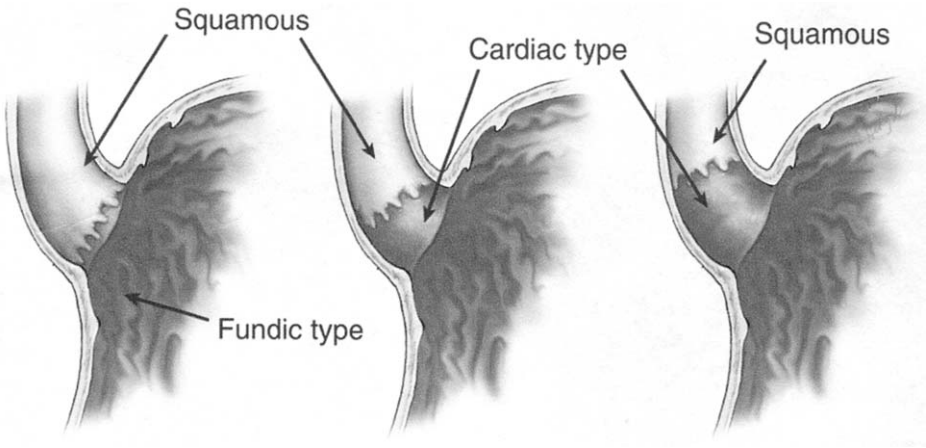

Fig. 4. Artist depiction of the replacement of squamous mucosa by cardiac-type columnar epithelium with increasingly severe GERD from left to right.

stem cells present in the proliferative layer of the esophageal epithelium are responsible. Biochemically, Barrett's tissue resembles colonic epithelia and is characterized by the lack of disaccharidase activity, high isomaltase/sucrase activity, low mucosal levels of glutathione, low mucosal protein synthesis, and abundant cytokeratin (CK-13).[30] Several provocative papers regarding the origin of Barrett's epithelium have been published. A unique surface cell at the junction of squamous and Barrett's epithelium notable for the presence of both squamous and columnar markers on its cell surface has recently been described.[31] This hybrid cell, which by electron microscopy is shown to be cuboidal with abundant microvilli and secretory vesicles, is present at the squamocolumnar junction in 40% of patients with Barrett's esophagus. This distinctive cell may represent an intermediate stage in the development of Barrett's epithelium. Cytokeratin profile studies of Barrett's epithelium reveal characteristics of both squamous and columnar epithelia.[30] Focal areas of multilayered epithelium adjacent to Barrett's epithelium often stain for both types of markers, suggesting a multipotential cell as the cell of origin of Barrett's epithelium.[32]

The biology of the metaplasia-dysplasia-carcinoma process is characterized by an increased rate of proliferation, loss of differentiation, expression of growth factors and adhesion molecules, and abnormal apoptosis. Quiescent, nondysplastic, Barrett's mucosa demonstrates higher proliferative indices[33] and expression of inducible nitric oxide synthetase (iNOS)[34] and cyclooxygenase-2 (COX-2).[35] As the metaplastic-dysplastic process progresses, epigenetic alterations produce increased expression of several gene products including epidermal growth factors and their receptors, thereby causing genetic instability and histologically recognizable cellular changes characteristic of dysplasia (Fig. 5). Finally, loss of regulatory genes such as p53 and cadherin/catenin complexes inhibits apoptosis leading to carcinoma and tissue invasion.[36,37] Chromosomal loss,[38] DNA mutation, and gene silencing via DNA methylation[39] have all been shown to occur in the metaplasia-dysplasia-carcinoma sequence.

It is now possible not only to identify these molecular events but to quantitate them as well, allowing more accurate diagnosis, staging, and treatment selection. With knowledge of the molecular events, clinical applications emerge including chemoprevention,[40] molecular staging, and biomarker prediction of an increased risk of disease progression.[41] Each of these is presently undergoing active clinical trials. For example, telomerase activation and the relatively increased messenger RNA expression of hTERT have been shown to correlate with the development of dysplasia and adenocarcinoma.[42] Abnormalities of p53 expression have been shown to predict both survival[43] and response to chemotherapy,[44] which translates into clinical use in selecting the extent of surgical resection and the use of adjuvant chemotherapy in those patients who develop Barrett's-related adenocarcinoma.

Conceptually, Barrett's mucosa represents a mosaic of clonal populations, many of which may have malignant potential. Prospective studies from the University of Washington have shown that the neoplastic evolution of clonal populations occurs in a complex,

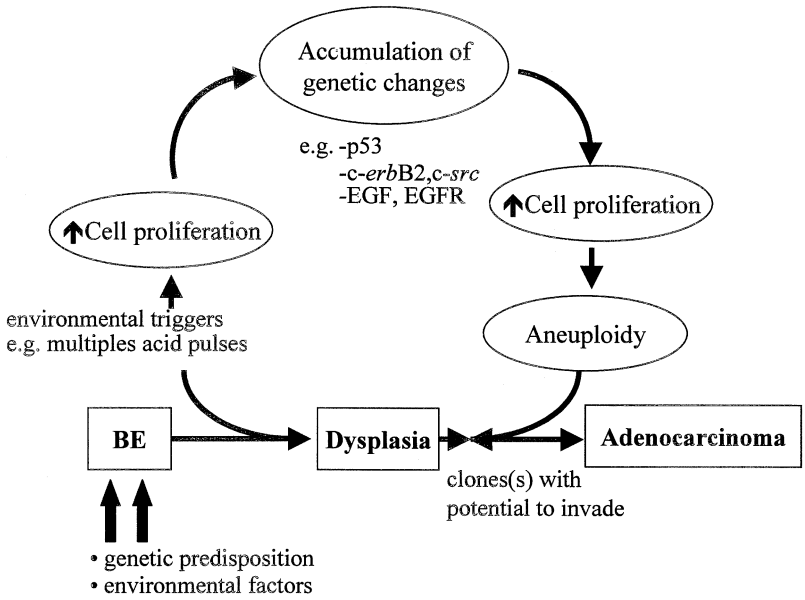

Fig. 5. Schematic depiction of the genetic events leading to cellular instability in the metaplasia-dysplasia-carcinoma sequence in Barrett's esophagus (*BE*) p53, c-erb B2, c-src = altered genes; EGF = epidermal growth factors; EGFR = epidermal growth factor receptors. (From Fitzgerald R, Triadafilopoulos G. Recent developments in the molecular characteristics of Barrett's esophagus. Dig Dis 1998;16:63–80. Reprinted by permission of the publisher.)

nonlinear fashion. Diploid cell progenitors containing genetic and/or epigenetic abnormalities in the p53 and p16 genes were shown to proliferate widely throughout large areas of Barrett's mucosa.[45] The subsequent premalignant progeny appear to undergo random mutation and loss of heterozygosity at various genetic loci leading to neoplastic progression. This fact has great bearing when considering treatment options including continued surveillance, mucosal ablation, or esophageal resection. Surveillance is complicated by sampling error within this mosaic, ablation by the need for elimination of all premalignant clones, and resection by its attendant morbidity and mortality.

SCREENING AND SURVEILLANCE

Screening refers to applying a diagnostic test to a group of individuals not known to have the disease. Its benefit is directly proportional to the prevalence of the disease in the population being screened.[46] Surveillance is the application of a diagnostic examination to patients already known to have the disease in an effort to detect its complications at an early stage. There are no current recommendations advocating screening for Barrett's esophagus, although results of preliminary studies suggest that it may be of benefit in high-risk populations.[47] In a rather remarkable recent study, Gerson et al.[14] provided insight into the prevalence of Barrett's esophagus in a largely white male (73% and 92%, respectively) population of veterans. A total of 110 patients undergoing sigmoidoscopy for colorectal cancer screening also underwent upper endoscopy to screen for the presence of Barrett's esophagus. Visible segments of Barrett's esophagus confirmed to harbor intestinal metaplasia on biopsy were found in 25% of these patients! Approximately two-thirds of them had short-segment (17%) and one-third had long-segment (7%) Barrett's esophagus. This remarkably high prevalence of asymptomatic patients with Barrett's esophagus almost certainly reflects the white male study population but is a strong argument favoring screening studies in high-risk populations.

The rationale for endoscopic surveillance in patients with Barrett's esophagus is twofold: (1) to detect progression of disease toward cancer and (2) to allow early intervention while cure is still likely.[48] Predicated on this rationale is the concept that the surveillance will be frequent enough that rarely, if ever, does the disease progress beyond an early, curable stage during the surveillance interval and that once disease progression is detected an intervention is performed. Controversy exists, however, about the point at which there has been sufficient progression to warrant intervention and what type of intervention should be done.

The recommended frequency for endoscopic surveillance in patients with Barrett's esophagus is based on available data concerning the likelihood and rapidity of progression including the presence or absence of dysplasia. Most advise patients with any length of intestinal metaplasia within the esophagus to undergo endoscopic examination every 1 to 2 years, with an increase in the frequency to every 3 to 6 months for patients with dysplasia. The Practice Parameters Committee of the American College of Gastroenterology has recently suggested that surveillance may be extended to every 2 or 3 years in patients with Barrett's esophagus who do not have dysplasia on biopsies from two annual endoscopies.[49] For patients with low-grade dysplasia, the American College of Gastroenterology recommendations are for surveillance endoscopy at 6-month intervals for the first year, and yearly thereafter if there has been no change. The optimal biopsy technique includes four-quadrant biopsies every 2 cm for nondysplastic epithelium and every 1 cm in the presence of dysplasia[50] (Table 2). Additional biopsy specimens should be taken from any visible lesions or ulceration.

The cost-effectiveness of surveillance endoscopy for Barrett's esophagus compares favorably to that of mammography for the detection of breast cancer.[51] The cost per cancer detected and the cost per patient cured are similar, but the cost per life-year saved is dramatically lower for surveillance endoscopy in patients with Barrett's esophagus than for mammography in women. This likely is because outside of a surveillance program, esophageal cancer often presents in an advanced stage and the clinical course is rapidly fatal. In contrast, the lag time between mammographic and palpable detection of breast cancer is shorter, and the clinical course of patients with breast cancer is often quite protracted.

Although there is increasing evidence that an antireflux operation alters the natural history of Barrett's esophagus, there is insufficient evidence to identify a point at which surveillance endoscopy no longer is necessary. Available data would certainly suggest that surveillance should be done routinely for the first 5 years, as it seems that this is the real risk period for patients with a functioning fundoplication. Additionally, the presence of recurrent symptoms or documented failure of the fundoplication would mandate continued surveillance endoscopy.

TREATMENT

Nondysplastic Barrett's Esophagus

There are four aims of therapy for patients with Barrett's esophagus, and they should be the same for both operative and nonoperative treatment. They include the following:

1. To provide long-term relief of symptoms
2. To allow healing of reflux-induced esophageal mucosal injury including stricture formation
3. To prevent progression to more advanced mucosal injury, dysplastic changes, or carcinoma
4. To induce regression of dysplastic to nondysplastic Barrett's esophagus or of intestinalized to nonintestinalized columnar epithelium

Achieving long-term success in the treatment of Barrett's esophagus, especially long-segment Barrett's, is difficult. The fact that it represents severe GERD and is usually associated with large hiatal hernias along with its premalignant nature combine to frustrate long-term success. Acid suppressive medication is increasingly recognized to be inadequate, and ablative therapies remain difficult, complicated, and investigational. This leaves antireflux surgery as arguably the best treatment option, provided long-term success can be shown.

The relief of symptoms remains the primary force driving antireflux surgery in patients with nondysplastic Barrett's esophagus. Healing of esophageal mucosal injury and the prevention of disease progression are important secondary goals. In this regard, patients with Barrett's esophagus are no different from the broader population of patients with gastroesophageal reflux. They should be considered for antireflux surgery when patient factors suggest severe disease or predict the need for long-term medical management, both of which are almost always true in patients with Barrett's esophagus.

Antisecretory Therapy

Once treatment is initiated, most patients with Barrett's esophagus will require life-long treatment with proton pump inhibitors (PPIs) both to relieve symptoms and control any coexistent esophagitis or

Table 2. Effect of biopsy protocol on endoscopic detection of early cancers in Barrett's esophagus

Biopsy protocol	Cancers detected	% of total
Visible lesions only	13/26	33
Every 2 cm without visible lesion	15/45	50
Every 2 cm and any visible lesion	32/45	71
Every 1 cm and any visible lesion	45/45	100

Adapted from Reid BJ, Blount PL, Feng Z, Levine DS. Optimizing endoscopic biopsy detection of early cancers in Barrett's high-grade dysplasia. Am J Gastroenterol 2000;95:3089–3096.

stricture. Although control of symptoms has historically served as the end point of therapy in patients with Barrett's esophagus, the wisdom of this approach has recently been questioned. Evidence suggesting that reflux control may prevent the development of adenocarcinoma and lead to regression of dysplastic and nondysplastic Barrett's segments (see below) has led many to consider control of reflux and rather than control of symptoms a better therapeutic end point. Complete control of reflux can be difficult, however, as has been highlighted by studies of both acid breakthrough during PPI therapy and persistent reflux after antireflux surgery. Katka and Castell[52] and Ouatu-Lascar and Triadafilopolous[53] have shown that 40% to 80% of patients with Barrett's esophagus continue to have abnormal esophageal acid exposure despite up to 20 mg twice daily of PPIs. Ablation trials have shown that mean doses of 56 mg of omeprazole were necessary to normalize 24-hour esophageal pH studies.[54] It is likely that antireflux surgery results in more reproducible and reliable elimination of reflux of both acid and duodenal content, although results of long-term outcome studies suggest that as many as 25% of patients who undergo Nissen fundoplication will have persistent pathologic esophageal acid exposure postoperatively confirmed by positive 24-hour pH studies.[55]

Antireflux Surgery

Antireflux surgery is an excellent treatment option for most patients with Barrett's esophagus. It must be remembered, however, that patients with Barrett's esophagus generally have severe GERD, with its attendant sequelae such as large hiatal hernia, stricture, shortened esophagus, and poor motility. These anatomic and physiologic features make successful antireflux surgery a particular challenge in this population. Indeed, recent data suggest that antireflux surgery in patients with Barrett's esophagus may not be as successful in the long term as it is in patients without Barrett's esophagus. Once the decision to perform surgery is made, the most important features to identify prior to surgery are the presence of esophageal shortening, failed esophageal body motility, and/or dysplasia. Each of these has significant bearing on the decision for surgical treatment as well as the approach and type of antireflux procedure selected.

Outcome of Antireflux Surgery in Barrett's Esophagus

Studies focusing on the symptomatic outcome after antireflux surgery in patients with Barrett's esophagus document excellent to good results in 72% to 95% of patients at 5 years after surgery.[55,56] Several investigators have compared medical and surgical therapy. Attwood et al.,[57] in a prospective but nonrandomized study, reported on 45 patients undergoing either medical (n = 12) or surgical (n = 19) treatment of Barrett's esophagus. The groups were similar in age, length of Barrett's segment, percentage of time with a pH <4, and length of follow up. Mean symptom scores improved dramatically after antireflux surgery. Symptoms of heartburn and/or dysphagia recurred in 88% of patients treated with medical therapy alone, and 21% after antireflux surgery. Reflux complications, largely the development of an esophageal stricture, occurred in 38% of medically treated and 16% of surgically treated patients ($P < 0.05$) over the 3-year follow-up period. One patient in each group developed esophageal adenocarcinoma. The investigators[7] concluded that antireflux surgery was superior to acid suppression for both the control of symptoms and the prevention of complications in patients with Barrett's esophagus.

Parilla et al.[58] recently reported an update of a study originally published in the *British Journal of Surgery* in 1996.[59] A total of 101 patients were enrolled over an 18-year period (1982 to 2000). Median follow-up was 6 years. Medical therapy consisted of 20 mg of omeprazole (PPI) twice daily since 1992 in all medically treated patients. Surgical therapy consisted of an open 1.5 to 3.0 cm Nissen fundoplication over a 48 to 50 F bougie with division of the short gastric arteries in 39% of patients and crural closure in all. Symptomatic outcome in the two groups was nearly identical, although esophagitis and/or stricture persisted in 20% of the medically treated patients compared to only 3% to 7% of those who had antireflux surgery. Fifteen percent of patients had abnormal acid exposure after surgery. Although pH data were not routinely collected from patients on PPI therapy, in the subgroup of 12 patients who did have 24-hour monitoring while on treatment, 3 (25%) of 12 had persistently high esophageal acid exposure and most (75%) had persistently high bilirubin exposure.

In contrast, Csendes et al.[60] suggested that the long-term results of antireflux surgery in patients with Barrett's esophagus may not be as good as previously thought. They reviewed their long-term results with "classic" antireflux surgery in 152 patients with both complicated and uncomplicated Barrett's esophagus. Fifty-four percent of those with uncomplicated Barrett's esophagus and 64% of those with Barrett's esophagus complicated by stricture or ulceration were classified as failures when symptoms were assessed 8 years postoperatively. Although this report challenges the long-term results of antireflux surgery in patients with Barrett's esophagus, it

is hampered by the fact that 85% of the patients were treated with a Hill repair and those results should not necessarily be extrapolated to patients who undergo Nissen fundoplication.

The outcome of laparoscopic Nissen fundoplication in patients with Barrett's esophagus has been assessed at 1 to 3 years after surgery. Hofstetter et al.[55] reported on our experience at the University of Southern California in 85 patients with Barrett's esophagus at a median of 5 years after surgery. Fifty-nine had long-segment and 26 had short-segment Barrett's esophagus, and a laparoscopic approach was used in 50. Reflux symptoms were absent in 67 (79%) of 85 patients. Eighteen (20%) developed recurrent symptoms, and four were back on daily acid suppressive medication. Seven patients underwent a secondary repair and were asymptomatic, raising the eventual successful outcome to 87%. Postoperative 24-hour pH was normal in 17 (81%) of 21 (Fig. 6). Ninety-nine percent of the patients considered themselves cured (77%) or improved (22%), and 97% were satisfied with the surgery.

Farrell et al.[56] also reported a symptomatic outcome after laparoscopic Nissen fundoplication in 50 patients with both long- and short-segment Barrett's esophagus. Mean scores for heartburn, regurgitation, and dysphagia all improved dramatically after the Nissen procedure. It is important to note that there was no significant decrement in symptom scores when 1-year results were compared to those at 2 to 5 years postoperatively. These investigators did find a higher prevalence of "anatomic" failures requiring reoperation in patients with Barrett's esophagus when compared to non-Barrett's patients with GERD. Others have reported similar results.[61,62]

Impact of Antireflux Surgery on the Metaplasia-Dysplasia-Carcinoma Sequence

Although by no means proved, there is a growing body of evidence attesting to the ability of fundoplication to protect against dysplasia and invasive malignancy. Several recent studies suggest that effective antireflux surgery may have an impact on the natural history of Barrett's esophagus (Table 3). The first such evidence came from an analysis of longitudinal follow-up of patients with Barrett's esophagus in the registry of the American College of Gastroenterologists. All patients had nondysplastic quiescent Barrett's esophagus at initial endoscopy. One hundred nineteen patients received medical treatment and 42 underwent antireflux surgery. Surveillance endoscopy was performed annually. Ten (19.7%) of 119 patients in the medically treated group and two (3.4%) of 42 in the surgical group developed dysplasia.[63] A retrospective review of 118 patients with Barrett's esophagus undergoing antireflux surgery at the Mayo Clinic between 1960 and 1990 revealed three cancers occurring over an 18.5-year follow-up period.[64] All were found within the first 3 years after surgery. The fact that the development of adenocarcinoma was clustered in the early years after antireflux surgery and not randomly dispersed throughout the follow-up period strongly suggests that antireflux surgery

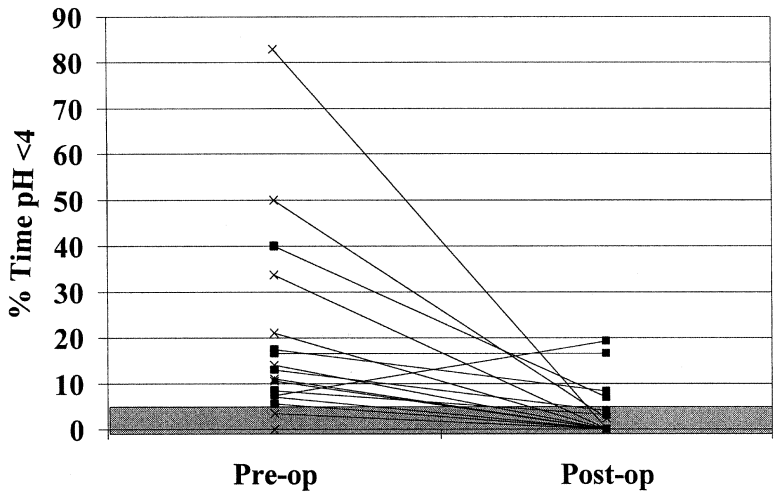

Fig. 6. Twenty-four-hour distal esophageal pH results before and after Nissen fundoplication in 24 patients with Barrett's esophagus studied pre- and postoperatively. (From Hofstetter WA, Peters JH, DeMeester TR, et al. Long term outcome of antireflux surgery in patients with Barrett's esophagus. Ann Surg 2001;234:532–539. Reprinted by permission of the publisher.)

Table 3. Clinical studies on the natural history of Barrett's esophagus

Reference	Year	Findings	Study design	Medical therapy	Surgical therapy
McCallum et al.[63]	1991	Progression to dysplasia more frequent after medical compared to surgical therapy	Retrospective	PPI and H_2; dysplasia in 20% of patients	Open Nissen; dysplasia 30% of patients
Ortiz et al.[59]	1996	Progression to dysplasia more frequent after medical compared to surgical therapy	Prospective	PPI and H_2; dysplasia in 22% of patients	Nissen or Collis-Nissen; dysplasia in 1 patient
McDonald et al.[64]	1996	No adenocarcinoma development later than 39 mo after antireflux surgery	Retrospective	N/A	Nissen ± Collis; 112 patients w/6.5-yr, F/U
Katz et al.[65]	1998	Antireflux surgery protected against development of dysplasia	Retrospective	Mostly H_2; dysplasia/adenocarcinoma in 40% of patients	Mostly Nissen; no dysplasia/adenocarcinoma in patients
DeMeester et al.[67]	1998	Complete regression of microscopic IM in 11/15 patients	Retrospective	N/A	Mostly/laparoscopic Nissen
Low et al.[69]	1999	Complete or partial regression in 12/14 patients	Prospective	N/A	Hill procedure
Spechler et al.[66]	2001	Esophageal adenocarcinoma developed after medical, but not surgical treatment for complicated GERD	Prospective	Multiple medications; adenocarcinoma in 4 patients	Nissen; 1 adenocarcinoma

PPI = proton pump inhibitor; H_2 = H_2 receptor blocker; N/A = not applicable; F/U = follow-up; IM = intestinal metaplasia; GERD = gastroesophageal reflux disease.

altered the natural history of the disease, particularly given the fact that once dysplasia has developed, prospective studies show that carcinoma ensues in an average of 3 years. The occurrence of all observed cancers in the first few years suggests that the point of no return in the dysplasia-cancer sequence had already occurred prior to the time of surgery.

Further evidence that antireflux surgery may alter the natural history of Barrett's esophagus was recently reported by Katz et al.[65] This outcomes study from the Veterans Administration retrospectively reviewed a group of 102 patients undergoing annual surveillance for Barrett's esophagus from 1970 to 1994, for a total of 563 patient-years of follow-up. All specimens with any degree of dysplasia were blinded and rereviewed. Nineteen patients developed new-onset low-grade dysplasia, four had high-grade dysplasia, and three had adenocarcinoma. Antireflux surgery was associated with a significant decreased risk of the development of dysplasia, the presence of which persisted in a multivariate analyses taking into account covariables such as age, sex, and smoking. None of the 15 patients in this study developed dysplasia after antireflux surgery. In the University of Southern California review, no patient developed high-grade dysplasia or cancer in 410 patient-years of follow-up.[55] Finally, two prospective randomized studies found less adenocarcinoma in the surgically treated groups. Parilla et al.[58] reported that although the development of dysplasia and adenocarcinoma was no different overall, the subgroup of surgical patients with normal postoperative pH studies developed significantly less dysplasia and had no adenocarcinoma. Spechler et al.[66] identified one adenocarcinoma 11 to 13 years after antireflux surgery compared to four after medical treatment. Most of these investigators concluded that there is a critical need for future trials exploring the role of antireflux surgery in protecting against the development of dysplasia in patients with Barrett's esophagus.

Regression of Barrett's Esophagus After Antireflux Surgery

The common belief that Barrett's epithelium cannot be reversed is likely false. DeMeester et al.[67] reported that after antireflux surgery, loss of intestinal metaplasia in patients with visible Barrett's esophagus was rare, but did occur in 73% of patients with nonvisible intestinal metaplasia of the cardia. This suggests that the metaplastic process may indeed be reversible if reflux is eliminated early in its process, that cardiac mucosa is dynamic, and that in contrast to intestinal metaplasia, which extends several centimeters into the esophagus, intestinal metaplasia of

the cardia is more likely to regress after antireflux surgery.

Gurski et al.[68] recently reviewed pre- and post-treatment endoscopic biopsies from 77 patients with Barrett's esophagus who were treated surgically and 14 who were treated with PPIs. Post-treatment histologic findings were classified as regression if two consecutive biopsies taken more than 6 months apart plus all subsequent biopsies showed loss of intestinal metaplasia or loss of dysplasia. Histopathologic regression occurred in 28 (36.4%) of 77 patients after antireflux surgery and in 1 of 14 (7.1%) treated with PPIs alone ($P < 0.03$). After surgery, regression from low-grade dysplastic to nondysplastic Barrett's esophagus occurred in 17 (68%) of 25 patients and from intestinal metaplasia to no intestinal metaplasia in 11 (21.2%) of 52 patients (Fig. 7). Both types of regression were significantly more common in short-segment (<3 cm) compared to long-segment (>3 cm) Barrett's esophagus, that is, 19 (58%) of 33 patients and 9 (20%) of 44 patients, respectively ($P = 0.0016$).

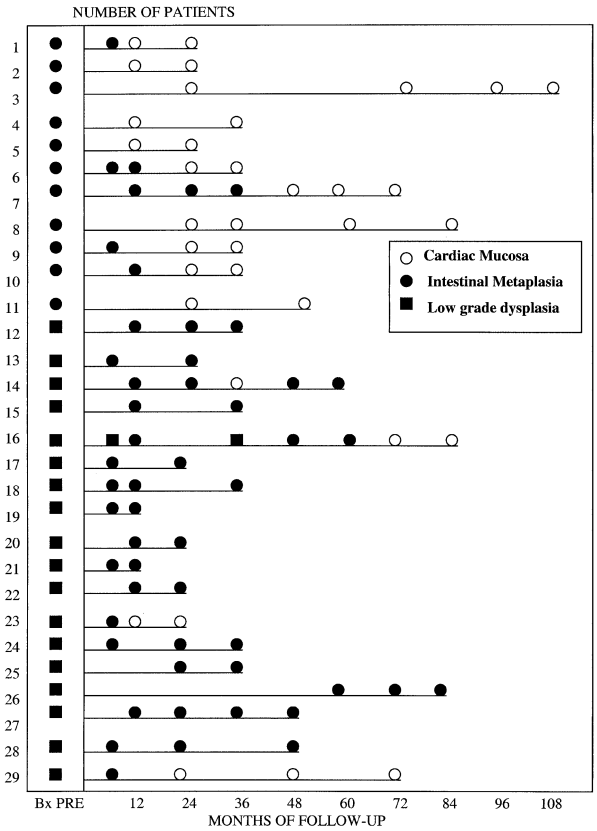

Fig. 7. Schematic representation of histopathologic regression in 29 patients with Barrett's esophagus. (From Gurski RR, Peters JH, Hagen JA, et al. Barrett's esophagus can and does regress following antireflux surgery: A study of prevalence and predictive features. J Am Coll Surg 2003;196:706–712; discussion 712–713. Reprinted by permission of the publisher.)

Eight patients progressed, five from intestinal metaplasia alone to low-grade dysplasia and three from low- to high-grade dysplasia. All those who progressed had long-segment Barrett's esophagus. On multivariable analysis, the presence of short-segment Barrett's esophagus and the type of treatment were significantly associated with regression; age, sex, surgical procedure, and preoperative lower esophageal sphincter and pH characteristics were not. The median time of biopsy-proved regression was 18.5 months after surgery with 95% occurring within 5 years.[68] Similar findings have been reported by the group from the University of Washington[69] and by Bowers et al.[70] Although these studies do not conclusively prove the ability of antireflux surgery to reverse the changes of early Barrett's esophagus, they do provide encouragement that given early changes, the process may indeed be reversible.

Recent evidence suggests that the development of Barrett's esophagus may even be preventable. Although this is a very difficult hypothesis to study, Oberg et al.[71] have followed a cohort of 69 patients with short-segment, nonintestinalized, columnar-lined esophagus over a median of 5 years of surveillance endoscopy. Forty-nine of the patients were maintained on PPI therapy and 20 had antireflux surgery. Patients with antireflux surgery were 10 times less likely to develop intestinal metaplasia in these segments of columnar-lined esophagus over a follow-up span of nearly 15 years (Fig. 8) than those on medical therapy. This rather remarkable observation supports the two-step hypothesis of the development of Barrett's esophagus (cardiac metaplasia followed by intestinal metaplasia) and suggests that the second step can be prevented if reflux disease is recognized and treated early and aggressively.

Ablation

The conceptual need to ablate Barrett's epithelium stems from its potential to degenerate into adenocarcinoma. This fact is important because the foundation on which ablation rests (in nondysplastic Barrett's esophagus) disappears if antireflux surgery is shown to prevent the development of cancer. The best use of ablative technology may be to eliminate Barrett's epithelium prior to antireflux surgery. Ablative methods currently in use include electrosurgery,[72] laser- and argon-directed light waves,[73] photodynamic therapy,[74] and endoscopic mucosal resections.[75] Electrosurgery, laser, and argon light generate heat, which is potentially dangerous to ratio of the techniques used has not been adequately evaluated, particularly in the absence of dysplasia. Furthermore, once the epithelium has been ablated, gastroesophageal reflux

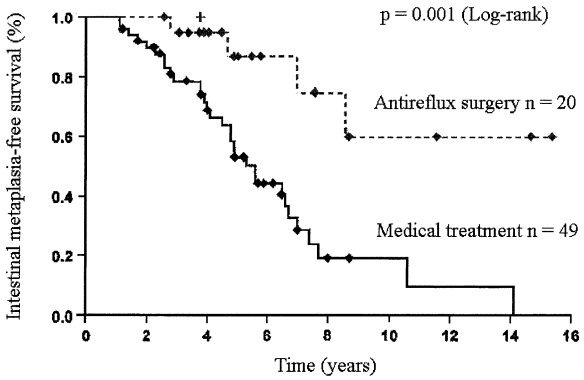

Fig. 8. Development of intestinal metaplasia in patients with nonintestinalized short segments of columnar-lined esophagus on medical therapy and after surgical treatment for Barrett's esophagus. (From Oberg S, Johansson H, Wenner J, et al. Endoscopic surveilence of columnar lined esophagus: Frequency of intestinal metaplasia detection and impact of antireflux surgery. Ann Surg 2001;234:619–626. Reprinted by permission of the publisher.)

must be controlled. Inadequate dosing of antisecretory drugs has resulted in recurrence of the intestinal metaplastic epithelium. All ablative methods are presently regarded as investigational.

Ablation of high-grade dysplasia or early adenocarcinoma has greater merit.[76] The critical issues yet to be resolved are whether the absence of a cancer can be accurately predicted and if cancer is present if the depth of the tumor (intramucosal vs. submucosal) and the presence of regional nodal metastases can be correctly predicted before surgery. The accuracy of endoscopic ultrasound imaging in determining the depth of tumors confined to the esophageal wall is questionable.[77] In most hands, the resolution of the present-day endoscopic ultrasonographic systems is not sufficient to predictably differentiate the fine detail of tumor infiltration when it is limited to the esophageal wall. In Japan, endoscopic mucosal resection has been used to resect dysplasia and early squamous cell carcinoma and relies on high-frequency ultrasound (20 MHz) to accurately determine the T-stage of the tumor.[78]

DYSPLASTIC BARRETT'S ESOPHAGUS

Dysplasia is defined as neoplastic epithelium that is confined within the basement membrane of the gland or epithelium within which it arose. The histopathologic classification of dysplasia in Barrett's epithelium relies on identification of cytologic and tissue architectural changes originally described in 1983 for ulcerative colitis[79] and subsequently modified for Barrett's esophagus.[80] Dysplasia is currently classified into the following four categories: (1) no dysplasia (intestinal metaplasia); (2) indefinite for dysplasia; (3) low-grade dysplasia; and (4) high-grade dysplasia. Prior to intervention, the diagnosis of high-grade dysplasia should be confirmed by at least two expert pathologists. Unfortunately, there is considerable interobserver disagreement among even expert gastrointestinal pathologists.[81] This is particularly true of the low-grade and indefinite categories. Repeat endoscopy with extensive biopsy should be performed if significant interobserver disagreement is encountered. Endoscopy with four-quadrant biopsies at 1 cm rather than 2 cm intervals within the visible columnar segment is recommended in the presence of dysplastic tissue.[82] Even using this 1 cm protocol, it is not possible to be certain that cancer is not present in patients with known high-grade dysplasia. Emphasizing this fact is the study by Cameron and Carpenter[83] in which they mapped esophagectomy specimens from 30 patients with high-grade dysplasia or early adenocarcinoma. The median surface area of the adenocarcinomas was 1.1 cm^2, and the three smallest cancers had surface areas of 0.02, 0.3, and 0.4 cm^2.

It can be difficult to distinguish between high-grade dysplasia and well-differentiated intramucosal adenocarcinoma. Most use the term high-grade dysplasia in the presence of neoplastic changes involving the epithelium but not extending into the lamina propria, i.e., superficial to the basement membrane.[84] Neoplastic disease involving the epithelium and lamina propria superficial to the muscularis mucosa is termed intramucosal adenocarcinoma. The term carcinoma-in-situ has largely been replaced by use of the term high-grade dysplasia.

The estimated prevalence of low-grade dysplasia in Barrett's esophagus ranges from 15% to 25%, whereas the prevalence of high-grade dysplasia is approximately 5%. The incidence of development of dysplasia is approximately 5% per year [85-87] (Table 4). O 'Conner et al.[88] prospectively followed 136 patients for a mean 4.2 years. Patients with both long-segment (≥3 cm, n = 106) and short-segment (<3 cm, n = 30) Barrett's esophagus were included. High-grade dysplasia developed in four patients (2.9%) and low-grade dysplasia developed in 24 patients (17.6%). The median time to development of low-grade dysplasia was 3.0 years (range 0.07 to 12.7 years). In another prospective study, Levine et al.[89] studied 62 patients with Barrett's esophagus for a mean 34 months. These investigators documented the development of low-grade dysplasia in 10 of 39 patients with no dysplasia on entry into the study, one new case of high-grade dysplasia, and one invasive

Table 4. Prevalence of the development of dysplasia in studies of Barrett's esophagus

Reference	Barrett's segment length	No. of patients	Mean follow-up (yr)	No. of patients developing dysplasia	% of patients developing dysplasia/yr
Hameetamen et al.[85]	Long	50	5.2	10	3.8
McCallum et al.[63]	Long	152	4	30	4.9
Ortiz et al.[59]	Long	27	4	6	5.5
Sharma et al.[86]	Short	32	3	5	5.2
Weston et al.[87]	Short	26	1.5	2	5.1
	Long	29	2	6	10.3
O'Conner et al.[88]	Short	30	4.2	4 (all LGD)	13.3
	Long	106	4.2	28 (4 HGD)	26

LGD = low-grade dysplasia; HGD = high-grade dysplasia.

carcinoma. Three patients with low-grade dysplasia at entry developed high-grade dysplasia.

Operative Management of High-Grade Dysplasia

There are three options for the management of patients with high-grade dysplasia; each of them has been advocated as the treatment of choice. They are as follows:

1. Endoscopic surveillance until carcinoma identified
2. Mucosal ablation
3. Esophagectomy

The optimal treatment is controversial partly because the natural history of high-grade dysplasia is uncertain. Prospective studies documenting that a minority of patients progress to detectable adenocarcinoma support a conservative approach to the management of these patients. Watchful waiting, however, involves a time-consuming, labor-intensive, expensive protocol that is impractical in most practice settings. Large cohorts of patients with high-grade dysplasia have now been prospectively followed at the University of Washington[89,90] and the University of Kansas[91] and retrospectively reviewed at the Hines Veterans Hospital in Chicago.[92] These data clearly show that cancer will be identified (identified is a more appropriate term than develop, as many of these patients may have had carcinoma for some time prior to its detection) in approximately 25% of patients at 1.5 years,[89] 50% at 3 years,[91] and up to 80% 8 years later.[90] The 80% figure should be interpreted in light of the fact that there is a 20% or so error rate in the pathologic diagnosis of high-grade dysplasia. Thus the natural history of high-grade dysplasia is becoming clear. Most patients will have an invasive adenocarcinoma identified during a 5- to 10-year surveillance period, although a significant minority may not. Although far from perfect, these facts, particularly when taken in association with p53 and cell cycle (flow cytometry) abnormalities, give the clinician significant information upon which to base clinical decisions.

Underscoring these points, a decision analysis study, to test whether esophagectomy or continued surveillance is the optimal treatment for patients with high-grade dysplasia, was recently reported in abstract form.[93] Seven strategies were tested, the first being immediate esophagectomy and the remaining six being surveillance for 3, 6, 12, 18, and 24 months and esophagectomy if cancer was identified, and finally no cancer ever identified. The simulation continued until all patients died from cancer or other causes. A 5-year estimate of the development of cancer in high-grade dysplasia of 20% to 50% was used (quite reasonable considering the data presented earlier) and included operative mortality and both short- and long-term disability associated with esophagotomy. Immediate esophagectomy was the preferred treatment for all values of cancer risk anywhere from 10% to 50%. Furthermore, immediate esophagectomy had the greatest gain in quality-adjusted life years. Esophagectomy remained the preferred treatment unless the incidence of cancer fell below 3% at 5 years, the operative mortality rose to more than 64%, or the quality-adjusted life years after esophagectomy declined to less than 0.5 (0 dead, 1 normal). These rather surprising data lend further credence to the decision to perform esophagectomy in patients with high-grade dysplasia.

Although efforts to achieve effective ablation of dysplastic Barrett's esophagus have been ongoing for more than a decade, major obstacles remain. Ablation of large segments of Barrett's epithelium is compromised by the fact that residual Barrett's epithelium remains in as much as half of the patients and 25%

to 30% will develop severe complications such as stricture or motility disturbances.[74,94] Effective ablation of small areas of dysplasia, whether by mucosal resection or thermal or photodynamic energy requires accurate localization. Localization of a nonvisible area containing high-grade dysplasia is presently not possible, although technologies looming on the horizon, such as optical coherence tomography, may make this a clinical reality. Finally, investigators at the Mayo Clinic Rochester have found that despite the histologic absence of dysplasia after ablation, genetic abnormalities characterizing a premalignant epithelium remain.[95] Ablation is still an elusive goal.

Evidence supporting the performance of esophagectomy in patients with high-grade dysplasia comes from studies of esophagectomy specimens from patients with a preoperative diagnosis of high-grade dysplasia without carcinoma.[96] A report from the Cleveland Clinic that included the use of a biopsy protocol employing jumbo forceps found adenocarcinoma to be present in 10 of 28 patients who had a maximum preoperative diagnosis of high-grade dysplasia.[97] Despite the use of a surveillance protocol that employed four-quadrant biopsies at 1 or 2 cm intervals within the Barrett's segment, a group from the the University of California San Francisco recently reported that adenocarcinoma was present in esophagectomy specimens in 4 of 11 patients with a maximum preoperative diagnosis of high-grade dysplasia.[61] Three of the cancer patients had disease that was not limited to the mucosa and died within 16 months of operation. Overall, between one-third and one-half of patients with a maximum diagnosis of high-grade dysplasia will have an occult adenocarcinoma. We believe that this fact justifies consideration of esophageal resection in all patients with a definite diagnosis of high-grade dysplasia. It is not possible with the present technology, including endoscopic ultrasound imaging, to differentiate between patients who do or do not harbor a cancer. It is important to note that the combination of regular surveillance and esophagectomy for patients with high-grade dysplasia has been shown to result in the detection of early-stage cancers and consequently high overall survival rates[98,99](Fig. 9). Five-year survival approaches 90% in this setting. When invasive cancer is found, most of these tumors will be limited to the wall of the esophagus, and few will have spread to regional lymph nodes.

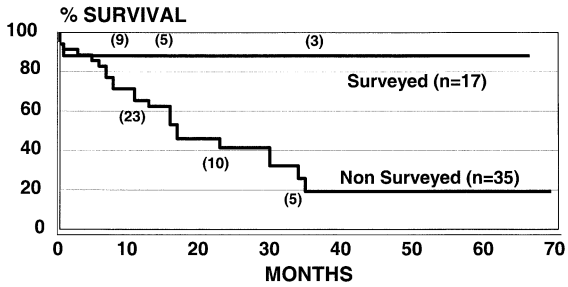

Fig. 9. Kaplan-Meier survival curves for patients enrolled and not enrolled in an endoscopic surveillance program for Barrett's esophagus. (From Peters JH, Clark GWB, Ireland AP, et al. Outcome of adenocarcinoma arising in Barrett's esophagus in endoscopically surveyed and non-surveyed patients. J Thorac Cardiovasc Surg 1994;108:813–822. Reprinted by permission of the publisher.)

Extent of Resection for High-Grade Dysplasia

The standard surgical resection for patients with high-grade dysplasia includes a total esophagectomy, removing all Barrett's tissue and any potential associated adenocarcinoma. This is generally accomplished via a transhiatal or transthoracic esophagectomy, with most expert centers favoring the transhiatal approach. Reconstruction is generally accomplished by means of a posterior mediastinal gastric "pull-up," with the anastomosis in the neck. Intrathoracic anastomoses are to be avoided because of the high prevalence of disabling reflux symptoms after an intrathoracic esophagogastrostomy. The mortality rate associated with this procedure should be less than 5%, and is less than 1% in centers experienced in esophageal surgery. Functional recovery is good to excellent in the vast majority of patients.

Recently we have used a transhiatal vagal-sparing esophageal stripping procedure, with colon interposition, as a more physiologic alternative to standard transhiatal esophagectomy in patients with high-grade dysplasia.[100] Sparing the vagal nerves improves functional outcome by eliminating one of the major sources of postoperative alimentary morbidity after esophagectomy, namely, the postvagotomy effects of gastric atony and postvagotomy diarrhea. Its use is limited, however, to patients in whom there is little or no likelihood of lymph node metastases. Selecting such patients can be difficult. Recent data from our experience indicate that given careful endoscopic examination and biopsy, in the absence of a visible lesion, nodal disease is rare.[101] The lymph node status of 10 patients with no endoscopically visible lesion, and a biopsy diagnosis of high-grade dysplasia or intramucosal adenocarcinoma, was retrospectively reviewed after en bloc esophagectomy. A total of 370 lymph nodes from these 10 patients were examined by both conventional histopathologic and immunohistopathologic methods. Only one lymph node contained metastatic disease. In contrast, five of nine patients with an endoscopically visible lesion and a preoperative diagnosis of either high-grade dysplasia

or intramucosal adenocarcinoma had metastasis to regional lymph nodes. If an endoscopically visible lesion is present, the frequency of submucosal disease is high. Because tumors that invade through the muscularis mucosa into the submucosa have a 60% or greater incidence of lymph node metastasis, it seems prudent to perform a regional lymph node dissection with esophagectomy for the treatment of visible lesions, regardless of the histologic findings on biopsy (i.e., high-grade dysplasia or intramucosal carcinoma). Recent studies indicate, however, that in early adenocarcinoma in Barrett's esophagus, metastases do not appear to involve the splenic artery nodes and the spleen. Splenic artery dissection and splenectomy are therefore not necessary in this circumstance, nor is extended gastric resection.

Without question, removing the esophagus is a major undertaking that is often fraught with significant morbidity and mortality. What is often underestimated is the intensity of resources and emotional burden associated with the decision to pursue surveillance every 3 months. When given the option, many patients prefer to eliminate the possibility of developing esophageal adenocarcinoma, even if esophagectomy is the price to do so. Our challenge is to improve the state of the art such that this can be accomplished with as little morbidity as possible. After esophagectomy, the average mortality rate has steadily decreased over the past two or three decades from over 25% to 2% to 4% in most centers. It approaches zero in large series of resection for benign disease[102] (in which patients with high-grade dysplasia can be included) and in units that have specifically focused on preventing death from esophageal resection, such as the unit in Hong Kong.[103] That being said, as these investigators aptly point out, it is arguably the most sensitive surgical procedure to volume-outcome relationships.

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Minimally Invasive Surgery for Achalasia: A 10-Year Experience

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Minimally invasive esophagomyotomy for achalasia has become the preferred surgical treatment; the employment of a concomitant fundoplication with the myotomy is controversial. Here we report a retrospective analysis of 53 patients with achalasia treated with laparoscopic Heller myotomy; fundoplication was used in all patients except one, and 48 of the fundoplications were complete (floppy Nissen). There were no deaths or reoperations, and minor complications occurred in three patients. Good-to-excellent long-term results were obtained in 92% of the subjects (median follow-up 3 years). Two cases (4%) of persistent postoperative dysphagia were documented, one of which was treated with dilatation. Postoperative reflux occurred in five patients, four of whom did not receive a complete fundoplication; these patients were well controlled with medical therapy. We suggest that esophageal achalasia may be successfully treated with laparoscopic Heller myotomy and floppy Nissen fundoplication with an acceptable rate of postoperative dysphagia. (J GASTROINTEST SURG 2004;8:18–23)

KEY WORDS: Achalasia, esophagomyotomy, minimally invasive surgery, dysphagia, gastroesophageal reflux

Achalasia, the most common motility disorder of the esophagus, has an incidence of 0.5 to 1 per 100,000.[1–3] The etiology of achalasia may involve autoimmunity and/or viral infection, but this is controversial.[4–6] Heller described the successful surgical treatment of chronic cardiospasm in 1913.[7] His anterior and posterior cardiomyotomy technique was modified in 1923 by Zaaijer[8] to include a single anterior myotomy. Since then, standard surgical treatment for achalasia has been this modified Heller myotomy performed through the abdomen or chest.[9] Heller myotomy is successful in 85% to 90% of patients with achalasia, which compares favorably with nonoperative treatment.[10–13] A minimally invasive approach to Heller myotomy became standard in the 1990s.[9,14,15] We began treating achalasia with minimally invasive Heller myotomy in 1992; this is a 10-year retrospective report of 53 consecutive patients treated with this procedure.

PATIENTS AND METHODS

Between January 1992 and August 2002, data were collected on patients who underwent laparoscopic Heller myotomy for esophageal achalasia, under the supervision of one of us (C.T.F.). Routine preoperative evaluation included a history and physical examination, esophagogastroduodenoscopy, manometry, and barium esophagram. Manometry was used

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to confirm the diagnosis of achalasia (i.e., presence of lower esophageal dysfunction). The barium esophagram (real-time fluoroscopic) was used as a semiquantitative measure of esophageal peristalsis. Traditionally achalasia has been associated with absence of peristalsis in the esophageal body. Because the pathophysiology of achalasia involves the lower esophageal sphincter, however, peristalsis in the esophageal body can still be present, even in advanced cases of achalasia.[16,17] Mild-to-moderate dysmotility was defined as zero to one peristaltic waves and severe dysmotility was defined as two or more peristaltic waves in the esophageal body during the course of the barium study. This fluoroscopic assessment of esophageal peristalsis provided an indicator of preoperative disease severity.

The technique of laparoscopic Heller myotomy has been well described[18]; briefly, a 6 to 7 cm myotomy on the anterior cardia (extending to the distal esophagus) was performed with an insulated hook electrocautery after mobilization of the gastroesophageal junction. The insulated hook cautery device (product under development) consisted of a conventional hook cautery with insulation covering the outer edge of the hook. This arrangement limits the application of electrical energy to the tissue, which is caught within the inner edge of the hook, minimizing any collateral injury to tissue that may inadvertently contact the outer edge of the hook. The myotomy is performed over the tapered tip of a 50 F lighted esophageal bougie (Fig. 1). After completion of the myotomy, the bougie is advanced so that the 50 F circumference is appropriately placed at the level of the gastroesophageal junction. The fundoplication is then performed with the bougie in proper position.

Either a floppy Nissen[19,20] or a Toupet (posterior 270-degree)[18] fundoplication was performed after the myotomy; the pertinent details of the Nissen fundoplication are given here. Division of the short gastric vessels is routine. The distal esophagus is mobilized downward so that 4 to 5 cm is intra-abdominal without tension. The esophageal hiatus is closed around the esophagus (with the 50 F bougie in place) using interrupted 2-0 polyester sutures if necessary. The fundus is carefully identified to avoid creating a wrap with the body of the stomach. The wrap is constructed using three 2-0 polyester sutures with the 50 F bougie in place and is 2 cm long when completed. The cephalad wrap stitch anchors the wrap to the diaphragm; none of the stitches incorporate the esophagus. The completed fundoplication is tested for laxity by inserting a 10 mm instrument alongside the esophagus. If the wrap is not loose, it is taken down and reconstructed.

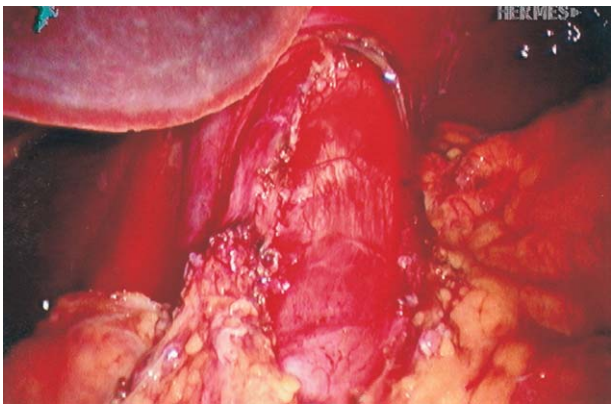

Fig. 1. Esophagomyotomy with lighted bougie in place.

Routine postoperative visits were scheduled at 1 week, 1 month, 3 months, and yearly after surgery; if a visit was not possible, the patient was contacted by phone. Each patient was questioned specifically about dysphagia, heartburn, and regurgitation. Routine postoperative testing included a barium esophagram within the first 3 months after the operation. Postoperative manometry and endoscopy were performed at the discretion of the referring gastroenterologist. Postoperative outcome was scored according to the method of Visick,[21] who described a method of classifying postgastrectomy outcome based on patients' symptoms. We have modified this scoring system to apply to our series and simply refer to the system as the modified Visick, or m-Visick. An excellent result (m-Visick I) was defined as the patient with rare (once per week or less) to no episodes of dysphagia or gastroesophageal reflux; these patients typically did not take medications for esophageal symptoms. A good outcome (m-Visick II) was defined as occasional episodes (several times per week) of dysphagia and/or reflux that may have required medication. A fair result (m-Visick III) was defined as more frequent symptoms controlled with (daily or near-daily) medical treatment. Unsatisfactory outcome and/or treatment failure (m-Visick IV) was defined as symptoms that were poorly controlled with medication and/or any patient undergoing reoperation.

RESULTS

All of the patients (n = 53; 37 men and 16 women; mean age 48 [range 21 to 75] years) in this series had preoperative dysphagia. The diagnosis, evaluation, and any endoscopic therapy for achalasia were performed by each patient's gastroenterologist prior to the surgical referral. Routine preoperative barium

esophagram revealed severe or mild-to-moderate dysmotility in 18 (34%) and 35 (66%) patients, respectively. Eighteen patients (34%) underwent preoperative pneumatic dilatation and nine patients (17%) had intrasphincteric botulinum toxin injections; 26 patients (49%) did not have any endoscopic therapy prior to esophagomyotomy.

All patients underwent a laparoscopic Heller myotomy. Forty-eight patients (90%) underwent concomitant Nissen fundoplication, four patients (8%) underwent Toupet fundoplication (patients 2, 6, 15, and 17, who all had severe esophageal body dysmotility), and one (patient 42, who had megaesophagus with severe dysmotility) did not receive a fundoplication. Average operative time was 87 ± 24 minutes (standard deviation [SD]; range 41 to 148 minutes); there were no open conversions or intraoperative complications. The median hospital stay was 2 days (range 1 to 4 days), and there were no deaths. Major postoperative complications consisted of one short-term reintubation and one case of pneumonia; the former patient was extubated prematurely and required reintubation in the recovery room, and the latter patient aspirated during induction of anesthesia. Minor complications consisted of urinary retention (n = 2), atelectasis (n = 2), and trocar site hematoma (n = 1).

Median follow-up was 3 years (range 6 months to 9 years). There were no reoperations. The m-Visick scores of postoperative outcome are presented in Table 1; the rate of the m-Visick I–II was 50/53 = 94%. Postoperative gastroesophageal reflux and dysphagia occurred in five (9%) and two (4%) patients, respectively. Four of the five patients with postoperative reflux had severe preoperative esophageal dysmotility, and one patient had moderate preoperative dysmotility; the concurrent antireflux procedure in these patients was a Nissen fundoplication (the one patient with moderate dysmotility), a Toupet fundoplication (n = 3), or no fundoplication (n = 1). These five patients opted for medical management of their postoperative reflux; four of these patients were classified as m-Visick II, and the fifth patient (the only person in this series without an antireflux procedure) had postoperative reflux severe enough to be placed in the m-Visick III category.

One of the two patients with postoperative dysphagia (see Table 1) had severe dysmotility on preoperative evaluation, and the other had mild-to-moderate dysmotility. Both of these patients had a Nissen fundoplication at the time of their myotomies, and both of their postoperative esophagrams indicated either an incomplete myotomy or an excessively tight wrap. The former patient declined any further intervention after the myotomy. This patient's postoperative dysphagia was improved compared to the preoperative state, so this patient was classified as m-Visick III. The latter patient with postoperative dysphagia (who preoperatively had mild-to-moderate dysmotility) underwent successful pneumatic dilatation of the distal esophagus. Even though his final outcome was good, he was classified as m-Visick IV because the surgical procedure was not successful.

DISCUSSION

In a total of 616 patients treated with minimally invasive esophagomyotomy culled from 12 publications on the subject, the rate of m-Visick I–II outcome was 90% (Table 2). This data summary is flawed, of course, because of the wide range of definitions, follow-up, and evaluation schemes used in these studies. Nevertheless, the results of our series are in concordance with the available results from patients undergoing minimally invasive esophagomyotomy for achalasia. This occurred despite our near-routine use of complete (Nissen) fundoplication. The addition of a fundoplication to an esophageal myotomy for achalasia is controversial. Many investigators advise selective utilization of fundoplication (especially partial) during esophagomyotomy for achalasia, because of the concern of wrap-induced dysphagia.[22,23] We attribute the low rate of dysphagia in our patients with complete wraps to our technique of floppy fundoplication, which is slightly modified from

Table 1. m-Visick scores in the follow-up period

m-Visick score	No. of patients	Severe preoperative dysmotility	Mild-to-moderate preoperative dysmotility	Fundoplication		
				N	T	None
I	46	11	35	46	0	0
II	4	3	1	1	3	0
III	2	2	0	0	1	1
IV	1	0	1	1	0	0
TOTAL	53	16	37	48	4	1

N = Nissen; T = Toupet.

Table 2. Published results of minimally invasive esophagomyotomy

Reference	No. of cases	m-Visick I–II (%)	Major complications (%)	Postoperative dysphagia (%)	Postoperative GERD (%)	Mean follow-up (mo)
Ancona et al.[30]	17	94	0	6	0	7
Rosati et al.[31]	25	96	4	4	NA	12
Delgado et al.[32]	12	83	1.6	16	0	3
Swanstrom and Penning et al.[33]	12	92	0	8	17	16
Hunter et al.[34]	40	90	7.5	10	5	12
Vogt et al.[35]	20	90	10	10	10	2
Wang et al.[36]	30	89	NA	22	54	18
Kjellin et al.[37]	21	100	9.5	29	21	22
Patti et al.[14]	133	90	9	11	17	23
Heniford et al.[38]	49	96	10	NA	NA	12
Luketich et al.[39]	57	92.5	14.5	9.4	9.4	19
Bloomston and Rosemurgy et al.[40]	100	86	9	16	14	22
Mean (total)	(516)	91.5	6.8	12.8	14.7	14

GERD = gastroesophageal reflux disease; NA = data not available.
Total number of cases = 516.

the classic description of Donahue et al.[19] (see Patients and Methods).

Early in the series we performed either a Toupet or no fundoplication in conjunction with the myotomy in five patients who had severe esophageal body dysmotility, because the convention had been to perform (at most) a partial fundoplication after the myotomy in patients with advanced disease. Four of these patients developed postoperative reflux. Subsequently we decided to perform a floppy Nissen fundoplication in conjunction with the myotomy in all patients. There have been several reports[24,25] of postmyotomy reflux being the cause of severe esophagitis, Barrett's metaplasia, and adenocarcinoma. In addition, 24-hour pH monitoring studies have revealed a decrease in the frequency of reflux when a fundoplication is performed.[26] To address these concerns, our standard approach has been to employ fundoplication routinely with myotomy for surgical treatment of achalasia. It is conceivable that there were cases of occult postoperative reflux in our series; because routine postoperative pH monitoring was not performed, however, these hypothetical cases were not diagnosed. The importance of reflux that can be detected with the pH probe, but which does not cause symptoms, is unclear.

The type of fundoplication to perform in conjunction with an esophageal myotomy is somewhat controversial. The Toupet fundoplication purportedly results in decreased postoperative reflux and dysphagia compared to a complete wrap[27]; the Dor fundoplication has been favored because it is an easily constructed partial wrap that covers the myotomy site.[28,29] Comparative data from various fundoplication methods in prospective trials are lacking. Our series suggests that a floppy Nissen fundoplication can be performed in conjunction with esophageal myotomy in patients with achalasia without incurring postoperative dysphagia.

Common causes of persistent dysphagia after Heller myotomy include underlying esophageal dysmotility, incomplete myotomy, preoperative error in diagnosis, esophageal stricture, improperly constructed fundoplication, spontaneous closure of the myotomy, or any combination of the above. The etiology of persistent postoperative dysphagia in the two patients in this series is not clear. Spontaneous closure as an etiology is unlikely because of a lack of a symptom-free interval; preoperative error in diagnosis is unlikely because of the extensive (endoscopic, manometric, and radiographic) preoperative evaluation obtained in these two patients, and there was no evidence of stricturing noted intraoperatively. An improperly constructed fundoplication or an incomplete myotomy is a more likely cause(s) of persistent dysphagia in these two patients. Because these patients did not undergo reoperation, the precise cause remains unknown.

We maintain that the technique of floppy fundoplication, as described in the Patients and Methods section, helped produce the low rate of postoperative dysphagia. In particular, we believe that our practice of anchoring the fundoplication to the crura, but not to the esophagus itself, facilitates the final product of a loose wrap. Another factor that likely contributed to our low rate of dysphagia was our use of the lighted

esophageal bougie during the myotomy. We particularly like how the bougie illuminates the muscle fibers during the myotomy, which we believe improves the precision of this procedure; there is a superb visualization of the interface between the muscularis propria and the submucosa. In addition, the surgeon can watch (via the laparoscope) the insertion of the lighted bougie past the gastroesophageal junction, and thereby advise the anesthetist on his/her progress with the insertion. We believe that this communication improves the safety of the potentially dangerous bougie insertion. In more than 600 laparoscopic esophageal procedures in which the lighted bougie was used, we have not had any insertion-related complications.

In most of the patients in this series, we treated achalasia with laparoscopic Heller myotomy and floppy Nissen fundoplication, and obtained long-term results comparable to those from other published series. Even though we used a complete wrap in the vast majority of our patients, the rate of postoperative dysphagia was at the low end of the published range. We support the concept that laparoscopic Heller myotomy with floppy Nissen fundoplication is a sound surgical option for achalasia.

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Esophageal Achalasia: Is the Herpes Simplex Virus Really Innocent?

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This study was designed to test the hypothesis that mononuclear cells in the myenteric plexus of patients with achalasia may be activated by herpes simplex virus type 1 (HSV-1). Strips of esophageal muscle were obtained from patients with achalasia and multiorgan transplant donors who served as control subjects. After muscle digestion, mononuclear cells were purified through a Percoll gradient and cultured in medium, either alone or containing ultraviolet-inactivated HSV-1 or poliovirus (multiplicity of infection 1:1.5). As an indicator of HSV-1–induced lymphocyte activation, we determined T-cell proliferation by means of ^{3}H-thymidine incorporation and interferon gamma release. DNA was extracted from esophageal muscle of achalasia patients and control subjects, and used as a template for PCR analysis using primer pairs specific for HSV-1. Circulating anti–HSV-1 and HSV-2 antibodies were detected by enzyme-linked immunosorbent assay on serum samples. Fifteen patients with naive achalasia and eight control subjects were studied. The prevalence of circulating anti–HSV-1 and HSV-2 antibodies proved similar in the two groups, and no HSV-1 DNA was detected by polyermase chain reaction in the esophageal muscle samples. The proliferative index in mononuclear cells from achalasia patients stimulated with HSV-1 showed a 3.4-fold increase in comparison with control subjects ($P < 0.01$). In addition, a 1.4-fold increase in interferon gamma release after incubation with HSV-1 was observed in cells from achalasia patients but not control subjects. The results of this study indicate that HSV-1–reactive immune cells are present in lower esophageal sphincter muscles of patients with achalasia. We hypothesize that the HSV-1–reactive lymphocytes in lower esophageal sphincter muscles of achalasia patients may contribute to damage of the neurons in the myenteric plexus and lead to the motor dysfunction. (J GASTROINTEST SURG 2004;8:24–30)

KEY WORDS: Achalasia, herpes simplex virus, lymphocyte activation

Esophageal achalasia is a relatively rare motor disorder characterized by the absence of esophageal peristalsis and incomplete relaxation of the lower esophageal sphincter (LES). These motor abnormalities cause a functional obstruction of the esophagus. Although the clinical picture has been recognized for more than 300 years[1] and the functional anomaly understood since the early 1970s,[2] the pathogenesis of this disease remains elusive. Achalasia is the result of a chronic inflammatory process involving the myenteric plexus. Neuritis and ganglionitis are evident in the early stages of the disease, leading to a progressive loss of ganglion cells and fibrosis.[3–5] Immunohistochemical studies have shown that most of the inflammatory cells infiltrating the myenteric plexus are activated by CD3-positive/CD8-positive lymphocytes.[6]

A myenteric plexus infiltrate rich in lymphocytes is consistent with an immune-mediated disease, but which antigen elicits the abnormal immune response remains an enigma.[7] Recent reports have clearly indicated a role for genetic factors because achalasia is associated with class II antigens of the major histocompatibility complex such as HLA DQw1.[8] Although the infectious hypothesis remains attractive, neither pathogens nor their DNA have been convincingly demonstrated in tissue specimens from patients

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with achalasia.[7] It may be, however, that the infectious agent represents only the factor initiating an immune reaction in the myenteric plexus, and this immune reaction eventually overcomes the infectious agent, but in genetically predisposed subjects it also damages ganglion cells. Among the potential infectious agents, human herpes simplex virus type 1 (HSV-1) presents some intriguing features with regard to achalasia; these include a preference for squamous epithelia–layered mucosa, high neuronal tropism, ability to persist in a latent state in neurons, and induction of a strong humoral and cell-mediated immune response.[9] Because a T-cell–mediated immune response is essential to eliminate viruses[10] and cytotoxic T lymphocytes are the predominant cell type infiltrating the esophagus of achalasia patients, we speculated that HSV-1–reactive lymphocytes may be involved in the ganglionitis in achalasia. To test this hypothesis, we evaluated whether mononuclear cells from the LES of achalasia patients would react to HSV-1 antigens.

MATERIAL AND METHODS

Patients and Sample Collection

Specimens from LES muscle were harvested during laparoscopic myotomy performed in the Department of Medical and Surgical Science at the University of Padova between April 2001 and March 2003. At the time of esophagomyotomy, a 5 mm wide strip of muscularis propria was obtained at the level of the cardia, on the right side of the myotomy, from patients who had not had previous endoscopic treatment for achalasia (dilatation or Botox injection). Control specimens were harvested from eight heart-beating cadaveric organ donors; in these cases a much larger piece of muscle (15 mm) was obtained. None of the cadaveric donors had clinical records consistent with a history of dysphagia. Three of the patients had received 8 to 24 mg of dexamethasone; corticosteroid therapy was suspended 24 hours before organ harvesting. Immediately after harvesting, the specimens were placed in ice-cold RPMI 1640 tissue culture medium supplemented with 10 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B and processed within 3 hours. A fragment of each tissue sample was snap-frozen in liquid nitrogen and stored at −80C to extract tissue DNA. At the same time as the surgical procedure, 15 ml of blood was obtained from achalasia patients and control subjects.

Isolation of Esophageal Lymphocytes

To purify lymphocytes infiltrating the LES, full-length specimens were cut into small pieces (2 × 2 mm) and then digested (37C for 45 minutes) in 10 ml of Hank's balanced salt solution (Invitrogen Italia SRL, San Giuliano Milanese, Italy) supplemented with 2.5 mg collagenase (Sigma-Aldrich, Milano, Italy), 150 μg DNase I (Calbiochem-Novabiochem, La Jolla, CA), penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml). To obtain a single cell suspension, digested tissues were forced through an 80 μm nylon mesh, and mononuclear cells were purified by centrifugation through a Percoll (Amersham Pharmacia Biotech Italia, Cologno Monzese, Italy) gradient as described elsewhere.[11] Cell viability, determined by trypan blue exclusion, was greater than 95% in all of the preparations tested. The purification procedure yielded 1 to 4 million cells from each patient and organ donor, although a larger piece of tissue was harvested from the latter.

Virus Preparation

HSV-1 strain 16 and wild-type poliovirus were used throughout our studies. Viral stocks were prepared and assayed on monolayers of Vero cells in modified Dulbecco's medium containing 10% heat-inactivated fetal calf serum.[12] Viral titer was determined by standard plaque techniques on Vero cells and expressed as plaque-forming units /ml. Aliquots (200 μl) of viral stocks (10^8 plaque-forming/ml) were subjected to ultraviolet inactivation, and absence of replication-competent viruses was confirmed in vivo on Vero cells. As control specimens, we used equal amounts of conditioned media from mock-infected Vero cells.

Esophageal Mononuclear Cell Proliferation Assay

Mononuclear cells isolated from LES muscles were seeded in 24-well cell culture plates in RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml), β-mercaptoethanol (50 μmol/L), L-glutamine (2 mmol/L), and 10% heat-inactivated fetal calf serum. Cells were seeded at a concentration of 10^6/ml and cultured at 37C in a 95% humidified atmosphere containing 5% CO_2 for 72 hours. Purified esophageal mononuclear cells were cultured for 48 hours in medium alone or supplemented with ultraviolet-inactivated HSV-1 or poliovirus suspension with a multiplicity of infection (MOI) of 1:1.5. Then 1 μCi/well of ^{3}H-thymidine (Amersham Pharmacia Biotech Italia, Cologno Monzese, Italy) was added to each well, and mononuclear cells were harvested after an additional 24 hours of incubation. Nonadhering cells (lymphocytes) were collected, transferred to microfuge tubes,

and washed twice by centrifugation to remove unincorporated ^{3}H-thymidine. Cell pellets were then dissolved by adding 200 µl of sodium dodecyl sulfate 10%. Incorporated ^{3}H-thymidine was quantified in a liquid scintillation gamma counter (LKG, EG&G Wallac Italia, Milan, Italy).

Interferon-γ Production by Esophageal Mononuclear Cells

To measure the production of interferon gamma (IFN-γ), purified esophageal mononuclear cells were stimulated in vitro with ultraviolet-inactivated HSV-1 or poliovirus suspension (MOI 1:1.5), as described earlier. After 72 hours' incubation, cells were harvested by centrifugation and the conditioned medium was stored at −20C. IFN-γ level was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Milan, Italy) in accordance with the protocol supplied by the manufacturer.

Serologic Assays

Blood samples (15 ml) collected from patients with achalasia and control organ donors were left for 2 hours at room temperature, and then the serum was collected by centrifugation (2000 rpm for 10 min at 4C). Serum was stored at −20C until it was used to determine the presence of anti–HSV-1 and anti–HSV-2 antibodies with a commercially available ELISA (Meridian Diagnostics Inc., Cincinnati, OH).

Tissue DNA Extraction and Polymerase Chain Reaction Analysis

Total DNA was extracted from the esophageal tissue samples harvested from achalasia patients and control subjects. Tissue samples (100 mg) were homogenized in Omnizol reagent (Euroclone), and DNA was extracted using standard procedures, precipitated by the addition of 100% ethanol, washed in 10% ethanol and 0.1 mol/L sodium citrate, and finally dissolved in 10 mmol/L NaOH. Two hundred micrograms of total tissue DNA were then used for polymerase chain reaction (PCR) analysis for human gamma globulin and HSV-1 thymidine kinase. PCR analysis was performed using the following primers pairs: 5′- TAGGAAATCCCATCACCATCTT-3′ and 5′-AGAGATGATGACCCTTTTGGCT-3′ for gamma globulin; 5′-TAGCCCGGCCGTGTGACA-3′ and 5′-CATACCGGAACGCACCACACAA-3' for thymidine kinase. The PCR conditions were as follows: a total of 40 PCR cycles, each consisting of 45 seconds of denaturing at 95C, 1 minute of annealing at 60C (gamma globulin) or 56C (thymidine kinase), and 45 seconds of extension at 72C. PCR amplification products were separated on 1.5% agarose gel and visualized by ethidium bromide staining using an ultraviolet transilluminator. As a positive control for PCR analysis, we used DNA extracted from the brain of rats injected intranasally with 10^{8} plaque-forming units of HSV-1, a condition known to induce HSV-1 latency in the central nervous system,[12] and from Vero cells infected with HSV-1 known to induce a lytic infection.[9]

Statistical Analysis

Data are expressed as median and range. Statistical analysis was performed using the Mann-Whitney U test and the Kruskal-Wallis test, as appropriate.

RESULTS

Patient Population

We studied 15 patients with achalasia, 10 men and five women, whose median age was 39 years (range 20 to 65 years). Achalasia was confirmed by clinical history, esophageal manometry, and barium swallow. Table 1 illustrates the main clinical characteristics of the patient population. Duration of disease ranged from 12 to 60 months (median 20 months). The control subjects included five men and three women whose median age was 42 years (range 23 to 66 years).

Serologic Studies

Serum samples from achalasia patients and control subjects were assayed to determine the presence of specific anti–HSV-1 and anti–HSV-2 antibodies by ELISA. As expected, anti–HSV-1 antibodies were detected in most of the subjects in this study (12 of 15 achalasia patients and seven of eight control subjects), whereas anti–HSV-2 antibodies were found in only a few patients (3 of 15). Thus 80% of patients with achalasia were positive for anti–HSV-1 antibodies compared to 87.5% of control subjects, whereas approximately 20% of both patients and control subjects were positive for anti–HSV-2 antibodies. However, the prevalence of anti–HSV-1 and anti–HSV-2 antibodies observed in patients and control subjects was not significantly different and was comparable to the prevalence previously reported in the Italian population.[13]

Detection of HSV-1 DNA in Tissue Samples

Total tissue DNA was extracted from a fragment of LES muscle obtained from achalasia patients and control subjects and assayed by PCR to detect the presence of HSV-1 DNA. As shown in Fig. 1, using

Table 1. Clinical, manometric, and radiologic features of achalasia patients studied

Patient number	Age (yr)	Sex	Duration of symptoms (mo)	Diameter of esophagus	LES pressure	LES nadir pressure
1	20	F	42	5	23	5
2	61	M	36	4	20	3
3	67	F	60	3.5	41	16
4	41	M	12	4	15	8.3
5	59	M	40	4	31	19
6	36	M	24	3.5	9	3
7	53	M	60	7	20	11.2
8	20	F	24	7	14	10
9	22	F	18	4.5	36	0.1
10	57	M	18	4	24	4
11	37	M	18	3.5	n.d.	n.d.
12	21	F	24	5	18	0
13	30	M	12	5	37	14
14	41	M	13	5	7	11
15	33	M	5	4	19	7
Mean	39.8		27.06	4.6	22.42	7.97
Median	37		24	4	20	7.6
Range	20–67		5–60	3.5–7	7–41	0–19

a primer pair specific for thymidine kinase of HSV-1, we did not obtain any specific amplification product for HSV-1 DNA in achalasia patients (0 of 15) or control subjects (0 of 8), whereas we obtained a specific amplification product using total DNA extracted from rat brain injected intranasally with HSV-1.

Mononuclear Esophageal Cells Proliferate in Response to HSV-1

Esophageal mononuclear cells obtained from the LES of achalasia patients and control subjects were cultured for 72 hours, and proliferation in response to HSV-1 was examined as an indicator of T-cell activation. Proliferation in response to an antigen is broadly used as a marker for the presence of specific immune

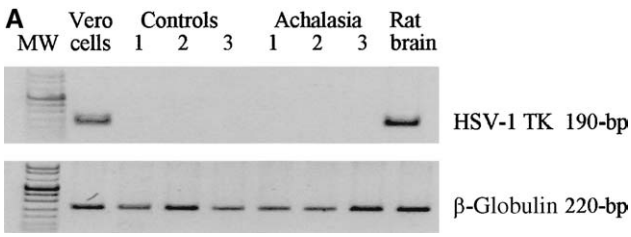

Fig. 1. Detection of HSV-1–specific PCR-amplified DNA products. DNA was extracted from strips of LES muscle of achalasia patients (n = 15) and control subjects (n = 8). PCR analysis was performed using 200 μg DNA as template and primers pairs specific for γ-globulin and HSV-1 TK. As positive controls we used DNA extracted from Vero cells and rat brain infected with HSV-1.

T cells.[14] As a positive control, we used phytohemagglutinin at a concentration of 5 μg/ml as a polyclonal T-cell stimulator. Data are expressed as proliferation index corresponding to the ratio of counts per minute obtained in antigen-specific stimulated versus unstimulated cells. As shown in Fig. 2, *A*, the median proliferation index of lymphocytes exposed to HSV-1 obtained from control subjects was 0.935 (range 0.3–1.29) as opposed to 1.85 (range 1.32–18.44) in lymphocytes extracted from achalasia patients ($P < 0{,}001$). Cells from achalasia patients and control subjects treated with phytohemagglutinin showed a median proliferation index of 125 (range 99–167) and 109 (range 101–175), respectively (P = NS). The median proliferation index observed in cells from achalasia patients and control subjects exposed to poliovirus antigens was comparable (1.36 [range 1.09–7.32] and 1.105 [range 0.48–4.23], respectively) (Fig. 2, *B*). Thus lymphocytes extracted from achalasia patients showed a specific 3.4-fold increase in antigen-driven proliferation by comparison with cells obtained from normal subjects exposed to HSV-1 but not to poliovirus.

Given the relatively small number of patients, no clear relationship was found between esophageal diameter, duration of symptoms, and proliferation index.

Mononuclear Esophageal Cells Release IFN-γ in Response to HSV-1

Mononuclear cells purified from LES muscle were seeded at a concentration of 10^6 cells/ml and stimulated as described earlier. After 72 hours, supernatants

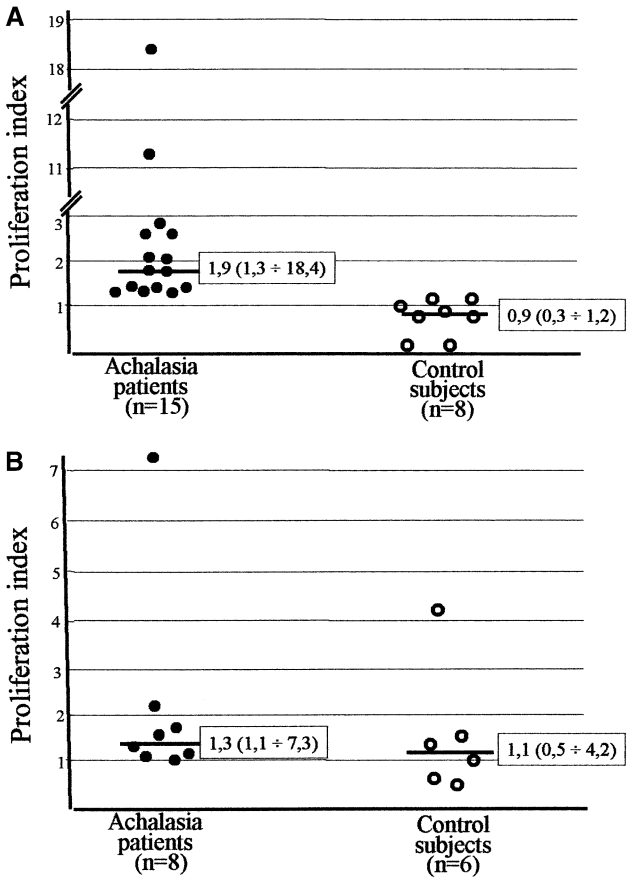

Fig. 2. LES mononuclear cells proliferation. Mononuclear cells were purified from LES specimens obtained from achalasia patients (*filled circles*) and control subjects (*open circles*). For the proliferation assays, cells were seeded at 10^6/ml and stimulated for 72 hours with ultraviolet-inactivated HSV-1 (**A**) or poliovirus (**B**) (MOI 1.5:1). Twenty-four hours before harvest, cells were pulsed with ^{3}H-thymidine. Then cells were collected and nonincorporated radioactivity was removed by centrifugation. T-cell proliferation was expressed as the proliferation index corresponding to the ratio of counts per minute obtained in antigen-specific stimulated versus nonstimulated cells. Samples were assayed at least in duplicate. $P < 0.001$ cells isolated from control subjects and exposed to HSV-1 as compared to achalasia patients exposed to HSV-1.

were collected and the IFN-γ level was determined in the conditioned medium by ELISA. As shown in Fig. 3, incubation of mononuclear cells from control subjects with ultraviolet-inactivated HSV-1 and poliovirus did not cause any substantial change in IFN-γ release. Indeed HSV-1, but not poliovirus, induced a modest (1.4-fold) increase in IFN-γ release from mononuclear cells from LES specimens of achalasia patients as compared to nonstimulated cells (358 pg/ml [range 162–651] vs. 270.5 pg/ml [range 184–371], respectively, P = NS, see Fig. 3). Indeed, a comparison between the amount of IFN-γ released from nonstimulated and HSV-1 exposed cells in the same patient revealed an increase in IFN-γ release in 75% of achalasia patients as opposed to 33% of control subjects.

DISCUSSION

Achalasia is a relatively uncommon disorder of the esophagus that is characterized by the disappearance of the intrinsic neurons in the myenteric plexus. Although several studies suggest immunomediated damage, the etiologic factor(s) remain elusive. The hypothesis that achalasia (or rather, the inflammatory process ultimately leading to achalasia) may be caused by an infectious agent is not new. Indeed, a disease in South America known as Chagas disease, which is characterized by denervation of the myenteric plexus involving both the esophagus and the lower gut, is the consequence of infection by a parasitic organism *Tripanosoma cruzii*.[7] The striking similarity between these two conditions has led investigators to hypothesize an infectious etiology in Western sporadic esophageal achalasia as well.[7] Thus occasional reports of viral infections preceding the onset of achalasia have supported a possible viral etiology.[15,16] However, to our knowledge no pathogens have been demonstrated by light or electron microscopy in the myenteric plexus of patients with primary achalasia. In addition, serologic studies have provided inconclusive results, as would be expected considering the high prevalence in the general population of the viral pathogens tested. Thus the incidence of serum antibodies against herpes or measles

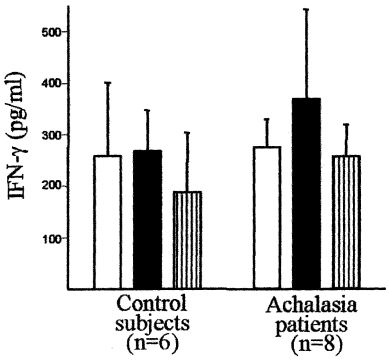

Fig. 3. IFN-γ secretion after stimulation in vitro of LES mononuclear cells. Mononuclear cells isolated from LES of achalasia patients or control subjects were seeded at 10^6/ml and incubated for 72 hours with medium alone (*open bars*), or with ultraviolet-inactivated HSV-1 (*filled bars*) or poliovirus (*striped bars*) at MOI. 1.5:1. Cells were harvested after 72 hours by centrifugation, and the IFN- γ level in the conditioned media was determined by ELISA. Results are expressed as means ± standard error.

viruses has been reported to be either increased or unchanged in different studies.[17,18] Therefore, because the HSV-1 infection is largely prevalent in our population,[13] it is hardly surprising that anti–HSV-1 antibodies were detected in the vast majority of our patients with achalasia and normal subjects.

In recent years, several studies have attempted to identify infectious agent(s) in the myenteric plexus of achalasia patients also taking advantage of molecular techniques. All of these studies produced negative results, however. Thus whereas Robertson et al.[18] identified specific HSV-1 DNA sequences in the myenteric plexus of three of nine patients with achalasia using an in situ hybridization technique, two other studies were unable to demonstrate any nucleic acid of herpes, measles, and human papilloma viruses by PCR in achalasia patients.[19,20] We were likewise unable to detect the presence of HSV-1 DNA by PCR in LES muscle specimens of achalasia patients and control subjects. Although the PCR analysis we used is among the most sensitive techniques available, we cannot rule out the possibility that the viral load in the samples was below the detection limit of our system. Indeed, after infection, less than 10% of neuronal ganglion cells became infected with HSV-1 and contained latent viral DNA.[21] Furthermore, neurons of the myenteric plexus, where the HSV-1 should be located, are lacking almost entirely at the time of sample collection,[3,5] making the identification of the viral genome extremely difficult.

Although several studies reported in the literature argue against an infectious etiology for primary achalasia, we still believe that a possible role for herpes viruses has not been completely ruled out. Indeed, the primary feature in achalasia is the selective loss of inhibitory myenteric neurons, and neurotrophic viruses such as HSV-1 can infect the nervous system selectively, as seen in trigeminal, facial, and vestibular neuritis.[8,12,21] The early phases of achalasia are characterized by a marked inflammatory infiltrate of the myenteric plexus, mainly represented by T lymphocytes. T cells are the main mediators of the protective immune response in recurrent herpes simplex.[10,22] Furthermore, T cells present in the myenteric plexus of achalasia patients have been identified as activated cytotoxic T cells.[6] The antigen driving these cells in the myenteric plexus is not known but could be a viral agent. The present study reports that lymphocytes isolated from tissue specimens of achalasia patients specifically replicate and release IFN-γ after exposure to ultraviolet-inactivated HSV-1 (see Figs. 2 and 3). These findings suggest that T cells in the myenteric plexus of patients with achalasia express TCR that is able to recognize HSV-1 antigens. Because HSV-1–reactive cytotoxic T cells can be isolated from mucosal and corneal lesions of patients recovering from HSV-1 infections,[22,23] we may speculate that the presence of HSV-1–reactive mononuclear cells in LES muscle of achalasia patients is the result of a specific immune response to HSV-1 eventually endowed in the myenteric neurons. Alternatively, it is possible that antigens expressed by neurons or muscle cells of the LES muscle may mimic HSV-1 moieties triggering the development of the T-cell–rich infiltrate.

Recent data indicate the existence of a common genetic background in achalasia patients. Thus the hypothesis of an immune-driven disease in genetically predisposed subjects is gaining consent. Indeed, an association between achalasia and the class II HLA antigen DQ1 was initially reported by Wong et al.[8] In addition, an association between achalasia patients and DQB1*0602 and DRB1*15 alleles was found in white patients using higher resolution molecular techniques[24]; such class II antigens have been previously associated with autoimmune diseases such as multiple sclerosis and Goodpasture syndrome. Finally, Storch et al.[25] found circulating anti–Auerbach plexus antibodies in the majority of patients with achalasia, and Ruiz-de-Leon et al.[26] found a strong association between the DQB1*0603 allele and circulating anti–Auerbach plexus antibodies in women with achalasia.

CONCLUSION

Our data support the hypothesis that immunologic mechanisms may, at least in part, have a role in the onset of achalasia. The demonstration of HSV-1–reactive mononuclear cells in the myenteric plexus of patients with achalasia suggests that an abnormal immunologic response to a viral agent may damage the esophageal intrinsic innervation. Thus it is possible to speculate that after the infection with a common neurotrophic agent such as HSV-1, subjects with a specific genetic background may develop a specific immune response that ultimately can lead to damage of the myenteric neurons. Further studies are warranted, however, to identify exactly which antigen is involved in initiating the inflammatory process that ultimately leads to achalasia, and whether these HSV-1–reactive mononuclear cells are effectively involved in the neuronal damage.

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Invited Discussion—Expert Commentator

Carlos A. Pellegrini, M.D. (Seattle, WA): The authors of this paper investigated the potential role of herpes simplex virus type 1 (HSV-1), in the pathogenesis of achalasia. They found that circulating anti–HSV-1 and HSV-2 antibodies as detected by ELISA on serum samples were present in these patients with the same frequency found in control subjects. Furthermore, no HSV-1 DNA was detected by PCR in the esophageal muscle samples of achalasia patients. However, the proliferative index in mononuclear cells from achalasia patients stimulated with HSV-1 was significantly higher than that of control subjects as was IFN-γ release after incubation with HSV-1. Based on these two observations, they *hypothesized* that mononuclear cells in the myenteric plexus of achalasia patients may have been activated by HSV-1, and as a consequence may have initiated the inflammatory process that led to the destruction of the myenteric ganglia. My concern with this study is that the basis for the authors conclusions are speculative and based on circumsntancial evidence. Indeed, they assume that the damage to the myenteric plexus is done by lymphocytes that have been activated by HSV-1 (or other virual infection) and that genetic factors are either the reason why in some individuals HSV-1 can stimulate lymphocytes or why the product of that activation damages the neural system. Having said this, I believe the study was well conducted and the paper offers a great review of the current knowledge on the relationship between viral infection and achalasia.

Disparity Between Symptomatic and Physiologic Outcomes Following Esophageal Lengthening Procedures for Antireflux Surgery

Edward Lin, D.O., Vickie Swafford, R.N., Rajagopal Chadalavada, M.D., Bruce J. Ramshaw, M.D., C. Daniel Smith, M.D.

Although esophageal lengthening procedures (Collis gastroplasty) have been recommended as an adjunct to antireflux surgery in patients with shortened esophagus, there are few data on physiologic outcomes in these patients. This study details the long-term outcomes in patients who underwent antireflux surgery with Collis gastroplasty. All patients undergoing esophagogastric fundoplication (EGF) with a Collis gastroplasty for the management of gastroesophageal reflux disease or paraesophageal hernia were identified from a prospectively maintained database. Symptom questionnaires were used during follow-up to assess symptomatic outcomes. Barium esophogram, upper endoscopy with biopsy, and catheterless esophageal acid monitoring (BRAVO system) were recommended for all patients. Patients with abnormal results of physiologic studies underwent further treatment based on a standardized algorithm. Between 1996 and 2002, a total of 68 patients underwent EGF with Collis gastroplasty. Twenty-seven (40%) had a large paraesophageal hernia, and 20 (30%) had undergone a prior EGF. Fifty-six (82%) of the procedures were performed laparoscopically. Mean follow-up time was 30 months, with 10 (15%) patients lost to latest follow-up. Symptomatic outcome data were available for 85% of patients, with significant improvements reported for heartburn (86%), chest pain (90%), dysphagia (89%), and regurgitation (91%). Most patients (84%) were off medications. Physiologic data were completed in 37% of the patients. Of those undergoing physiologic follow-up studies, 17% had recurrent hiatal hernia, and 80% had endoscopically identified esophagitis and pathologic esophageal acid exposure on pH testing. Despite this, 65% of the patients with objectively identified abnormalities reported significant symptomatic improvement compared to their preoperative symptoms. Two patients developed changes associated with Barrett's esophagus that were not present preoperatively. Distal esophageal injury can persist after EGF with Collis gastroplasty, despite significant symptomatic improvements. Appropriate follow-up in these patients requires objective surveillance, which should eventuate in further treatment if esophageal acid is not completely controlled. Although the Collis gastroplasty is conceptually appealing, these results call into question the liberal application of this technique during EGF. (J GASTROINTEST SURG 2004;8:31–39)

KEY WORDS: Esophagitis, Collis gastroplasty, short esophagus, hiatal hernia, paraesophageal hernia

Laparoscopic Nissen (360-degree) fundoplication is the most widely applied antireflux procedure, accounting for 87 of every 100,000 hospital discharges in 1999 according to the National Inpatient Sample.[1] This represents an almost eightfold increase in this procedure over a 10-year period, and is attributed to the increased use of esophageal pH and motility testing,[2] increasing awareness of the significant impairment in quality of life resulting from gastroesophageal disease (GERD), and the advent of minimally invasive antireflux surgery in 1990. The best outcomes with follow-up of 5 years or longer report patient satisfaction ranging from 86% to 96%, making the laparoscopic Nissen fundoplication the "gold standard" for antireflux procedures.[3–6]

The true failure rate following Nissen fundoplication for uncomplicated GERD documented by objective studies is estimated to range from 2% to 5%,

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and even fewer patients require reoperations.[6,7] Our group's reoperation rate for uncomplicated GERD is less than 3%.[8] However, the surgical failure rate in patients with Barrett's esophagus or severe esophagitis preoperatively approaches 8%,[9] and anatomic failure after paraesophageal hernia repair is 26%.[10] The most common pattern of failure for these complicated cases of GERD has been transdiaphragmatic wrap migration into the chest.[11] One predisposition to herniation is the presence of a shortened esophagus, a condition associated with more advanced GERD, as evidenced by erosive esophagitis, strictures, and changes associated with Barrett's esophagus.[11–16]

Other abnormalities that should raise suspicion of a short esophagus include a hiatal hernia on esophagram that is not reducible when the patient is in the upright position, a hiatal hernia larger than 5 cm, or an esophageal length of less than 35 cm from the incisors as determined by endoscopy.

Although the diagnosis of shortened esophagus is uncommon (Table 1), its high association with failure after Nissen fundoplication necessitates preoperative recognition and intraoperative confirmation. Adequate esophageal length is determined intraoperatively by attaining at least 2 cm of intra-abdominal esophagus after full mediastinal esophageal mobilization. This confirmation can only be accurate when intraoperative endoscopy is used to identify the squamocolumnar junction in relation to the hiatus.[17] It is perhaps the adequate esophageal mobilization performed in patients with suspected short esophagus that has reduced the frequency of esophageal lengthening procedures being performed.

Once a shortened esophagus is confirmed intraoperatively, several options have historically been proposed for lengthening the esophagus. The most commonly accepted method for esophageal lengthening has been the Collis gastroplasty,[18] which was readily adapted to a laparoscopic technique in the mid-1990s.[19] Over the past decade, the Collis gastroplasty has been applied with patient satisfaction usually exceeding 90%, and with this, some have advocated its routine use for the conditions described earlier, particularly in the repair of large paraesophageal hernias.[20–25]

Although many have reported good outcomes after Collis gastroplasty for GERD, most report primarily symptom resolution as a measure of outcome. Although this may adequately reflect the response to treatment for uncomplicated GERD, it is unclear if patients with more advanced GERD can be followed in the same manner, especially because there may be an element of esophageal insensitivity or symptom denial/acceptance that has allowed such advanced esophageal pathology to develop. This study was undertaken to determine the physiologic and symptomatic outcomes after esophageal lengthening in antireflux surgery and to develop an algorithm for follow-up.

MATERIAL AND METHODS

Patients

The institutional review board of Emory University School of Medicine approved this study. Between 1996 and 2002, a total of 1579 patients underwent antireflux surgery (ARS) through the Emory Endosurgery Unit and Gastroesophageal Treatment Center. During this time, 68 (4.3% of all EGFs performed) Collis-Nissen fundoplications were performed (Table 2). These procedures were performed by two different surgeons, and the decision to use a Collis gastroplasty at the time of EGF was based on the surgeon's own judgment at the time of surgery as to the presence of esophageal shortening or the need for lengthening. Follow-up information was obtained by phone interviews and office visits.

Symptom Outcomes

All patients underwent preoperative and postoperative symptom assessment for chest pain, dysphagia,

Table 1. Reported incidence of short esophagus requiring esophageal lengthening

Group	Year	Reference	Incidence (%)	Total number
Legacy	1996	20	14%	238
USC	1999	12	15.6%	236
Pittsburgh	2000	24	27%	100 (PEH only)
Emory	2001	8	2.9%	1000
Nebraska	2001	22	5%	260
Pittsburgh	2002	25	56%	200 (PEH only)
Emory	2003	—	4.3%	1579

PEH = paraesophageal hernia.

Table 2. Preoperative demographics and symptom data in 68 patients undergoing Collis-Nissen fundoplication

Males:Females	33:35
Age (yr; mean ± SEM)	57.6 ± 1.6
Symptom duration (mo; mean ± SEM)	59.8 ± 8
Patients with esophagus	29 (43%)
Patients with Barrett's esophagus	16 (23.5%)
Patients with strictures	16 (23.5%)
Combined Barrett's and strictures	4 (5.9%)
Patients with delayed gastric emptying	4 (5.9%)

heartburn, and regurgitation. Severity of symptoms was reported as none, mild, moderate, or severe. In addition, use and dosage of antisecretory medications were recorded.

Physiologic Studies

Barium swallow was performed by standard methods. All esophagogastroduodenoscopies (EGD) and appropriate biopsies were performed by our group's surgeons. Esophageal pH testing was performed using a wireless system (BRAVO; Medtronic, Minneapolis, Minnesota). Patients with endoscopic evidence of erosive esophagitis during follow-up had biopsies, but they did not undergo esophageal pH testing.

Statistical Analysis

Comparisons of preoperative and postoperative data were made with the Wilcoxon signed-rank test. Statistical significance was set at $P < 0.05$ for each symptom.

RESULTS

Over a 7-year period, 68 patients underwent Collis-Nissen fundoplication. Twenty-one patients (30%) underwent primary EGF for GERD and 27 patients (40%) underwent EGF for paraesophageal hernias, whereas 20 patients (30%) underwent reoperations for failed prior EGF (Fig. 1). Fifty-six operations (82%) were completed laparoscopically.

Symptom evaluations were obtainable from 58 patients (85%) over a mean follow-up period of 30 ± 4 months. There was significant improvement in the following symptoms: chest pain (90%), dysphagia (89%), heartburn (86%), and regurgitation (91%) (Fig. 2). Eighty-four percent of the patients reported no use of antisecretory medications.

Twenty-five patients (37%) were able to return for in-office follow-up and objective physiologic testing with EGD, barium swallow and esophageal pH testing. Among these 25 patients, 16 (65%) reported significant improvement in symptoms and were satisfied with their surgical outcomes. However, 20 (80%) of the 25 patients had abnormal pH studies (Fig. 3) or severe esophagitis on EGD. Four patients had transdiaphragmatic wrap herniation and two patients had new Barrett's-associated changes that were not present before surgery.

DISCUSSION

Although some are advocating routine use of the Collis gastroplasty for patients with complicated reflux and paraesophageal hernia, anecdotal experience with our own patients and those undergoing esophageal lengthening procedures elsewhere suggested that outcomes were not comparable to those of patients undergoing EGF for uncomplicated GERD. These observations prompted us to undertake a comprehensive review of our experience with Collis-Nissen fundoplication.

The need for esophageal lengthening procedures is rare in most large foregut surgery experiences, and

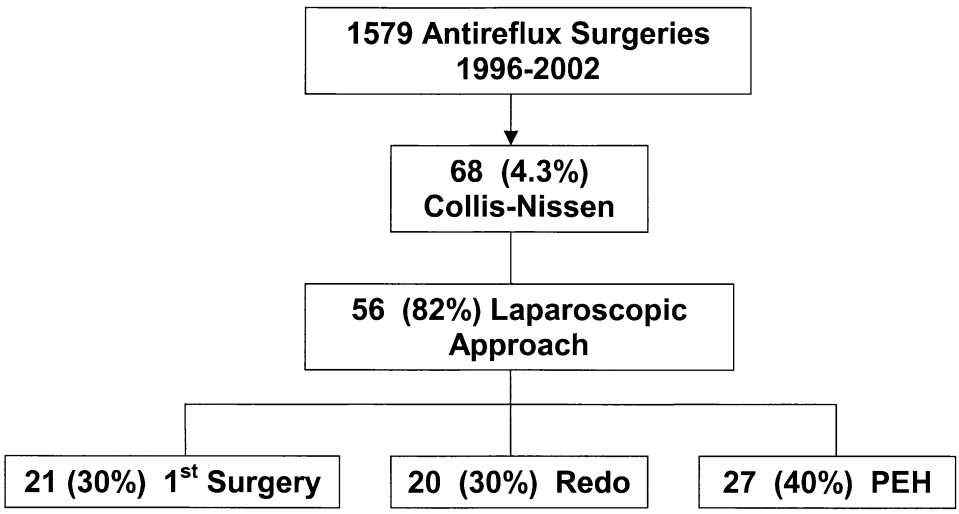

Fig. 1. Breakdown of patients who underwent Collis-Nissen fundoplication in a 7-year period. Most cases were completed laparoscopically. Redo = revisional EGF; PEH = paraesophageal hernia; 1st Surgery = no prior antireflux surgery.

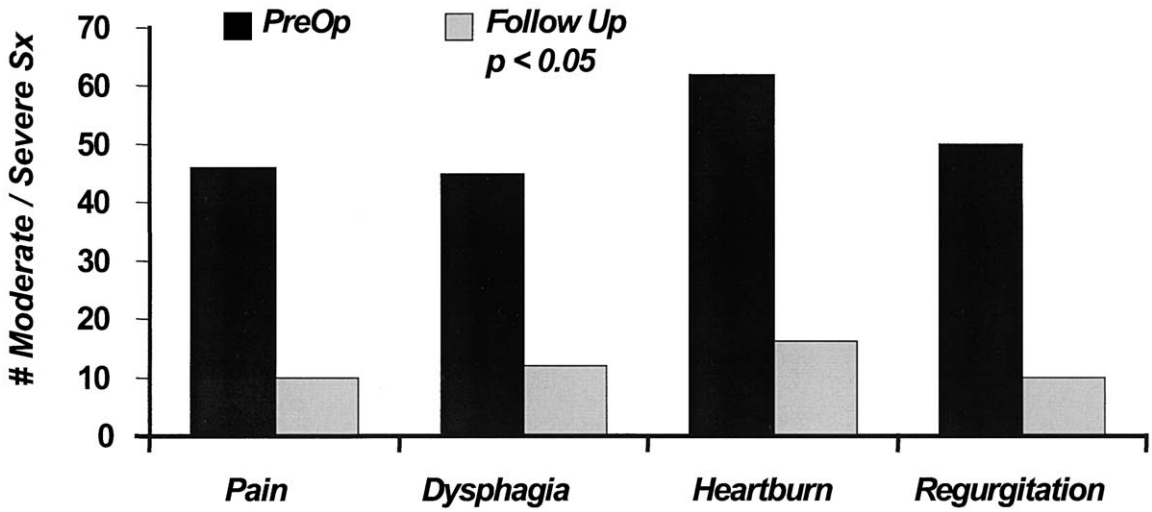

Fig. 2. Symptom follow-up for 58 (85%) of 68 patients who underwent Collis-Nissen fundoplication. Mean follow-up time was 30 months. Postoperative symptoms were significantly changed from preoperative symptoms.

the incidence is 4.3% in this series (see Table 1). If the diagnosis of short esophagus is suspected preoperatively, adequate mediastinal mobilization of the esophagus during laparoscopic antireflux surgery can often increase the intra-abdominal length by 4 to 5 cm and obviate the need for an esophageal lengthening procedure. Furthermore, endoscopic confirmation of an intra-abdominal squamocolumnar junction is essential for accurate evaluation of esophageal length. In cases of complicated reoperative antireflux surgery or large paraesophageal hernias, we routinely use intraoperative esophagoscopy to localize the esophagogastric junction and assess esophageal length, and use this selectively when short esophagus is suspected from preoperative testing.

If an esophageal lengthening procedure is necessary, it is recommended that the neoesophagus not exceed 3 cm in length. This minimizes the amount of parietal cell mass in the neoesophagus that may potentially secrete acid (Fig. 4). In experiences gathered

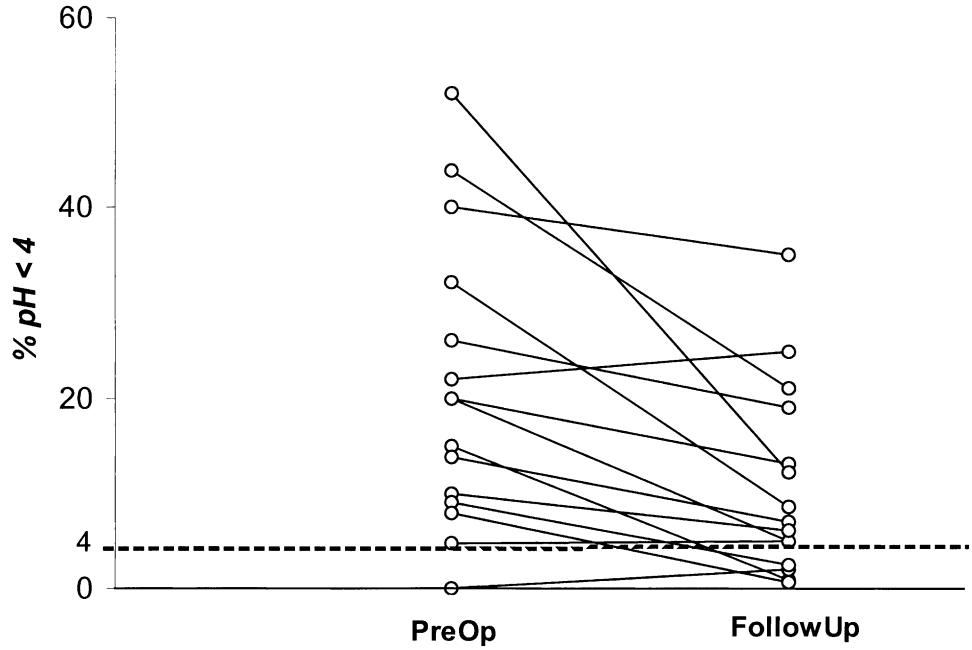

Fig. 3. Total percentage of time with pH <4 in 15 patients who underwent Collis-Nissen fundoplication. There is an overall reduction in acid reflux at follow-up (21.1 ± 4 vs. 10.9 ± 2.6, $P < 0.04$, Wilcoxon's matched-pair test), but most are still outside of the normal range.

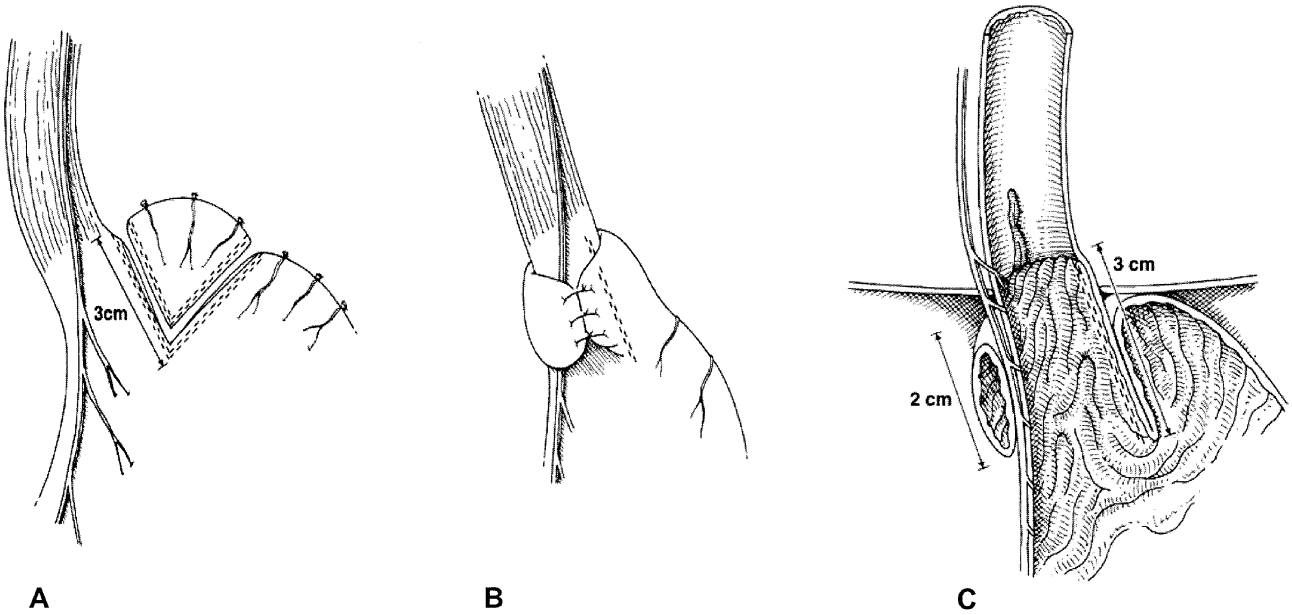

Fig. 4. Method of creating a neoesophagus presently used. **A**, A 3 cm long wedge resection at the angle of His is made using a linear cutting stapler to create the neoesophagus. **B** and **C**, The gastric fundus is wrapped around the neoesophagus to prevent dilation of the tubularized stomach and create an antireflux valve.

from patients undergoing Roux-en-Y gastric bypass with small vertical pouches of less than 15 ml capacity, the amount of acid produced by the gastric pouch is negligible.[26] However, attempts to add to the length of a neoesophagus poses the risk of creating a tubularized stomach with acid-producing capacity. Indeed, follow-up of patients after Collis gastroplasty with biopsies has demonstrated acid-producing parietal cells within the neoesophagus proximal to the fundoplication.[27] This may create a "double valve," bordered proximally by the lower esophageal sphincter and distally by the fundoplication (Fig. 5). The

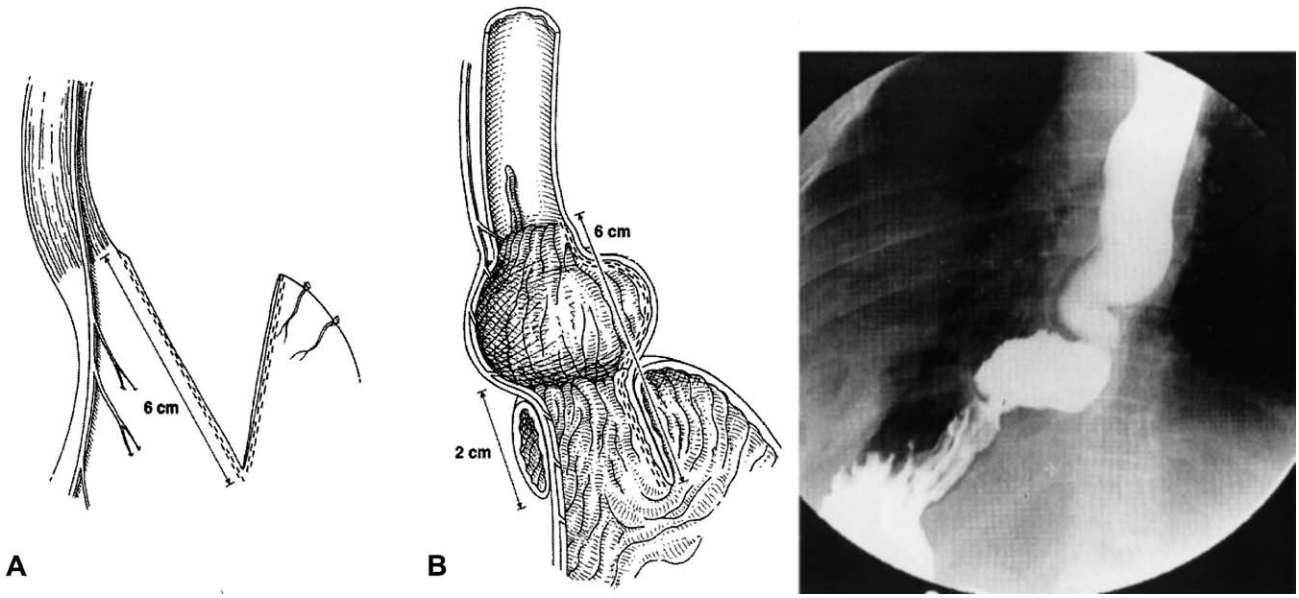

Fig. 5. A, An excessively long neoesophagus resulting in excessive parietal cell mass and potential acid secretion creates a "double valve" with the lower esophageal sphincter and the fundoplication. **B**, The proximal portion potentially expands, creating a reservoir of acid-secreting neoesophagus. **C**, Barium swallow of a long neoesophagus and a gastric reservoir demonstrating the "double-valve" effect.

perpetual acid secretion and inherent dysmotility within the long segment of neoesophagus has the potential for dilating and creating an acid reservoir that can exacerbate distal esophagitis—particularly if there is an outlet obstruction created by the fundoplication. When lengthening the esophagus by 3 cm is inadequate, we recommend a thoracic approach for additional esophageal mobilization or leaving the fundoplication above the diaphragm.

Whether a Collis-Nissen gastroplasty causes regression of esophagitis or Barrett's changes remains to be fully elucidated. The University of Montreal group followed 45 patients with Barrett's esophagus who had Collis-Nissen fundoplications over a 3-year period.[28] They demonstrated a reduction in acid reflux and augmentation of lower esophageal sphincter pressure. Although healing of esophagitis was demonstrated in most patients, there was no reversal of preexisting columnar mucosa with intestinal metaplasia. In fact, 10 (22%) of 45 patients still exhibited pathologic acid exposure in the distal esophagus after surgery. In an earlier study by the same group, erosive esophagitis healed in patients who had uncut Collis-Nissen gastroplasties, but recurred in 2 of 27 patients within 3 years.[29] These investigators concluded that physiologic improvements and an anatomically tension-free repair can be achieved with the Collis-Nissen gastroplasty, but the procedure does not fully reverse the preexisting abnormal esophageal mucosa.

In our own experience, 13 patients had Barrett's esophagus preoperatively. However, only four of these patients were followed regularly by our group. At last follow-up, two of these patients had esophagitis but no intestinal metaplasia. The other nine patients chose to be followed by their local gastroenterologists, and to date four of these nine patients have informed us that they have been discharged from the care of their gastroenterologists because of normalization of symptoms. We caution that the lack of histologic identification of intestinal metaplasia and resolution of symptoms does not necessarily indicate resolution of Barrett's esophagus. Given our postoperative findings of persistent severe esophagitis, abnormal pH studies, and identification of two previously undiagnosed cases of Barrett's esophagus, the lack of histologic evidence of intestinal metaplasia postoperatively can likely be attributed to sampling error. Furthermore, not all clinicians are accustomed to managing patients with Barrett's esophagus or those

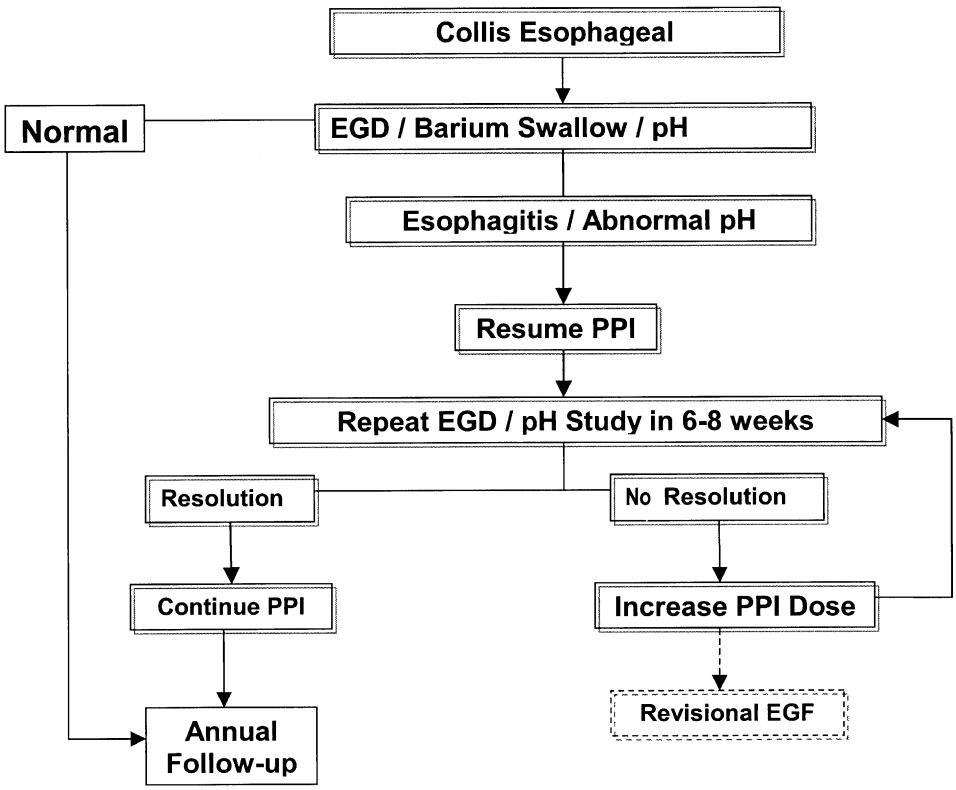

Fig. 6. Present algorithm lthat is followed for patients who have undergone esophageal lengthening procedures as part of their EGF. The two principle objectives are to provide regular objective follow-up and to prove acid suppression in those with pathologic acid reflux.

with Collis-gastroplasty because of the relative rarity of these encounters.

The experience reported here underscores the fact that symptomatic outcomes are inadequate for following patients with complex GERD. Although 84% of the patients undergoing Collis-Nissen fundoplication were off antisecretory medications, 80% of those submitting to EGD and pH testing had evidence of persistent acid injury to the distal esophagus despite a significant reduction in acid reflux at follow-up (see Fig. 3). One possible explanation may be that patients do experience relief relative to the severity of preoperative symptoms and accept their current improved state as the best possible outcome. Another explanation may be that the presence of columnar mucosa or severe esophagitis renders the distal esophagus insensate to the chronically acidic environment.[30,31] This can be inferred by the lack of correlation between pathologic acid reflux episodes and symptom recordings in our patient diaries (data not shown).

We do not recommend the liberal application of esophageal lengthening procedures during antireflux procedures, even though it can create an anatomic tension-free fundoplication. If the procedure is necessary, it is performed fully appreciating the potential sequelae we have presented from our experience.

In light of our findings, we presently follow an algorithm for patients who have undergone Collis-Nissen gastroplasty with the goals of providing regularly scheduled objective evaluations and suppressing pathologic acid exposure to the distal esophagus (Fig. 6). Our initial treatment strategy in those with evidence of persistent esophageal acid reflux has primarily relied on the use of high-dose proton pump inhibitors. Available surgical options for the containment of pathologic esophageal acid reflux may include revisional EGF as well as endoluminal antireflux techniques currently in development (e.g., Stretta or Enteryx).

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Discussion

Dr. L. Swanstrom (Portland, OR): This is a great paper. We were just looking at posters on the value of symptomatic follow-up in patients and how important it is to get objective follow-up. I think your paper just emphasizes it in a particularly difficult group of patients. With regard to your conclusion that these patients should undergo reoperation to reposition the wrap, maybe you could explain a little bit better how that would eliminate this finding?

Dr. E. Lin: Thank you for your kind comments. In fact, I think you started us on the road toward the physiologic follow-up of patients undergoing collis gastroplasty. Regarding the redo operation, we have not had to do one yet, but that is a potential arm that we are willing to pursue if these patients do not respond to proton pump inhipitors. We have tried other modalities, such as the Stretta, but it is way too early for me to report on an outcome for that.

Dr. Swanstrom: I have another question. You very quickly mentioned a new occurrence of Barrett's esophagus in these patients. This is something that we have not seen. Our patients do have mild esophagitis, but we found no Barrett's esophagus. What was your incidence of that and what are you planning to do with that group of patients?

Dr. Lin: There were two new cases of Barrett's esophagus that were not diagnosed before surgery, and this was after going through the patients workups' and having pathologists confirm the diagnosis. These patients did not have any dysplasia. At this point we are only going to treat their esophagitis with proton pump inhibitors and rigorous follow-up.

Dr. J. Hunter (Portland, OR): Thank you very much for carrying out such a great follow-up of these patients. They certainly needed it. I have two questions: (1) How did you select the 25 patients you studied, out of the 68 patients in the series, and (2) how many of those were studied because they were symptomatic, and how many who were studied were asymptomatic?

Dr. Lin: Just about every patient receives symptomatic follow-up according to our protocol. Two patients prompted the physiologic workup initially. They exhibited symptoms such as heartburn, 5 or 6 years after the procedure. Why did we only perform physiologic studies in 25? For many asymptomatic patients it was very difficult to convince them.

Dr. Hunter: I certainly understand that. Was there any correlation between outcome and the time at which the patients underwent Collis gastroplasty? Was there any indication that the patients who were more likely to having reflux had surgery earlier than those who had surgery later?

Dr. Lin: Do you mean earlier in the learning curve?

Dr. Hunter: I mean in the 1996 to 1999 time frame as opposed to the 2000 to 2001 time frame.

Dr. Lin: No, I did not look at that, but you raise an excellent point. We do have data available comparing wedge gastroplasty vs the EEA anastomosis and will certainly explore it.

Dr. Hunter: You might look at that because one of the things we tried to do was to place the highest stitch of the fundoplication on the native esophagus so that we were not leaving parietal cells above the fundoplication. If a patient is asymptomatic and does not have esophagitis, why put them on proton pump inhibitors? I can understand the logic of treatment if they have esophagitis. In other words, do you believe that one should treat a positive pH study in the absence of esophageal injury or symptoms?

Dr. Lin: Frankly most of these patients did have some form of esophagitis, and we do treat it because we believe that many of these patients with progressive acid injury will probably end up with esophagitis of some form. That is why we proceeded with proton pump inhibitor treatment. We need to keep in mind that these patients had extreme reflux disease before surgery, and the ultimate goal of treatment was to reduce pathologic acid exposure.

Dr. C. Pellegrini (Seattle, WA): I remember a long time ago when I read about the Collis gastroplasty, I could not understand the rationale for doing it. Bringing

the esophagus down into the abdomen during an antireflux procedure makes all the sense in the world physiologically. Dividing the stomach and creating a tube of stomach that may look like the esophagus but still produces acid does not. Dr. Swanstrom presented the results from his carefully followed patients showing that reflux persisted in 50% of them. So my first question is, when are esophageal surgeons going to abandon this operation? Having experienced the same results reported by Dr. Swanstrom. I have stopped using it for the most part.

You have shown again today that this is a totally ineffective operation in patients with gastroesophageal reflux. You showed that esophagitis recurs, pH monitoring is abnormal postoperatively, and even your reported rate of development of Barrett's esophagus is about 6%, which is 2 in 37 patients. My question is, why would you still have this operation in the algorithm? There are other ways to deal with reflux besides trying to force a Nissen fundoplication.

Dr. Lin: I do agree with you. We emphasize again that we do not advocate the liberal use of the procedure. I do not think it is a bad operation if you make that neoesophagus short. You bring up another great point. There is an argument for creating a wrap and letting it sit in the chest, or at least low in the mediastinum, rather than going through a procedure like this.

The University of Montreal experience demonstrated that patients do very well initially, and they have a significant reduction in acid reflux, but I share your doubts. Some of these patients had recurrent esophagitis 3 to 5 years after this procedure was done.

Dr. G. Zaninotto (Padova, Italy): Did you find any difference between those who are doing well with normal pH monitoring and those who are not, with regard to the length of the neoesophagus?

Dr. Lin: Did I find a difference between what?

Dr. Zaninotto: Those who are doing well—that is, the 20% with normal pH monitoring after the operation—and those who were not?

Dr. Lin: We basically just took all-comers, and most of them did not have symptoms or were significantly improved.

Dr. Swanstrom: Just having gotten pegged as the guy who killed the Collis gastroplasty, I just wanted to mention that this is not reflux that we are talking about. This procedure is great at stopping gastroesophageal reflux. This is just an abnormal 24-hour pH; it is a local type of phenomenon.

Dr. S. Horgan (Chicago, IL): You conclude that you are calling into question the liberal application of the Collis gastroplasty. I do not see how you are planning to change this. How are you planning to approach the patients differently now? That is probably correlated with what Dr. Pellegrini was asking.

Dr. Lin: When we encounter what we think is a short esophagus, the first thing we always do is mobilize the esophagus into the mediastinum, which I do not believe everyone does.

Dr. Horgan: You were not doing that before?

Dr. Lin: Yes, we were doing it.

Dr. Horgan: So what is different now?

Dr. Lin: Right now we continue to mobilize the esophagus up into the mediastinum up to the aortic arch, and we add a G-tube gastropexy in the large paraesophageal hernias. When necessary, we do add thoracic mobilization. These maneuvers have reduced the number of esophageal lengthening procedures we perform.

Insulin-Like Growth Factor-2 Activation of Intestinal Glutamine Transport Is Mediated by Mitogen-Activated Protein Kinases

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Insulin-like growth factor-2 (IGF-2) plays a pivotal role in regulating intestinal epithelial metabolism, growth, and proliferation, but its regulatory effects on mucosal cell amino acid transport have not been well studied. The purpose of this in vitro study was to investigate the regulatory mechanisms and intracellular signaling pathways involved in the regulation of IGF-2 on glutamine transport in cultured intestinal cells. Continuous incubation with IGF-2 stimulated glutamine transport activity in cultured IEC-6 cells in a dose- and time-dependent fashion. Prolonged incubation (up to 48 hours) resulted in a 50% increase in transport activity (0.81 ± 0.21 nmole/mg protein/min in IGF-2 cells vs. 0.57 ± 0.15 nmole/mg protein/min in control cells) and a threefold increase in glutamine transporter ATB^0 mRNA levels. IGF-2 stimulated transport activity by increasing transport maximal capacity (V_{max} 4.31 ± 0.36 nmole/mg protein/min in IGF-2 cells vs. 2.51 ± 0.23 nmole/mg protein/min in control cells) without affecting the transport affinity (K_m 0.31 ± 0.03 mmol/L glutamine in IGF-2 cells vs. 0.28 ± 0.03 mmol/L glutamine in control cells). This IGF-2–induced glutamine transport activity was attenuated by actinomycin-D or cycloheximide. The levels of mitogen-activated protein kinases p42/44, MEK1/2, and p38 as well as protein kinase C levels were elevated in IGF-2–treated cells and inhibitors of mitogen-activated protein kinase MEK1 (PD 98059), mitogen-activated protein kinase p38, and protein kinase C (chelerythrine chloride) individually attenuated the IGF-2–induced glutamine transport. These data suggest that IGF-2 stimulates intestinal glutamine uptake in cultured rat intestinal epithelial cells via a mechanism that involves transcription and translation of the transporter. Activation of mitogen-activated protein kinases and protein kinase C cascades are involved in the regulation. This increase in glutamine uptake may occur to support intestinal cell growth and proliferation. (J GASTROINTEST SURG 2004;8:40–47)

KEY WORDS: Insulin-like growth factor, glutamine, intestine, proliferation

Small intestinal epithelia undergo rapid proliferation and differentiation along the crypt-villus axis.[1–4] Peptide growth factors such as insulin-like growth factors (IGF-1 and 2) are important mitogenic polypeptides in intestinal epithelial cell proliferation and growth.[5–9] The action of IGFs is thought to be through IGF-1 receptor interaction and modulated by various IGF binding proteins.[10–12] As members of the IGF family, IGF-1 and IGF-2 exhibit a diverse spectrum of biological activities.[5–9] Although the biological activities of IGF-1 have been extensively investigated, the effects of IGF-2 are much less known.

Amino acid glutamine is the preferred metabolic fuel as well as a major precursor for biosynthesis of biological compounds for intestinal epithelia. Glutamine has profound effects on gut-related immune functions and an anabolic effect on host protein synthesis.[13] Demand for amino acids increases in the

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rapidly proliferating state. Luminal glutamine transport across the intestinal membrane into enterocytes, via discrete amino acid transport systems, is an essential step in maintaining host glutamine homeostasis.[14] The intestinal glutamine transport is regulated by various local and systemic factors including luminal substrate concentrations, hormones, growth factors, and metabolic byproducts.[15]

The rat intestinal epithelial cell (IEC) line IEC-6, which is derived from rat jejunal crypts, proliferates and grows to a homogeneous population under standard cell culture conditions.[16] The IEC-6 cell line has been successfully used as an in vitro enterocyte model for proliferation studies.[17,18] IEC-6 cells express high-level IGF receptors and proliferate in response to exogenous insulin and insulin-like growth factors. IGF-2 exhibits a significant autocrine effect on rapid IEC-6 proliferation.[18]

The regulatory effects of IGF-2 on mucosal cell amino acid transport have not been well studied. The purpose of this in vitro study was to investigate the regulatory mechanisms and intracellular signaling pathways involved in the regulation of IGF-2 on glutamine transport in proliferating cultured rat intestinal epithelial cells.

MATERIALS AND METHODS

Cell Culture

The IEC-6 cell line was obtained from American Type Culture Collection (Rockville, MD) at passage 13. Cells were routinely maintained in T-150 flasks in a 37C humidified incubator in 10% CO_2/90% O_2. Cells were routinely grown in Dulbecco's modified Eagle medium (DMEM) containing 25 mmol/L glucose and 0.4 mol/L sodium bicarbonate, supplemented with 5% fetal bovine serum, 4 mmol/L glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 μg/L insulin. The stock cells were passaged weekly at a ratio of 1:5 after treatment with 0.05% trypsin and 0.02% EDTA. Cells were reseeded at a density of 4.5×10^6 cells per T-150 flask for future subculturing, seeded in six-well cluster Costar tissue culture plates at a density of 10^5 cells per well for Northern blot or Western blot analysis, or seeded in 24-well cluster Costar tissue culture plates at a density of 1×10^4 cells per 12 mm well for transport experiments. Near-confluent cells (day 5, passages 16 to 30) were used for experiments. The day of seeding was designated as day 0. The growth medium was changed every other day, and cultures were inspected daily with the use of a phase-contrast microscope.

Cell Treatments

Prior to treatment, the cell monolayer was washed three times with serum-free medi and reincubated in serum-free and insulin-free media (i.e., DMEM supplemented only with penicillin and streptomycin, but lacking fetal bovine serum) for 6 hours at 37C. The cell monolayer was washed three times again with serum-free media and then exposed to each agent at various times and concentrations as described below. Treatment media were replenished every 6 hours. Cells were treated individually with insulin-like growth factor-2 (IGF-2; 0 to 1 μmol/L) for various periods of time (minutes to 72 hours) in a 37C 10% CO_2/90% air humidified incubator. Cells were also treated with the following individual mitogen activated protein kinase (MAPK) inhibitors: PD 98059 (0 to 100 μmol/L) for MEK 1, SB 203580 (0 to 10 μmol/L) for p38, chelerythrine chloride (0 to 6.6 μmol/L) for protein kinase C, actinomycin-D (act-D; 0.1 μmol/L), or cycloheximide (CHX; 10 μmol/L) for various periods of time (minutes to 72 hours). Equal amounts of dimethylsulfoxide or water served as the control medium. Caco-2 cells remained healthy (viability >99% by dye exclusion) during at least 72 hours of treatment in serum-free media.

L-Glutamine Uptake Measurements

L-Glutamine transport activity was measured at 37 ± 1.0C. Following pretreatment of cells with the various agents described earlier, cells were rinsed three times with "uptake buffer" (37C) comprised of 137 mmol/L NaCl (or 137 mmol/L choline chloride), 10 mmol/L HEPES/Tris buffer (pH 7.4), 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, and 2.5 mmol/L $CaCl_2$. Transport was initiated by simultaneously adding 1 ml of this buffer containing also L-[^{3}H]glutamine (2 μCi/ml, 1 μmol/L to 10 mmol/L) into each transport plate (24 wells). Each transport plate contained cells from both control and treatment groups. Cell culture plates were continuously shaken by an orbital shaker (1 Hz) during the uptake period. Uptake was arrested by discarding the uptake buffer and washing the cells three times with ice-cold uptake buffer lacking substrate. Radioactivity of isotope extracted from the cells with 1 ml of 1N NaOH was neutralized with acetic acid, and assayed by liquid scintillation spectrometry. Protein in the NaOH extract was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Initial rates of transport activity were determined during the linear uptake period (2 minutes), with zero time points serving as blanks.[19,20] Uptake rates are expressed as nmoles glutamine per minute per mg of cell protein. Sodium-dependent system B

glutamine transport was obtained by subtracting total glutamine transport measured in choline chloride buffer from that in NaCl buffer.

Northern Blot Analysis of System B ATB^0 mRNA

Following pretreatment of cells with various agents (described above), cells were rinsed three times with phosphate-buffered saline solution Total RNA was isolated from control and treated Caco-2 cells using the "Totally RNA" isolation kit (Ambion, Inc., Austin, TX). Total RNA (10 μg) was separated on a 1% formaldehyde gel and transferred to GeneScreen membrane (New England Nuclear, Boston, MA) in 20X standard saline citrate. The membrane was hybridized with an antisense oligonucleotide probe specific to human ATB^0 (5′-TTACATGACTGATTCC TTCTCAGAG-3′), and then stripped and rehybridized with an oligonucleotide probe specific for 18 S ribosomal RNA (5′-GTTATTGCTCAATCTCGG GTG-3′). Autoradiographs were scanned with a laser densitometer and the ATB^0 signal was normalized to 18S RNA. The ATB^0 probes were 3′ end-labeled using terminal transferase and ^{32}P-dATP and the 18S probe was 5′ end-labeled using T_4 polynucleotide kinase and ^{32}P-ATP.

Western Blot Analysis of Phospho-Protein Kinase C and Mitogen-Activated Protein Kinases

Following pretreatment of cells with various agents (described), cells were rinsed three times with phosphate-buffered saline solution. IEC-6 whole-cell lysate was obtained by incubating cells in lysis buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 1.0 mmol/L EGTA, 100 mmol/L NaF, 0.2 mmol/L Na_3VO_4, 1 mmol/L phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin). An equal amount of protein from control and treated cells was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to Immobilon-P transfer membrane (Millipore, Medford, MA). The transfer membrane was then hybridized with phospho-protein kinase C antibody and MAPK antibodies (Cell Signaling Technology, Beverly, MA) overnight at 4C and rehybridized with horseradish peroxidase–conjugated secondary antibody (1:2000). Protein was detected using the ECL system (Amersham, Piscataway, NJ).

Statistical Analysis

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

RESULTS

Effect of Insulin-Like Growth Factor-2 on L-Glutamine Uptake Activity

Transport time course of L-glutamine into IEC-6 cell monolayer was first performed over transport periods of 0 to 15 minutes. The L-glutamine (1 μmol/L and 10 mmol/L) transport rate was linear up to the 10-minute uptake period. A 2-minute uptake period was selected for the remainder of the study. To test the effect of IGF-2 on glutamine transport activity, glutamine transport was measured in IEC-6 cells after the cells had been incubated in IGF-2 (0 to 1 μg/ml) for various times (minutes to 72 hours) (Fig. 1). IGF-2 stimulated the glutamine transport activity in a time- and dose-dependent manner. At least 24 hours of continuous incubation was required for IGF-2 to exhibit stimulatory effect. A prolonged continuous incubation (48 hours) of IGF-2 (500 ng/ml) resulted in a 50% increase in glutamine transport activity. Pulse IGF-2 stimulation, where cells were exposed to IGF-2 for up to 6 hours and reincubated in IGF-2–free medium for the remaining period of incubation (42 hours), did not affect the glutamine transport activity compared to control cells. IGF-2 stimulated glutamine transport activity in a dose-dependent manner. Significant stimulation

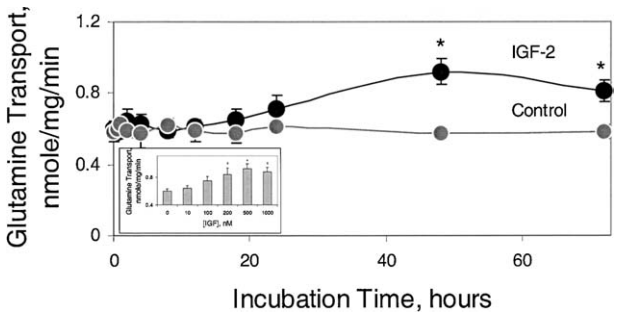

Fig. 1. Effect of IGF-2 on glutamine transport activity. Uptake of glutamine (50 μmol/L) was measured in cells incubated in IGF-2 (0 to 1000 ng/ml) for various periods of time (minutes to 72 hours). Transport values are means ± SD (n = 6; *$P < 0.05$).

was observed at [IGF-2] > 100 ng/ml (see Fig. 1). Therefore a 48-hour IGF-2 (500 ng/ml) treatment point was selected for the subsequent experiments in this study.

Glutamine transport kinetics was then measured. Transport of glutamine in various concentrations (1 μmol/L to 10 mmol/L) was measured in control and IGF-2–treated cells. Fig. 2 shows the Eadie-Hofstee transformation of the Na^+-dependent glutamine transport kinetics. IGF-2 treatment stimulated the Na^+-dependent system B glutamine transport maximal velocity (V_{max}, 2.51 ± 0.23 nmole/mg/min control vs. 4.31 ± 0.36 nmole/mg/min IGF-2 treatment; $P < 0.01$). However, the transporter apparent affinity (K_m) was not affected by the IGF-2 incubation (K_m, 280 ± 30 μmol/L glutamine control vs. 310 ± 30 μmol/L glutamine IGF-2 treatment; P = NS) (see Fig. 2).

These data suggest that prolonged IGF-2 exposure stimulates IEC-6 glutamine transport activity via a mechanism that involves an increase of functional transport units as indicated by the transport kinetic parameters.

Involvement of De Novo Transcription and Translation Processes in the IGF-2 Stimulation of Glutamine Transport Activity

To assess the effect of IGF-2 on glutamine transport system B transporter gene ATB^0 expression, ATB^0 mRNA levels were measured in control and IGF-2–treated cells. The mRNA level was increased threefold after 48 hours of continuous IGF-2 incubation (relative levels: 1.0 control vs. 3.2 ± 0.3 IGF-2 group; $P < 0.001$) (Fig. 3).

To test whether the IGF-2 stimulation required de novo transcription and/or translation, glutamine transport activity was measured in control and IGF-2–treated cells with actinomycin D (Act-D; 0 to 0.1 μmol/L), or cycloheximide (CHX; 0 to 1 μmol/L) in the incubation medium. Act-D and CHX individually blocked the IGF-2–induced system B glutamine uptake (Fig. 4). The concentration of Act-D and CHX was selected so that baseline control cell transport activity was not affected to minimize the nonspecific inhibititory effect of Act-D and CHX. The protein content and cell numbers of the 48-hour Act-D– or CHX–treated cells were comparable to the pretreatment levels. The viability (by dye exclusion) of both control and Act-D/CHX–treated cells was greater than 99%. Compared to the control group (with only DMEM treatment), the Act-D/CHX–treated cells had 10% less protein and 20% fewer cells. The inhibitory effect of Act-D or CHX on the system B glutamine uptake was likely due to inhibition of new protein synthesis rather than a cytotoxic effect.

These data suggest that IGF-2 stimulation of glutamine transport involves elevation of transporter

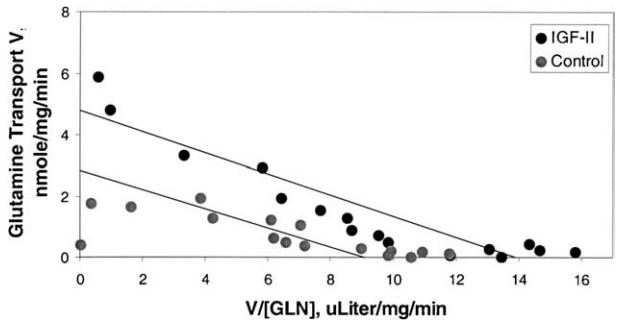

Fig. 2. Eadie-Hofstee transformation of system B glutamine uptake kinetics. Uptake of glutamine (1 μmol/L to10 mmol/L) was measured in cells incubated in IGF-2 (0 to 500 ng/ml) for 48 hours. Transport values are means ± SD (n = 9).

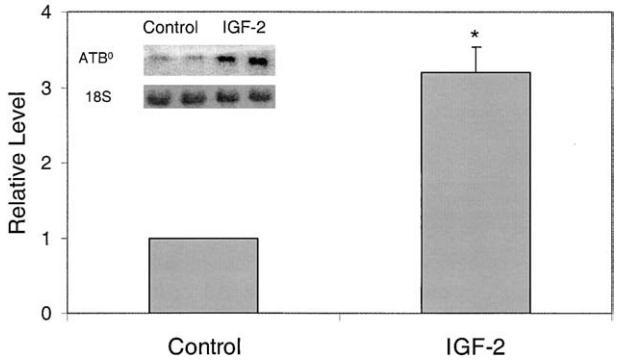

Fig. 3. Northern blot of system B mRNA (ATB^0). Glutamine transporter ATB^0 levels were measured in cells incubated in IGF-2 (0 to 500 ng/ml) for 48 hours.

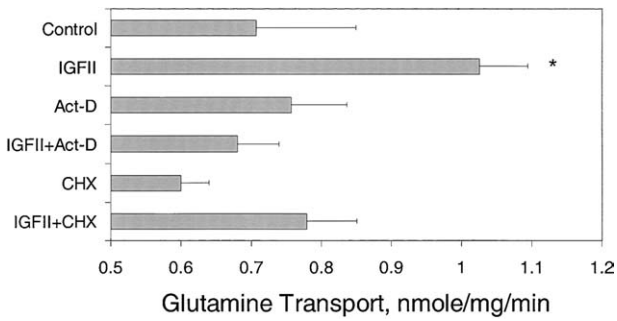

Fig. 4. Effect of actinomycin-D (*Act-D*) and cycloheximide (*CHX*) on IGF-2–stimulated glutamine transport activity. Uptake of glutamine (50 μmol/L) was measured in cells incubated in IGF-2 (0 to 500 ng/ml) ± Act-D (0 to 0.5 μmol/L) and CHX (0 to 10 μmol/L). Transport values are means ± SD (n = 6).

mRNA levels and a de novo transcription and translation process.

Involvement of Protein Kinase C Activation in the IGF-2 Stimulation of Glutamine Transport Activity

To assess the effect of IGF-2 on cellular protein kinase C (PKC) activity, phospho-PKC (pan) activity was measured by Western blot analysis using commercially available phospho-PKC (pan) antibody in control and IGF-2–treated cells. Phospho-PKC levels were elevated in the IGF-2–treated cells (Fig. 5), suggesting IGF-2 stimulated activation of PKC activity.

To further define the involvement of PKC activation in the IGF-2 stimulation of glutamine transport, glutamine transport activity was measured in both control and IGF-2–treated Caco-2 cells in the presence and absence of the specific PKC inhibitor chelerythrine (0 to 6.6 μmol/L; DMSO as control). Chelerythrine abolished the IGF-2–induced glutamine transport activity (Fig. 6) without affecting the baseline transport levels. The concentration of chelerythrine was selected so that baseline control cell transport activity was not affected to minimize the possible nonspecific inhibition effect of chelerythrine. The protein content and cell numbers of the cells treated for 48 hours with chelerythrine were comparable to that in the control cells.

These data suggest that IGF-2 stimulates intracellular PKC activity, and the IGF-2 stimulation of system B glutamine transport activity is mediated by intracellular PKC activation.

Involvement of Mitogen-Activated Protein Kinases in the IGF-2 Stimulation of Glutamine Transport Activity

To assess the effect of IGF-2 on MAPK activities in Caco-2 cells, MAPK p44/42 and MAPK phospho-p44/42 levels were measured by Western blot analysis using commercially available MAPK p44/42 and MAPK phospho-p44/42 antibodies in cells treated with IGF-2 (0 to 500 ng/ml) for 48 hours. IGF-2 elevated the active form MAPK phospho-p44/42 activity but not the total MAPK p44/42 (Fig. 7), suggesting that IGF-2 activated the MAPK ERK 1 cascade.

To further define the role of MAPKs in the IGF-2 activation of glutamine transport activity, glutamine transport activity was measured in cells treated with IGF-2 with or without coincubation of the specific MAPK MEK1 inhibitor PD 98059 (0 to 50 μmol/L, DMSO as control). PD 98059 (50 μmol/L) blocked the IGF-2–induced activation of glutamine transport in a dose-dependent fashion without affecting the control cells (Fig. 8). The concentration of PD 98059 was selected so that baseline control cell transport activity was not affected to minimize the nonspecific inhibitory effect of PD 98059. The protein content and cell numbers in the cells treated for 48 hours with PD 98059 were comparable to levels in the control cells.

These data indicate that IGF-2 stimulates the MAPK MEK1/2 cascade that mediates the IGF-2 stimulation of glutamine transport activity in IEC-6 cells.

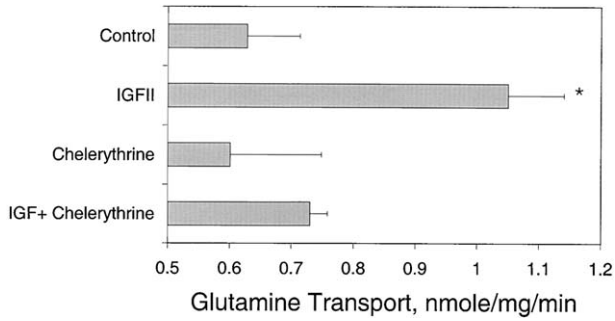

Fig. 6. Effect of inhibitor of protein kinase C on IGF-2 stimulation on glutamine transport uptake of glutamine (50 μmol/L) was measured in cells incubated in IGF-2 (0 to 500 ng/ml) ± protein kinase C inhibitor chelerythrine chloride (0 to 6.6 μmol/L). Transport values are means ± SD (n = 9; $^*P < 0.01$).

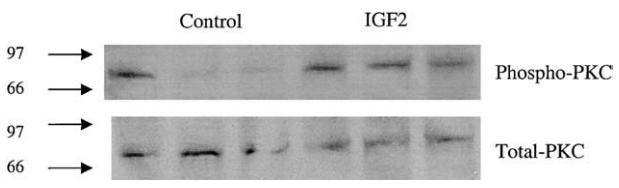

Fig. 5. Western blot of phospho-protein kinase C (*PKC*). Whole-cell PKC and phospho-PKC (pan) levels were measured using monoclonal PKC and phospho-PKC antibodies in cells incubated in IGF-2 (0 to 500 ng/ml) for 48 hours.

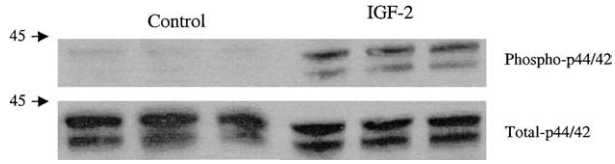

Fig. 7. Western blot of mitogen-activated protein kinase p44/42 and phospho-p44/42. Whole-cell p44/42 and phospho-p44/42 levels were measured using monoclonal mitogen-activated protein kinase p44/42 and p44/42 antibodies in cells incubated in IGF-2 (0 to 500 ng/ml) for 48 hours.

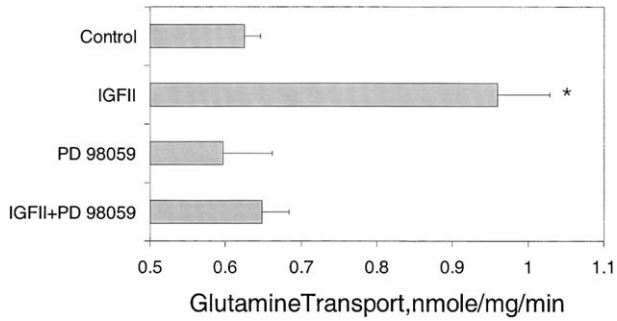

Fig. 8. Effect of inhibitor of mitogen-activated protein kinase MEK on IGF-2 stimulation in glutamine transport uptake of glutamine (50 μmol/L) was measured in cells incubated in IGF-2 (0 to 500 ng/ml) ± MEK 1 inhibitor PD 98059 (0–50 μmol/L). Transport values are means ± SD (n = 9; *$P < 0.01$).

DISCUSSION

The objective of this study was to investigate in vitro the regulation of intestinal epithelial membrane glutamine transport by the peptide growth factor IGF-2 and associated intracellular signaling pathways such as PKC activation and MAPK activation.

The small intestinal epithelia, exposed to various stimuli including luminal growth factors, which regulates epithelial cell growth, proliferation, and differentiation,[6–10] undergo rapid proliferation and differentiation along the crypt-villus axis. IGF-1 and IGF-2 are important mitogenic polypeptides in intestinal epithelial cell proliferation and growth. The action of IGF is thought to be through IGF-1 receptor interaction and modulated by various IGF binding proteins.[10–12] IGF-2 elicits its functions through binding to the IGF receptor, a tyrosine kinase in the plasmic membrane, which regulates many biological activities.[10–12] The IGF receptor activates phospholipase, MAPK, ras, and phosphoinositol-3 kinase.[10–12]

The intestinal epithelial cell line (IEC-6), which is derived from rat small intestine crypt cells, undergoes proliferation and grows as a homogeneous population under standard cell culture conditions.[16] IEC-6 cells express high levels of receptors for IGF and they proliferate, but do not differentiate, in response to exogenous insulin and IGFs. The response of intestinal epithelial cells is consistent with mediation primarily by the IGF receptors, making it a suitable model for studying growth factors and cell proliferation.[17,18] Proliferating IEC-6 cells secrete significant amounts of IGF-2 during proliferation; this IGF-2 exhibits an autocrine effect by stimulating further rapid cell proliferation. However, the effect of this autocrine function on amino acid absorption is still unknown.

Glutamine is the most abundant amino acid in the body; it has profound effects on intestine-related immune functions and has an anabolic effect on host protein synthesis.[13] Luminal glutamine transport across intestinal brush-border membrane into enterocytes, via discrete amino acid transport systems, is an essential step in maintaining host glutamine homeostasis and is regulated by various local as well as systemic factors.[14,15] Amino acid transport systems were traditionally characterized on the basis of criteria such as substrate selectivity, Na^+ dependency, pH sensitivity, amino acid analog inhibition profile, and kinetic analysis.[21,22] Glutamine transport across IEC-6 plasma membrane occurs via three components: passive diffusion, a Na^+-independent facilitated transport system L, and a Na^+-dependent transport system B. At a low glutamine concentration (50 μmol/L), passive diffusion accounts for less than 1% of the total transport, Na^+-independent system L transport accounts for 30% transport, and the Na^+-dependent system B accounts for nearly 70% of the total transport activity.

IGF-2 elicits its biological activities through two classes of mechanisms: an acute phase mechanism (in minutes) and a chronic phase (in hours).[10–12,16–18] The acute phase involves intracellular phosphorylation, triggering rapid responses. On the other hand, the chronic phase normally involves intracellular cascades and protein synthesis to provide slow but sustained responses. In our study, IGF-2 stimulated the glutamine transport activity in a time- and dose-dependent manner. As shown in Fig.1, prolonged IGF-2 exposure (>24 hours) was required for IGF-2 to stimulate glutamine transport activity. It is well known that IEC-6 cells produce IGF-2 as well as IGF binding proteins that inhibit IGF bioavailability. To minimize the possible endogenous autocrine IFG-2 effect, during the IGF-2 continuous treatment the incubation medium was changed every 6 hours to ensure a consistent IGF-2 exposure and minimize the possible involvement of a paracrine effect that may be associated with IGF-2 exposure. Transient exposure of IGF-2 does not exhibit the same stimulatory effect. These data suggest the IGF-2–induced glutamine transport stimulation participates in the chronic phase of IGF-2 activity rather than triggering an acute effect.

Transport activity can be altered by modulation of existing transporter and/or synthesis of new transporter units. Analysis of transport kinetic parameters can predict how transport activity is altered. Fig. 2 represents the Eadie-Hofstee transformation of Na^+-dependent glutamine transport kinetics. Our kinetic analyses revealed that maximal capacity V_{max} was increased by IGF-2, while transport affinity K_m was

unaffected. Taken together with the increase in glutamine transporter mRNA ATB^0 by IGF-2 exposure (see Fig. 3), the data suggest that IGF-2 stimulates glutamine uptake by increasing functional copies of system B transport units rather than modifying transport affinity. The elevation of transporter ATB^0 mRNA after IGF-2 treatment (see Fig. 3) indicates that IGF-2 stimulates system B glutamine transport activity by either specifically enhancing the transcription of the system B transporter ATB^0 transcription or stabilizing the transcribed mRNA.

Act-D or CHX in the incubation medium each blocked the IGF-2–induced glutamine uptake (see Fig. 4), indicating the possible involvement of transcription and de novo protein synthesis. Low concentrations of Act-D and CHX were selected to minimize the nonspecific inhibitory effect that Act-D and CHX might have on cells. Because a system B antibody is currently not available, it is unclear whether the observed increase in transport activity V_{max} reflects de novo protein synthesis of the transporter protein itself or another regulatory protein.

IGF-2 exhibits its biological activity via binding to IGF receptor, which activates a cascade of intracellular pathways. IGF-2 binds to IGF-1 receptors, which show approximately 84% homology with the insulin receptor. IGF receptor is a tyrosine kinase that catalyzes various protein kinases cascade including ras and MAPK ERK cascade.[16–18]

PKC is a family of intracellular enzymes that mediates diverse biological intestinal mucosal functions including amino acid transport.[16,18] As shown in Fig. 5, IGF-2 incubation increased the phospho-PKC level indicating an increase in PKC activity. CHX, a specific PKC inhibitor that specifically inhibits the catalytic domain of PKC,[23,24] blocked the IGF-2–induced glutamine uptake demonstrating the involvement of PKC in signaling events associated with IGF-2 system B induction in IEC-6 cells.

MAPKs are a family of kinases that mediate various biological activities and regulation of gene expression in response to various stimuli.[25–27] There are at least four distinctly regulated groups of MAPKs: extracellular signal-related kinases (ERK)1/2, Jun amino-terminal kinases (JNK1/2/3), p38 protein (p38α/β/γ/δ), and ERK5. Each group is activated by specific MAPK kinases, such as MEK1/2 for ERK1/2 or MKK3/6 for p38.[25–28] IGF-2 initiates many of its biological activities via activation of intracellular MAPK pathways.[16–18] The intracellular signaling cascade for IGF-2–induced system B glutamine transport in IEC-6 cells is unknown.

As shown in Fig. 6, IGF-2 stimulated MAPK phospho-p44/42, the active form of MAPK p44/42, without altering total MEK1/2, indicating that IGF-2 activates MAPK p44/42 cascade. To delineate the relationship among IGF-2, MAPK, and system B glutamine transport, glutamine transport activity was measured in the presence of MAPK ERK 1 inhibitors. 2′-amino-3′-methoxyflavone (PD 98059). As shown in Fig. 8, PD 98059 blocked IGF-2–stimulated glutamine transport activity without affecting the baseline activity, suggesting that IGF-2 induced upregulation of glutamine transport activity involved MAPK ERK cascade.

CONCLUSION

IGF-2 stimulates intestinal glutamine transport activity and transporter ATB^0 mRNA expression via a mechanism that involves a de novo synthesis of new transporters. This IGF-2 stimulation of glutamine transport is mediated by intracellular PKC and MAPK MEK1/2 pathways.

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Serum Fat-Soluble Vitamin Deficiency and Abnormal Calcium Metabolism After Malabsorptive Bariatric Surgery

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Weight loss after biliopancreatic diversion or duodenal switch is due to decreased calorie absorption secondary to fat malabsorption. Fat malabsorption may also cause essential fat-soluble vitamin deficiencies, which may have severe clinical consequences and alter calcium metabolism. Serum vitamins A, D, E, and K, zinc, parathyroid hormone, corrected calcium, and alkaline phosphatase levels were measured in a cohort of patients who had previously undergone biliopancreatic diversion. Two bariatric surgery units were involved in the study: New York University School of Medicine (New York, NY), and the Wesley Medical Center (Brisbane, Australia). A total of 170 patients completed the study. The incidence of vitamin A deficiency was 69%, vitamin K deficiency 68%, and vitamin D deficiency 63% by the fourth year after surgery. The incidence of vitamin E and zinc deficiency did not increase with time after surgery. The incidence of hypocalcemia increased from 15% to 48% over the study period with a corresponding increase in serum parathyroid hormone values in 69% of patients in the fourth postoperative year. There is a progressive increase in the incidence and severity of hypovitaminemia A, D, and K with time after biliopancreatic diversion and duodenal switch. Calcium metabolism is affected with an increasing incidence of secondary hyperparathyrodisim and evidence of increased bone resorption in 3% of patients. Long-term nutritional monitoring is necessary after malabsorptive operations for morbid obesity. (J GASTROINTEST SURG 2004;8:48–55)

KEY WORDS: Biliopancreatic diversion, duodenal switch, nutrition, bariatric surgery, calcium metabolism, dietary supplements, obesity, morbid [surgery], vitamin A, vitamin D, vitamin E, vitamin K, zinc

Decreased vitamin and nutrient absorption is a well-recognized complication of bariatric surgery. Standard Roux-en-Y gastric bypass is known to cause vitamin B_{12}, iron, and folate deficiencies, and most surgeons would recommend daily multivitamin supplementation for these patients. Long-limb gastric bypass, biliopancreatic diversion, and biliopancreatic diversion with duodenal switch are more aggressive forms of bariatric surgery; in addition to restricting intake, these procedures induce weight loss by fat malabsorption. Fat malabsorption may also affect the absorption of fat-soluble nutrients such as vitamins A, D, E, and K, zinc, and essential fatty acids.

Biliopancreatic diversion and duodenal switch decrease intestinal fat absorption by delaying the mixing of gastric and pancreatic enzymes with bile until the final 50 to 100 cm of the ileum. Caloric intake is markedly reduced as a consequence of fat malabsorption, and rapid sustained weight loss ensues with a mean long-term percentage excess weight loss (%EWL) of 78%.[1] Fat absorption is markedly decreased. Biliopancreatic diversion was originally devised and popularized by Scopinaro et al.[2], who found that only 28% of ingested fat is absorbed after the surgery. It is likely that fat-soluble vitamin absorption is similarly affected.

Case reports have documented metabolic disorders attributable to fat-soluble vitamin deficiency after malabsorptive surgery, but the true incidence of vitamin deficiency is not know. This study examines the

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incidence of fat-soluble vitamin deficiency after malabsorptive bariatric surgery and its effect on calcium metabolism.

METHODS

Institutional review board approval was not sought for this study because regular biochemical analysis, including serum vitamin levels, is part of the standard follow-up protocol that our patients consent to prior to surgery.

Patients who had undergone malabsorptive surgery at two bariatric surgery centers were included in the study. Indications for primary bariatric surgery were based on the National Institutes of Health recommendations for bariatric surgery. All patients qualified with a body mass index greater than 40 kg/m^2, or greater than 35 kg/m^2 in association with a recognized comorbid condition. A further indication for biliopancreatic diversion was for revision after laparoscopic adjustable gastric band failure. Prior to surgery, all patients underwent full psychological and medical evaluation with subsequent dietary education. All patients were prescribed the following supplements as mandatory postoperative care: 1 multivitamin daily; iron (325 mg with B_{12} and folate) daily; calcium citrate (1800 mg) daily; and fat-soluble vitamin supplements (10,000 IU of vitamin A, 1200 IU of vitamin D, 60 IU of vitamin E, and 300 μg of vitamin K) daily. All patients were placed on empiric H_2-blockers. All operations were performed by two surgeons (C.A.R. and G.A.F.) who used similar techniques. Biliopancreatic diversion was performed with the use of a 200 cm alimentary limb and a 50 cm common channel, either as a laparoscopic or an open procedure. Biliopancreatic diversion with duodenal switch was performed with a 175 to 200 cm alimentary limb and a 50 to 75 cm common channel.[3] Patients were seen every 3 months for the first postoperative year and every 6 to 12 months subsequently. Demographic and outcome data including body mass index and %EWL were collected prospectively.

In October 2002, all patients who were at least 12 months after surgery—either biliopancreatic diversion or duodenal switch—were queried from a prospective database, which each program maintained. These patients were asked to undergo a series of serum laboratory tests. In addition to comprehensive electrolytes, hematology panel, and iron levels, serum vitamin A, D, E, and K, parathyroid hormone, alkaline phosphatase, corrected calcium, and zinc levels were measured. All results were entered into a computerized spreadsheet (Microsoft Excel) and tabulated for mean value with 95% confidence interval (CI), range, and percentage of abnormal (Statview; Abacus Concepts, Inc., Berkeley, CA).

RESULTS

From July 1999 to December 2002, a total of 376 patients underwent biliopancreatic diversion with or without duodenal switch. Two hundred two patients were found to be 12 months or longer postoperative after surgery. Of these 202 eligible patients, 170 completed the study, reflecting an overall follow-up rate of 84%. Forty six patients (27%) were 12 months after surgery, 59 (35%) were 24 months after surgery, 37 (22%) were 36 months after surgery and 28 (16%) were 48 months after surgery. Biliopancreatic diversion or duodenal switch was performed as a primary operation in 117 patients, and 53 patients underwent malabsorptive surgery as a revision after previous laparoscopic adjustable gastric banding. Ninety-four patients underwent biliopancreatic diversion and 76 patients underwent duodenal switch.

The mean age of the patients at the time of surgery was 45 years (range 17 to 68 years). There were 140 females and 30 males. Mean weight at presentation was 122.7 kg (range 63 to 218 kg) with a mean body mass index of 43 kg/m^2 (range 24 to 73 kg/m^2). There were 46 patients 1 year after surgery, 59 patients 2 years after surgery, 37 patients 3 years after surgery, and 28 patients 4 years after surgery. Mean %EWL at 1, 2, 3, and 4 years after surgery was 48% EWL, 68% EWL, 59% EWL, and 59% EWL, respectively. The number of serum values measured, the mean and 95% CI, standard normal ranges, the incidence of low serum values, and the mean (95% CI) for the low values are shown for each of the nutrients measured in tabulated and graph form in Tables 1 and 2 and Figs. 1 and 2.

The incidence of low serum vitamin A levels was 52% in the patients 1 year after surgery and increased annually to 69% by 4 years after surgery. Similarly, vitamin K levels were abnormal in 51% of the patients 1 year after surgery and by the fourth year the incidence had increased to 68%. By the fourth year, 42% of patients had serum vitamin K levels that were unquantifiable with serum levels less than 0.1 nmol/L. Serum vitamin E levels were normal in all of the patients less than 1 year after surgery and remained normal in 96% of the patients up to 4 years after surgery. Serum zinc levels were abnormally low in 51% of the patients at 1 year and remained at a similar level with a 50% incidence of low zinc levels at 4 years after surgery (see Table 1 and Fig. 1).

Serum vitamin D levels were abnormal in 57% of the patients 1 year after surgery and increased so that

Table 1. Serum fat-soluble vitamin and zinc levels

Nutrients	Years after bariatric surgery			
	1 yr	2 yr	3 yr	4 yr
Vitamin A (1.6–2.3 nmol/L)				
Normal	22 (48%)	25 (42%)	11 (30%)	8 (31%)
Low	24 (52%)	34 (58%)	26 (70%)	19 (69%)
Total No. of patients	46	59	37	27
Mean (95% CI) for low values	1.21 (range 1.08–1.34)	1.21 (range 1.08–1.34)	1.02 (range 0.89–1.16)	0.88 (range 0.71–1.05)
Vitamin E (>7 μmol/L)				
Normal	44 (100%)	36 (86%)	34 (97%)	23 (96%)
Low	0	6 (14%)	1 (3%)	1 (4%)
Total No. of patients	44	42	35	24
Mean (95% CI) for low values		5.17 (range 3.36–6.97)	6	4
Vitamin K (0.3–2.6 nmol/L)				
Normal	17 (49%)	15 (38%)	10 (31%)	6 (32%)
Low	13 (37%)	16 (41%)	18 (56%)	5 (26%)
Very low (<0.1 nmol/L)	8 (14%)	8 (21%)	4 (13%)	8 (42%)
Total No. of patients	35	39	32	19
Mean (95% CI) for low values	0.13 (range 0.09–0.17)	0.14 (range 0.09–0.18)	0.19 (range 0.15–0.24)	0.11 (range 0.06–0.17)
Zinc (12–28 μmol/L)				
Normal	21 (49%)	28 (50%)	25 (71%)	13 (50%)
Low	22 (51%)	28 (50%)	12 (29%)	13 (50%)
Total No. of patients	43	56	35	26
Mean (95% CI) for low values	10.6 (range 9.62–11.7)	10.6 (range 9.61–11.5)	11 (range 10.3–11.8)	9.6 (range 7.79–11.38)

by the fourth year 63% of the patients had vitamin D deficiency. Hypocalcemia was present in only 15% of the patients 1 year after surgery and increased to 48% by the fourth postoperative year. The incidence of secondary hyperparathyroidism was 31% 1 year after surgery and increased to 69% at 4 years. By year 4, 27% of patients were considered to have clinically significant hyperparathyroidism, with serum parathyroid hormone levels more than 50% above the upper limit of normal. Of these patients, 6% had raised alkaline phosphatase levels, but only 3% had normal results for all of the remaining liver function tests (see Table 2 and Fig. 2)

No patient in this cohort suffered symptoms of night blindness or visual abnormality, bone pain or fracture, excessive bleeding, or any metabolic sequelae associated with these fat-soluble vitamin derangements.

DISCUSSION

Malabsorptive operations for morbid obesity have been shown to result in significant weight loss that is sustained for up to 20 years.[1] The initial weight loss is attributable to moderate gastric restriction. However, weight loss durability is thought to be due to the malabsorption created by the diversion of pancreatic enzymes and bile from the alimentary tract and food bypassing the jejunum and proximal ileum. The last 50 to 75 cm of distal ileum, also known as the common channel, is where food is exposed to bile and pancreatic enzymes. This results in absorption of only 28% of ingested fat and 57% of ingested protein.[2]

This study confirms that fat malabsorption not only results in weight loss but also affects the absorption of fat-soluble nutrients, and with time increasing numbers of patients develop fat soluble hypovitaminemia. Prior to this study, the incidence of fat-soluble vitamin deficiency after biliopancreatic diversion and biliopancreatic diversion with duodenal switch was not known.

Before this study, it was not our usual practice to measure serum fat-soluble nutrients before surgery, and consequently it was not possible to compare the results of this study with preoperative control values. Obesity is known to affect fat-soluble vitamin biochemistry,[4] and in this study nearly half of the patients had low serum zinc, vitamin A, vitamin K, and vitamin

Table 2. Calcium metabolism

	Years after bariatric surgery			
	1 yr	2 yr	3 yr	4 yr
Vitamin D (50–300 nmol/L)				
Normal	20 (43%)	27 (45%)	20 (54%)	10 (37%)
Low	26 (57%)	33 (55%)	17 (46%)	17 (63%)
Total No. of patients	46	60	37	27
Mean (95% CI) for low values	37.6 (range 33.12–42.1)	30.9 (range 26.5–35.38)	34 (range 28.21–40.02)	37.6 (range 31.5–43.8)
Corrected calcium (2.25–2.65 mmol/L)				
Normal	38 (85%)	44 (73%)	25 (69%)	14 (52%)
Low	7 (15%)	16 (27%)	11 (31%)	13 (48%)
Total No. of patients	45	60	36	27
PTH (6–40 ng/L)				
Normal	29 (69%)	32 (57%)	13 (38%)	8 (31%)
High	9 (21%)	20 (35%)	11 (31%)	11 (42%)
SH	4 (10%)	4 (8%)	11 (31%)	7 (27%)
Total No. of patients	42	56	35	26
Alkaline phosphatase (30–150 μ/L)				
Normal	43 (93%)	53 (86%)	33 (89%)	26 (96%)
High	3 (7%)	5 (14%)	4 (11%)	1 (4%)
Total	46	58	37	27

PTH = parathyroid hormone; SH = significantly high, greater than 50% above upper limit of normal.

D levels within 1 year of surgery, suggesting that fat-soluble vitamin deficiency was already present in many of our patients before surgery. Previous small studies in nonoperated obese subjects have found the incidence of low serum vitamin D levels to be approximately 16%,[5] and in a study by Bell et al.[6] the mean serum vitamin D level was 29 ng/ml in obese patients compared with 37 ng/ml in nonobese patients. Similarly, in obese boys Decsi et al.[7] found that median serum vitamin E (3.41 vs. 7.46 mg/L) and vitamin A (0.038 vs. 0.078) mg/L) levels were reduced compared to control values. Preoperative testing of fat-soluble nutrients is now performed routinely in our practices.

Although the incidence of vitamin deficiency was not known, the metabolic consequences of these vitamin derangements after biliopancreatic diversion have been reported. Scopinaro et al.[1] observed a 2.8% incidence of night blindness. Two case reports of night blindness caused by vitamin A deficiency after biliopancreatic diversion have been published,[8,9] and a single case report has documented temporary neonatal blindness in an infant born to a mother with vitamin A deficiency after malabsorptive bariatric surgery.[10] High-dose supplementation appears to be effective in reversing this condition. Our study clearly shows that the incidence of vitamin A deficiency increases with time such that 70% of patients are affected by the fourth postoperative year despite dietary vitamin A supplementation.[11]

Vitamin K levels are also affected by an observed increase in the incidence of deficiency over time, as well as an increase in the severity of the deficiency. By the fourth year of the study, 42% of patients had serum vitamin K levels below the measurable range of 0.1 nmol/L compared with 14% at the end of the first year. The clinical significance of this low level is not known; there have been no published studies of blood clotting after bariatric surgery, and no case reports have documented altered clotting or spontaneous hemorrhaging after malabsorptive operations. The personal experience of the authors has observed increased sensitivity to warfarin after biliopancreatic diversion and duodenal switch, which resulted in spontaneous gastric bleeding requiring significant lowering of warfarin dosing. This should warn surgeons to counsel their patients on the potential increased risk of bleeding should they undergo future interventions or have warfarin prescribed for them.

Although vitamin E levels are known to be affected by obesity,[12] in our study the incidence of vitamin E deficiency was low and did not increase with time. Despite this, oral vitamin E supplementation has been shown to normalize the results of liver function tests in obese children with nonalcoholic steatohepatitis,

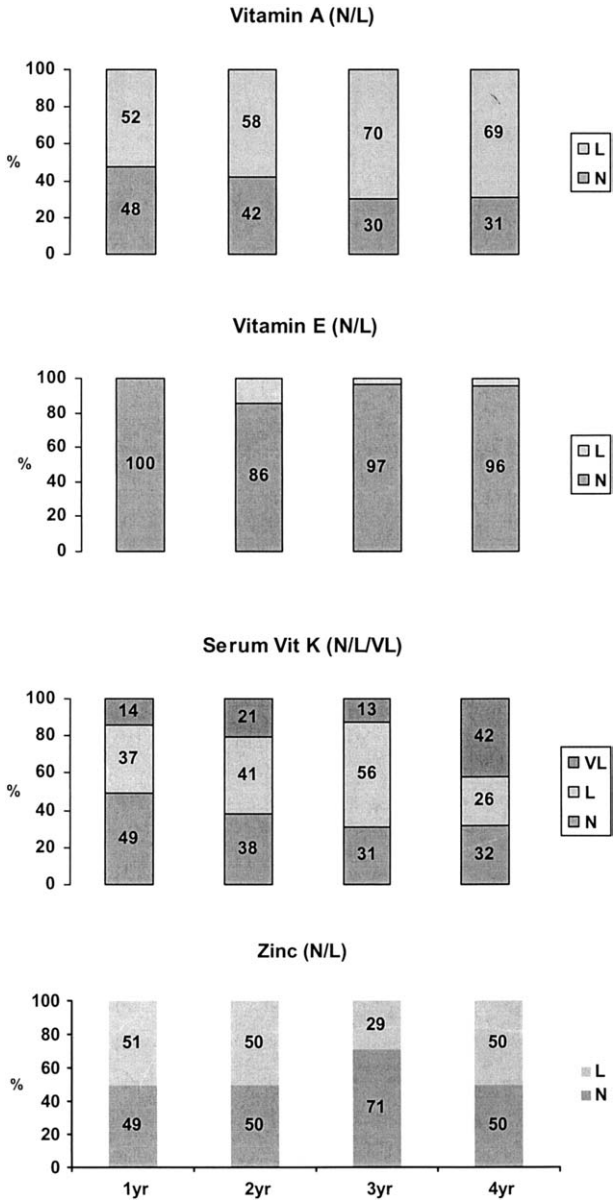

Fig. 1. Annual percentage normal and low serum vitamin A, E, K, and zinc levels after biliopancreatic diversion.

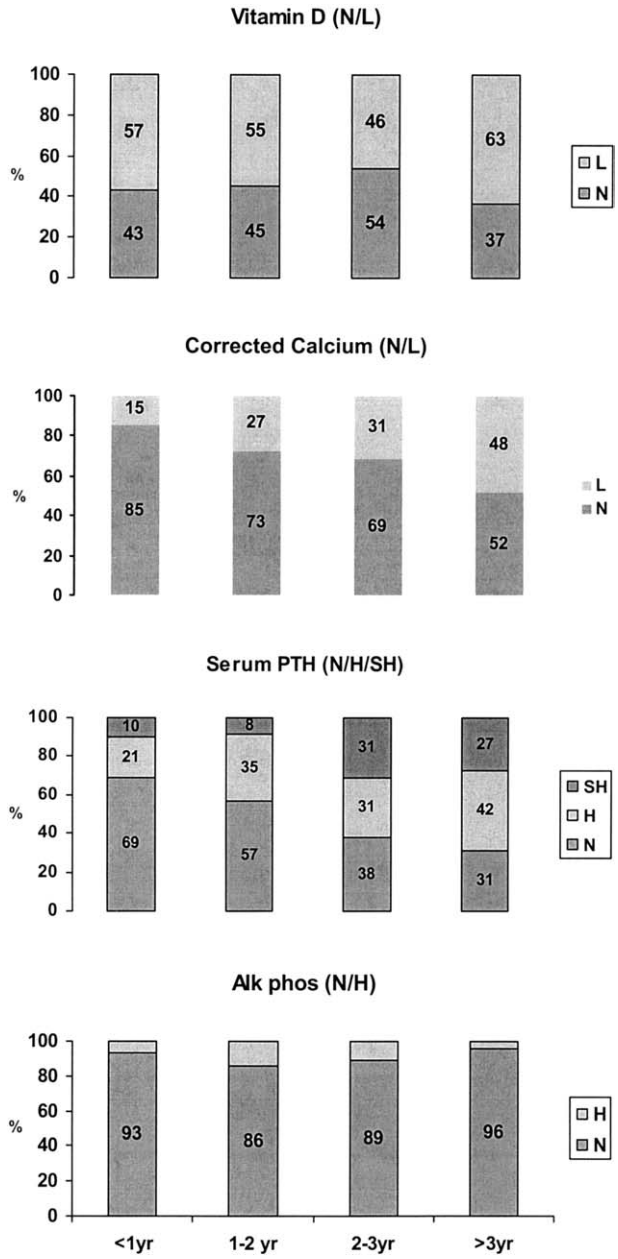

Fig. 2. Annual percentage normal and low serum.

and it would seem sensible to continue advising patients to supplement their diets with vitamin E.[13] Zinc deficiency has not been well described, and its metabolic sequelae have not been clearly delineated. However, as a nutrient that depends on fat absorption, zinc was observed to be deficient in up to 50% of patients and therefore confirms yet another nutrient that can be affected by malabsorptive operations.

Hypocalcemia is a well-recognized complication of malabsorptive operations. Marceau et al.[14] observed a 20% incidence of hypocalcemia, with 2% of patients suffering bone fractures. Similarly, Scopinaro et al.[1] reported a 6% incidence of bone loss. Malabsorption of vitamin D interferes with intestinal absorption of calcium, leading to secondary hyperparathyroidism. Osteomalacia and secondary hyperparathyroidism has been reported previously after biliopancreatic diversion but the long-term sequelae are not known.[15] The results of this study have similarly shown an increase in the incidence of hypocalcemia with time; 48% of patients had hypocalcemia by the fourth postoperative year. In parallel with the development of hypocalcemia, the incidence of secondary hyperparathyroidism increases with time. An increase in

serum parathyroid hormone to a serum level 50% above the upper limit of normal is considered to be clinically important and by the fourth year, one fourth of the patients were at this level. It is likely that only those patients with secondary hyperparathyroidism and an isolated increase in serum alkaline phosphatase have demineralization of their bony skeletons and are at risk of pathologic fracture. In this study, 3% of patients had raised parathyroid hormone and alkaline phosphatase levels with otherwise normal liver function tests, which correlates closely with Marceau's 2% incidence of pathologic fracture after malabsorptive surgery.[14] Bone scans were not performed routinely but may be indicated in high-risk subgroups, particularly postmenopausal women, on an annual basis.

Patient education is a critical component of both preoperative preparation and continual postoperative care. This is incorporated into the bariatric programs by the surgeons, with the knowledge that nutritional deficiencies are a rare but distinct complication after these operations. Despite counseling, there continues to be noncompliance by patients, typically up to 40%,[16] although in a previous study by the Australian arm of this study patient compliance with vitamin supplementation after biliopancreatic diversion was greater than 80%.[11] Patient compliance is a major concern after malabsorptive operations mainly because it is an entity over which surgeons have no control. This study reflects the true incidence of fat-soluble vitamin deficiencies after malabsorptive surgery performed within two comprehensive bariatric surgery programs that involve comprehensive nutritional counseling, monitoring, and supplementation.

A recent survey of American bariatric surgeons found that many of them do not routinely measure fat-soluble vitamin levels beyond 1 year after biliopancreatic diversion.[16] Our observational study shows that these vitamin deficiencies actually can worsen after 1 year from surgery. Because long-term sequelae and their incidence are unknown, we support the life-long annual measurement of fat-soluble nutrients after malabsorptive operations for morbid obesity, with continued nutritional counseling and education. In the absence of a proven regimen for vitamin supplementation after malabsorptive surgery, our current practice is life-long daily supplementation of fat-soluble vitamins at the following minimum dosages: 10,000 IU vitamin A; 1200 IU vitamin D; 300 μg vitamin K, and 1800 mg calcium citrate, along with standard supplementation of multivitamin, zinc, and iron/folate therapy. H2-blockers should be given empirically. We would also recommend regular monitoring of serum nutrient levels. Vitamin deficiencies may need to be supplemented with higher doses of the respective vitamin, and may even require intramuscular or intravenous administration. Patients with abnormal laboratory values should be retested after additional supplementation until values improve. Failure to respond to additional vitamin supplementation could be considered an indication for revision of the biliopancreatic diversion, although in the absence of clinical symptoms associated with the vitamin deficiency this may not be justified.

CONCLUSION

Malabsorptive bariatric surgery results in a high incidence of vitamin A, D, and K deficiency with altered calcium metabolism. It is our opinion that these patients need frequent, long-term follow-up with measurement and replacement of these essential nutrients. Surgeons undertaking these operations as a treatment for morbid obesity need to be prepared to follow these patients long-term and to address these nutrient deficiencies. Further study is necessary to investigate the actual incidence of long-term metabolic sequelae and how best to prevent them.

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Discussion

Dr. E. Denham (Chicago, IL): Of all these deficiencies that you identified with the laboratory tests, were you able to see how many patients had symptoms?

Dr. C. Ren: We did not specifically ask for symptoms; however, there were no episodes of night blindness or bone pain. Granted, the bone density studies were done selectively on several patients, but we have not yet collected data on symptoms.

Dr. B. Wolfe (Sacramento, CA): I think this is very interesting data which clearly demonstrate that these deficiency states can and do occur. Of greatest concern is that the deficiencies progressively worsened over the 4 years.

Is this preventable by replacement, or has compliance diminished over the 4 years? Despite ongoing compliance, are these deficiencies progressive, in which case a longer period than 4 years needs to be studied?

Dr. Ren: That is an excellent question, and I think that compliance is a very big issue in this operation. We have seen in the subcohort of patients of more than 2 years an 80% compliance rate. Having said that, you still need to take into consideration that there are going to be patients who are noncompliant. I think this study is important because we have no control over their compliance, and it must be assumed that some patients are not going to be compliant.

Dr. M. Murr (Tampa, FL): The hypocalcemia and the increased serum levels of parathyroid hormones are very striking. I think we need to perform bone densitometry and not just study alkaline phosphatase levels to confirm that weight loss is affecting bone density, and if that is the case. What are your thoughts on how to fix that?

Dr. Ren: I agree. The only person who has addressed bone densitometry is Dr. Marceau in Canada, who has performed many duodenal switch procedures. He finds an increase in bone resorption, but it stabilizes after 10 years. We do not test bone density on a regular basis. However, having seen these data, we will start doing annual bone densitometry testing, because it is not reversible.

Dr. J. Kral (Brooklyn, NY): We are seeing everything reinvented. If you go back in time, more than the 5 years of your literature search, you will find papers on various kinds of supplementation, types of vitamin D, parathyroid hormone levels, and so forth. Scopinaro himself has published papers on fat-soluble vitamins and bone morphometry. In the bad old days of intestinal bypass with "pseudoresearch" done by surgeons with the "Hey! I can do this" mentality, creating monsters of malabsorption and malnutrition, there are numerous reports of night blindness, bone remodeling, and the importance of vitamin E for gut immune function.

The message of your paper is clear and important. Yes, you must have life-long follow-up! But do not try to compare your data with Fielding's in the Australian system! Insurance circumstances, affordability of laboratory testing supplements, office visits, time off from work—the costs to the individuals are so different, it is ridiculous to compare the two. Your follow-up of 61% will likely decrease further, based on decades of experience following postoperative obese patients. You are repeating the mistakes of the 1970s and 1980s.

If we do not find a mechanism to pay for good follow-up after malabsorptive obesity operations, we are doomed to the disdain of the medical community and society at large for performing poor research and providing unacceptable patient care.

Invited Discussion—Expert Commentator

Bruce D. Shirmer, M.D. (Charlottesville, VA): The authors have measured levels of fat-soluble vitamins D, A, parathyroid hormone, alkaline phosphatase, calcium, and zinc following either biliopancreatic diversion or duodenal switch operations. They are to be commended for adding data to what until now has been a relatively poorly studied aspect of these malabsorptive operations. These operations by nature lend themselves to the malabsorption of fat-soluble vitamins, but data as to how severely have been scarce. Vitamin A deficiency, with its potential implications for vision loss, was present in more than half of the patients at 2 years. Elevated parathyroid hormone and alkaline phosphatase levels were found in more than 40% of patients at 2 years, and vitamin D deficiency in nearly that many. The implications for severe long-term bone disease are significant. Finally, in the few patients for whom essential fatty acid levels were measured, they were all deficient.

The manuscript is unclear as to how much supplementation these patients had received or been recommended as part of their routine postoperative care. In light of these data, it would be appropriate for the authors to make specific recommendations for supplementation amounts, in addition to the obvious appropriate recommendation of the need for long-term follow-up with continued measurement of these vitamin, mineral, and nutrient levels. Finally, all surgeons performing or contemplating performing these malabsorptive operations should assure themselves that their patients comply with long-term follow-up and documentation of the need for and efforts to achieve compliance with such follow-up is performed. Although this is true for all bariatric operations, it is particularly important for the malabsorptive ones.

Predicting the Node-Negative Mesorectum After Preoperative Chemoradiation for Locally Advanced Rectal Carcinoma

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Preoperative chemoradiation therapy (CRT) in patients with locally advanced rectal cancer allows for radical surgery with sphincter preservation in many patients. To determine whether patients downsized with preoperative CRT may be potential candidates for local excision, we investigated residual disease patterns after neoadjuvant treatment. A retrospective analysis was carried out of patients with T3 or T4 rectal adenocarcinoma who were treated with neoadjuvant CRT. Clinical and pathologic data were analyzed to (1) determine the response rates to preoperative CRT in the tumor bed and regional nodal basin and (2) identify the incidence of residual disease in the mesorectum in patients downsized to ⩽T2. A total of 219 patients met the inclusion criteria. Preoperatively 193 patients (88%) were staged as T3, and 99 patients (47%) had clinical N1 disease. The pathologic complete response rate was 20% (43 of 219 patients). T stage was downsized in 64% of the patients (140 of 219), and 69% (67 of 97) of the patients with clinical N1 disease were rendered node negative. Seventeen percent (21 of 122) of patients downsized to ⩽T2 had residual disease in the mesentery. With a median follow-up of 40 months, 182 patients (83%) remain alive and free of disease. Nine patients (4.1%) have had a local recurrence. Although tumor response rates to preoperative CRT within the bowel wall and lymph node basin are similar, one in six patients with pT0–2 tumors will have residual disease in the rectal mesentery and nodes. Despite a substantial reduction in tumor volume with neoadjuvant CRT, local excision should be recommended with caution in patients with locally advanced rectal cancer. (J GASTROINTEST SURG 2004;8:56–63)

KEY WORDS: Rectal cancer, chemoradiation, lymph node, metastasis, neoadjuvant

Multimodality therapy has become the standard treatment for patients with locally advanced (T3/T4) rectal carcinoma. In particular, preoperative chemoradiation therapy (CRT) followed by surgery is frequently favored in this group of patients. The potential benefits of this neoadjuvant approach include sphincter preservation, reduction in risk of radiation injury to the small bowel, and improved function of the nonirradiated neorectum.[1–4]

Most patients achieve at least a partial response after preoperative CRT, with published rates ranging from 26% to 62%.[5–8] Pathologic complete response (pCR) rates have been reported in 7% to 30% of patients.[2,5,7–10] Despite these rates of tumor response, radical resection of the primary tumor and draining lymph nodes remains the standard recommendation. This surgery is associated with a significant risk of urinary and sexual dysfunction as well as anastomotic leakage. There is interest, therefore, in considering a less radical approach to resection of residual disease, particularly in patients with a good response to preoperative CRT. This approach has been evaluated in a number of recent series.[3,11–13] However, these studies have reported on a small number of patients,[3,11–13] have included earlier stage tumors,[3,11,12] and have had a short duration of follow-up.[11] To assess the

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feasibility of a local resection approach in patients downsized after preoperative CRT, we looked at the incidence and pattern of residual disease in 219 patients with T3 or T4 rectal tumors after neoadjuvant treatment.

METHODS

Patient Population

Using a departmental colorectal database, a search was performed to identify patients who met the following criteria: (1) resectable rectal tumor 12 cm or less from the anal verge; (2) T3 or T4 as staged by endorectal ultrasound (ERUS); (3) preoperative CRT; (4) no evidence of metastatic disease either by preoperative imaging or intraoperative assessment; and (5) primary curative surgical treatment received at The University of Texas M.D. Anderson Cancer Center. From a database of 624 patients, 219 patients met these criteria. The patients were accrued from January 1992 through August 2001. Most of these patients were treated by a single surgeon.

Preoperative Chemoradiation Therapy Regimens

Radiation treatment was delivered using a three-field belly-board technique to either a total dose of 45 Gy in 25 fractions or with an additional boost of 7.5 Gy in five fractions to the primary site. Concomitant 5-fluorouracil–based therapy was delivered by continuous infusional or oral (capecitabine) routes.

Assessment of Clinical Response

After completion of preoperative CRT, and just before surgery, patients underwent proctoscopic evaluation. Appearance of the tumor at this time was classified as follows: (1) mucosal ulceration; (2) scar/induration; or (3) no visible abnormality. Additional preoperative staging studies such as CT scan and ERUS were not routinely performed.

Pathologic Evaluation

The resected specimen was staged according to the tumor node metastasis (TNM) classification. Complete pathologic response was defined as the absence of viable tumor in the specimen. The presence of mucin alone was not considered to be positive for residual disease. Any residual tumor was further compartmentalized into disease limited to the bowel wall, disease in the regional nodes, and tumor deposits in the mesentery (combined as mesorectal disease). Patients without tumor in the bowel wall were classified as pT0, and residual mesorectal disease was scored separately. Patients with tumors limited to the bowel wall and with additional separate disease in the mesentery were staged according to the depth of invasion into the bowel wall (T1–2); again the mesenteric deposits were scored separately. Patients with contiguous disease extending through the bowel wall were staged as T3 or T4.

Statistical Analysis

Analysis of local failure and survival was performed using the Kaplan-Meier method, and the curves were compared using Pearson's chi-square test. All quoted significance levels are two-sided.

RESULTS

Patient Population

A total of 219 patients met the criteria for inclusion in this analysis. The majority of patients (88%) had T3 tumors, and nearly half had ultrasound evidence of nodal disease (Table 1). The median distance from the anal verge was 5 cm. Overall, 90% of patients completed their proscribed course of preoperative CRT. Most patients (75%) were able to undergo sphincter-preserving operations. Nine patients did not wish to pursue the recommended radical extirpation of their tumors and instead underwent local excision (n = 6) or Kratzke procedure (n = 3).

Primary Tumor Response to Preoperative Chemoradiation Therapy

Response to preoperative CRT was determined by comparing T stage as determined by ERUS with the final pathologic tumor size. Forty-three patients

Table 1. Characteristics of patient cohort

No. of patients	219
Median age (yr)	58 (range 22–79)
Male: female ratio	126:93
ERUS T3	193 (88%)
ERUS T4	26 (12%)
ERUS N1	99 (47%) (excluding NX)*
Distance from anal verge (median)	5 cm
Completed preoperative CRT	197 (90%)
Sphincter preservation	164 (75%)

ERUS = endorectal ultrasound; T = tumor; N = node; CRT = chemoradiation therapy.
*Nodal status (ERUS NX) could not be ascertained in seven patients.

were found to have no evidence of residual tumor in the resected specimen for a complete pathologic response rate of 20%. In addition, in 140 (64%) of 219 patients, the tumor was downsized by at least one T stage when compared to preoperative ERUS images (Table 2). There was no change in T stage in 71 patients (32%), and progression of local disease was seen in six patients (3%). Patients with ERUS T3 and ERUS T4 tumors were equally likely to have downsizing of their primary tumors (123 [64%] of 193 ERUS T3 patients and 17 [65%] of 26 ERUS T4 patients, respectively) (see Table 2).

Incidence of Nodal Disease

Preoperative nodal staging was complete in 212 of 219 patients. Ninety-nine (47%) of 212 patients were determined to have ultrasound evidence of nodal disease (see Table 1 and Fig. 1). Fifty-one (24%) of 210 patients were found to have pathologic evidence of nodal metastasis (see Fig. 1). Overall, sterilization of the nodal basin occurred in 69% of the patients in this cohort (Table 3), a rate similar to the response seen in the primary tumors (see above).

Time Interval From Completion of Preoperative Chemoradiation Therapy to Surgery

To determine whether the complete pathologic response to preoperative CRT seen in 20% of the cohort may have been influenced by the timing of surgery, we looked at the interval from completion of preoperative therapy to date of surgery. The median interval from completion of preoperative CRT to surgery was 49 days (range 32 to 130 days) in patients without evidence of residual disease and 48 days (range 24 to 205 days) in the remainder of the cohort.

Table 2. Tumor response to chemoradiation therapy. Preoperative endorectal ultrasound stage compared to postoperative pathologic tumor (T) staging

	ERUS T3 (n = 193)	ERUS T4 (n = 26)	All patients (n = 219)
pT0	45 (23.3%)	2 (8%)	47 (21%)
PTIS	2 (1%)	0	2 (1%)
pT1	16 (8.3%)	1 (4%)	17 (8%)
pT2	60 (31.1%)	3 (11%)	63 (29%)
pT3	62 (32.1%)	11 (42%)	73 (33%)
pT4	6 (3.1%)	9 (35%)	15 (7%)
pTX*	2 (1%)	0	2 (1%)

ERUS = endorectal ultrasound; T = tumor.
*Pathologic tumor stage was not available for two patients.

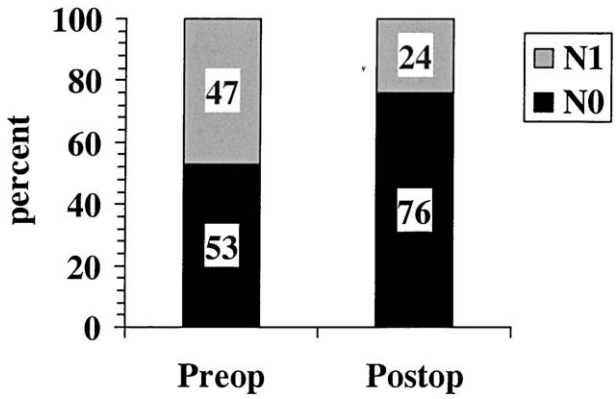

Fig. 1. Incidence of nodal disease before and after preoperative chemoradiation therapy. Preoperative nodal staging by endorectal ultrasound compared to postoperative pathologic nodal evaluation. Data exclude seven patients whose nodal stage could not be determined preoperatively.

Residual Mesorectal Disease After Preoperative Chemoradiation Therapy

We next looked at the pattern of residual mesorectal disease in patients treated with preoperative chemoradiation who underwent the recommended radical resection. Table 4, *A* demonstrates the compartmentalized breakdown of residual disease in patients downsized to ⩽T2. Overall, 17% (21 of 122) of these patients were found to have tumor beyond the bowel wall (lymph nodes plus mesenteric deposits). Specifically, 9% (4 of 45) of patients with pT0 tumors, 20% (3 of 15) of patients with pT1, and 23% (14 of 62) of patients with pT2 tumors had residual tumor in the mesorectum. Exclusion of poorly differentiated tumors did not alter the incidence of mesorectal disease in this subgroup of patients. The overall incidence of residual malignancy beyond the bowel wall in patients with well to moderately differentiated tumors was 17% (19 of 113 patients), 5% (2 of 41) of pT0 patients, 20% (3 of 15) of pT1 patients, and 25% (14 of 57) of pT2 patients.

To improve the selection of patients with node-negative, pT0–2 tumors after preoperative CRT,

Table 3. Downstaging of nodal disease after chemoradiation therapy

	pN0†	pN1
ERUS N0*	88 (83%)	18 (17%)
ERUS N1	67 (69%)	30 (31%)

ERUS = endorectal ultrasound; N = node.
*Excludes seven patients who were staged ERUS NX.
†Excludes nine patients who underwent transanal or Kratzke excision.

Table 4. Incidence of mesorectal disease

All patients	Mesorectal disease	Excluding patients with poorly differentiated tumors	Mesorectal disease (excluding poor differentiation)
A. After Preoperative CRT			
pT0 (n = 45)*	4 (9%)	41	2 (5%)
pT1 (n = 15)†	3 (20%)	15	3 (20%)
pT2 (n = 62)‡	14 (23%)	57	14 (25%)
Overall pT0–T2 (n = 122)	21 (17%)	113	19 (17%)
B. After preoperative CRT in ERUS N0 patients			
pT0 (n = 22)*	2 (9%)	20	1 (5%)
pT1 (n = 11)†	2 (18%)	11	2 (18%)
pT2 (n = 35)‡	7 (20%)	32	7 (22%)
Overall pT0–T2 (n = 68)	11 (16%)	63	10 (16%)

CRT = chemoradiation therapy; ERUS = endorectal ultrasound; N = node; T = tumor.
*Excludes two patients who underwent transanal excision.
†Excludes two patients who underwent transanal excision.
‡Excludes one patient who underwent transanal excision.

we looked at the incidence of residual mesenteric disease in patients who were staged as N0 by ERUS prior to neoadjuvant treatment. Of the 68 patients in this subgroup, 16% were found to have nodal disease at the time of resection (Table 4, *B*). Further selection of patients with ERUS N0, well to moderately differentiated tumors downsized to pT0–2, did not alter the pattern of residual disease seen (Table 4, *B*).

Correlation Between Clinical Appearance and Pathologic Response

Prior to surgical resection, patients underwent proctoscopic evaluation, and the appearance of the tumor was characterized in one of three ways: mucosal ulceration/tumor, healed scar/induration, or no visible mucosal abnormality. Correlation between the clinical appearance of the site of the primary lesion and the final pathologic response assessment (gross residual disease, microscopic disease only, or no residual tumor) is outlined in Table 5. Eighty-seven percent (160 of 184) of patients with a clear mucosal abnormality had residual disease confirmed by pathologic sectioning compared to a 41% incidence (13 of 32 patients) of pathologically verified residual disease in patients with either no visible mucosal abnormality or mucosal scar/induration. However, more than half of the patients (24 of 43; 56%) with a complete pathologic response had gross mucosal abnormalities, and 39% (17 of 43 patients) had scar/induration at the primary site. Only two patients with a complete pathologic response to preoperative CRT had no visible mucosal abnormalities at the time of surgical resection. Sensitivity and specificity of clinical evaluation (presence of ulceration) for predicting any residual pathologic disease (gross and microscopic) was 92% and 44%, respectively.

Local Recurrence

The overall crude local recurrence rate for all patients in the series was 4.1%. Median time to recurrence was 32 months (range 11 to 98 months). There was no statistically significant difference in local recurrence between patients rendered pathologically free of disease and those with residual tumor after preoperative CRT ($P = 0.511$) (Fig. 2, *A*). Of the nine patients with local recurrence, seven had pT3 tumors, one had a pT2 tumor, and one patient had no evidence of any residual tumor in the surgical specimen. One patient had undergone transanal excision. Mesorectal disease was present in four of these nine patients.

Patient Survival

With a median follow-up of 40 months (range 2 to 148 months), 182 patients (83%) remain alive and

Table 5. Correlation between clinical appearance of primary site after chemoradiation therapy and pathologic extent of residual disease*

	Gross residual disease (n = 114)	Microscopic disease (n = 59)	No residual disease (n = 43)
Mucosal ulceration (n = 184)	113	47	24
Scar/induration (n = 29)	1	11	17
No visible abnormality (n = 3)	0	1	2

*Data excludes three patients for whom clinical response information was not available.

free of disease. There were no statistically significant differences in overall survival and disease-free survival between the patients with a complete response to preoperative CRT and those with residual disease on pathologic evaluation of the resected specimen (Fig. 2, *B* and *C*).

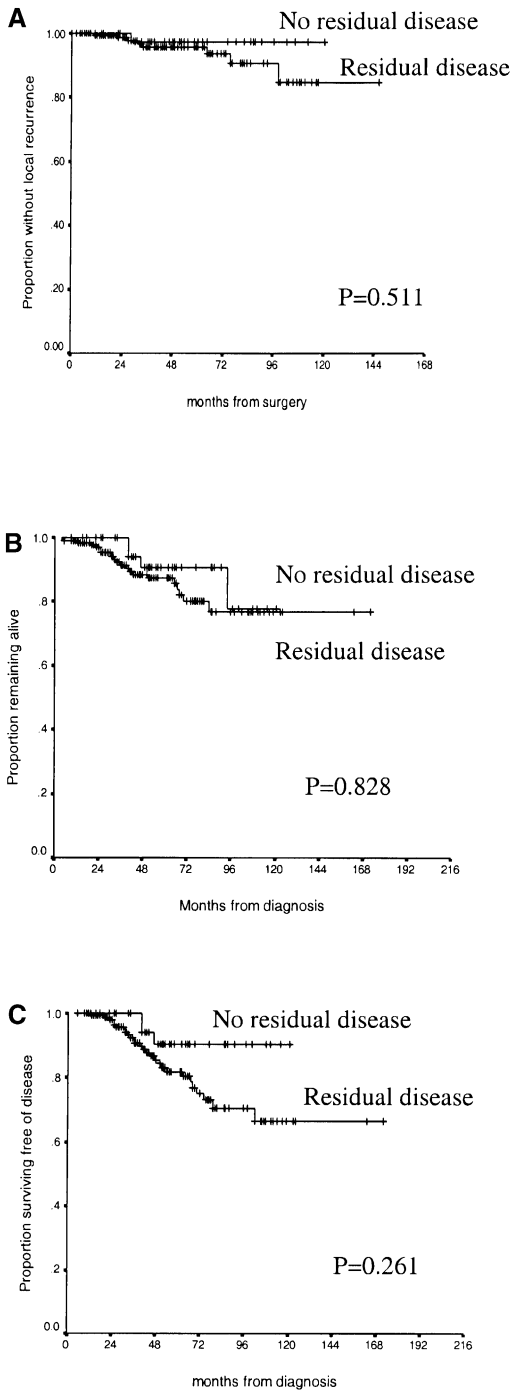

Fig. 2. Local recurrence (**A**), overall survival (**B**), and disease-free survival (**C**) in patients with a complete pathologic response to preoperative chemoradiation therapy compared to those with either gross or microscopic residual tumor. There were no statistical differences between the two groups in any of these parameters.

DISCUSSION

Local excision for rectal cancer has been prospectively evaluated for selected patients with early-stage tumors.[14] The results from this study found that in patients with T1 tumors, local excision appeared to be adequate and in patients with T2 tumors, the outcome was favorable when local excision was combined with postoperative radiation and chemotherapy.

There has been no prospective trial evaluating the role of neoadjuvant CRT followed by local excision. However, there have been a few single-institution reports evaluating the role of local excision in advanced rectal tumors downsized to ⩽T2 with preoperative radiation alone or preoperative CRT.[3,11,12] These studies suggest that patients downsized to ⩽T2 with radiation therapy or CRT have acceptable local recurrence and 5-year survival rates (10% to 13% and 83%, respectively).[3,12] However, these studies have reported on a small number of patients,[3,11–13] have included earlier stage tumors,[3,11,12] and have had a short duration of follow-up.[11] To assess the feasibility of local excision in patients with rectal cancer after preoperative CRT, a better understanding of the patterns of residual disease after such treatment is necessary.

Our data are consistent with previously published reports, including those from our own institution, showing high rates of response to preoperative CRT,[6–8,15] with 64% of patients downsized at least one T stage. This response rate appears to be consistent across ERUS T stages (T3 vs. T4). A similar proportion of patients (69%) with preoperative ultrasound evidence of nodal disease were rendered node negative by neoadjuvant CRT. This analysis of preoperative nodal staging by ERUS needs to be taken with some caution. Recent reports demonstrate that the accuracy of this approach may be as low as 64%, with 25% of patients overstaged and 11% understaged.[16] In our own series 17% of patients staged clinically as N0 were found to have nodal metastases in the resected specimen. Although these cases may represent disease progression after completion of clinical staging, more likely they represent true false negative results of ERUS. These limitations of ERUS certainly have an impact on the accurate determination of nodal response rates to preoperative CRT. Nonetheless, it appears that a substantial proportion of patients do experience sterilization of the nodal basin.

Despite the similar response rates to preoperative CRT within the bowel wall and the mesorectum,

assessment of tumor response in a compartmentalized fashion demonstrates that there are, in fact, discordant responses seen. Specifically, of the 45 patients with no evidence of residual tumor in the bowel wall, four of them (9%) had extramural disease present. This is similar to the 13% incidence of lymph node metastases in pT0 patients reported by Onaitis et al.[9] In addition, even among patients with pT1 tumors, 20% had residual nodal disease, a rate much higher than expected with primary T1 tumors.[17] These discrepancies underscore the fact that in a significant number of patients, the tumor and nodal responses to preoperative CRT are not uniform.

Overall, 17% (21 of 112) of patients with ERUS T3 or T4 tumors who were downsized to ⩽T2 had tumors in the mesentery. Our findings are similar to the those of the Memorial Sloan-Kettering Cancer Center investigators who recently reported that a significant number of patients with T2 or T3 rectal cancer had persistent nodal disease after preoperative radiation therapy.[18] In our series, exclusion of patients with poorly differentiated tumors or those with clinically node-negative staging prior to CRT did not alter this proportion. However, exclusion of poorly differentiated tumors had the greatest impact in those patients with complete sterilization of disease in the bowel wall (T0); among these patients, exclusion of poorly differentiated tumors reduced the incidence of residual extramural disease from 9% to 5%. Therefore, in this highly selected group of patients, with well to moderately differentiated tumors and no residual disease in the bowel wall, the risk of residual disease in the mesorectum may be sufficiently low as to justify a local excision approach.

In the remainder of the cohort of patients downsized to T1–2, more than one in five had residual mesorectal disease that could not be predicted by the differentiation status of the tumor. The persistence of such disease in the nodal basin after neoadjuvant radiation therapy has been shown to increase the risk of local recurrence to as high as 40%.[3,6,7] Therefore, in the absence of reliable criteria to select those patients downsized and rendered node negative, the risk of inadequate, limited excision and subsequent local recurrence appears to be high.

Our study also confirms the difficulty of clinical determination of the extent of the response to preoperative CRT. When we correlated mucosal appearance to pathologic residual disease, we found that more than half of the patients with no residual tumor had mucosal abnormalities suspicious for persistent disease. Other series have similarly reported on the difficulty in using clinical, radiologic, or pathologic criteria in predicting patients with a pathologic complete response.[9,19,20] With no difference in the median time to surgery between patients with pathologic complete responses and all others in the cohort, it is unlikely that the lack of correlation between clinical appearance and pathologic response is simply a function of time. Although patients with a complete pathologic response are potentially overtreated with radical surgical excision, the difficulties in predicting patients with a complete pathologic response currently preclude proper selection of these patients for more limited excision. Furthermore, the long-term impact of limited excision on local recurrence and survival of patients with rectal cancer who are downsized with neoadjuvant CRT is unknown. Therefore treatment with local excision, even in patients with a pathologic complete response after neoadjuvant CRT, should be performed as part of clinical protocols.

CONCLUSION

Despite the high response rate to preoperative CRT, the tumor response in the bowel wall and nodal basin is not uniform, and nearly 20% of patients with pT0–2 tumors have residual extramural disease. In addition, accurate presurgical assessment of the pathologic response remains challenging. Radical surgery, therefore, remains the standard of care for patients downsized by neoadjuvant CRT. Better markers are needed to identify the subgroup of patients with complete pathologic responses in the bowel wall and mesentery who may be candidates for more limited surgical therapy.

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Invited Discussion—Expert Commentator

David Fromm, M.D. (Detroit, MI): Dr. Bedrosian and her associates emphasize the technological limits of preoperative staging of rectal cancers invading the muscularis propria or through the serosa. Downstaging of rectal cancers with neoadjuvant therapy is appealing because local resection is associated with low morbidity and excellent functional results. However, it should be noted that the authors are careful to state that neoadjuvant chemoradiation *downsizes* tumors. They do not use the term downstaging. I believe an unresolved question is the value of neoadjuvant therapy with regard to survival in the present era of total mesorectal excision. There are data suggesting that residual disease in the regional lymphatic vessels, rather than microscopic involvement of the surgical margin, is a major cause of failure after local excision. We must be mindful of the observation that salvage surgery for recurrence after local excision means we are usually dealing with advanced disease, with many patients being unresectable. In those who are resectable, there is a relatively low incidence of 5 year cure (25% in the recently reported aggressive approach used at the Mayo Clinic).

Discussion

Dr. H. Chen (Madison, WI): In your pathologic specimens after resection, of the patients who started out at N0, 16% had disease in the mesorectum. Is it possible that there were even more patients who actually had disease before therapy?

Dr. I. Bedrosian: Absolutely. What we rely on is endorectal ultrasound (ERUS), and it is well documented that ERUS, in particular for assessing regional nodes, is not terribly accurate. Certainly our nodal analysis is limited by that fact, and I am sure that there were patients in our study who were probably microscopically node positive but were just not seen preoperatively.

Dr. K. Kelly (Scottsdale, AZ): Could you comment on the continence of patients after the method of treatment you administered? Did the radiation and chemotherapy have any effect on their long-term continence?

Dr. Bedrosian: I think in general we have seen pretty good results after preoperative chemotherapy and radiation, but I am sorry, I do not have specific numbers.

Dr. M. Zenilman (Brooklyn, NY): Did you define complete response as the shrinkage of the primary tumor and the nodes as well, and was the rate of the shrinkage the same for the nodes and the primary tumor? How do you actually prove that you have no tumor in the nodes, even though they appear to be on the CAT scan, but you never really have a pathologic diagnosis? Did you perform any adjuvant studies such as transanal biopsies or positron emission tomography to see if that was true?

Dr. Bedrosian: To answer your last question first, we did not perform any biopsies to determine whether or not the sonographic suspicion of malignancy in the nodes was actually confirmed pathologically. So, yes, that is a limiting factor in this study and in any study that uses ERUS only to stage nodal disease preoperatively.

We define a pathologic complete response as the complete absence of tumor anywhere in that specimen, whether bowel wall or mesorectum. It would have to be both. We found no viable tumor. That was a complete response. And, yes, the rate of shrinkage is the same. So when you look at the rate of shrinkage of the tumor, we observed that approximately two thirds of the patients did experience shrinkage of the primary tumor. We saw the same number in terms of patients who went from N1 disease, and I grant you that this is N1 disease based on radiographic criteria, to N0 disease based on pathology. The rates were the same.

Electromyographic Biofeedback Can Improve Subjective and Objective Measures of Fecal Incontinence in the Short Term

Peter Beddy, M.D., Paul Neary, M.D., Emmanuel I. Eguare, M.D., Ruth McCollum, B.Sc., James Crosbie, M.D., Kevin C. Conlon, M.D., Frank B.V. Keane, M.D.

Electromyographic biofeedback therapy has demonstrated subjective improvement in patients with fecal incontinence that is comparable to surgery. We assessed the efficacy of biofeedback therapy in a consecutive heterogeneous group of patients using both subjective and objective assessment criteria. These 28 patients with fecal incontinence were studied retrospectively. Patients were assessed using a quality-of-life questionnaire (QOL), the Vaizey and Wexner incontinence scoring systems, and anorectal manometry for efficacy of treatment, before and after biofeedback therapy. Eighty-six percent of patients completed the study. Median follow-up was 18 months. Eighty percent of patients demonstrated significant improvements in their Vaizey and Wexner scores ($P < 0.001$ and $P < 0.001$, respectively). The mean QOL score improved from 62 to 77 ($P < 0.01$). Significant improvements were also demonstrated in the mean resting pressure ($P < 0.01$), peak amplitude of squeeze ($P < 0.01$), and the duration of squeeze pressure ($P < 0.05$). The deferred 15-minute evacuation time also significantly increased ($P < 0.001$). This study reported significant short-term improvement in fecal incontinence with electromyographic biofeedback therapy using validated subjective and objective scoring systems. Similarly, this treatment also significantly improved anorectal manometric findings. Our data confirm the role of biofeedback therapy in the multimodality approach to patients with fecal incontinence. (J GASTROINTEST SURG 2004;8:64–72)

KEY WORDS: Biofeedback, manometry, incontinence

Fecal incontinence is the uncontrolled passage of fecal material through the anus.[1] It is estimated to affect between 7% and 18% of the general population.[2,3] It is a debilitating and often underreported condition.[4] The therapeutic options for restoration of continence in these patients are largely dependent on the underlying etiology. Patients with an external sphincter disruption secondary to trauma, whether obstetric related or otherwise, do benefit from early surgical intervention. In a significant number of patients, however, the underlying etiology is not remediated by surgery and the therapeutic options are limited. Biofeedback has been used in the treatment of fecal incontinence in which surgical intervention is not possible for almost 30 years.[5] Several studies have shown that it can achieve a subjective improvement in a patient's overall symptoms with a reported efficacy rate ranging from 40% to 100%.[6]

Biofeedback aims to improve the ability of the patient to voluntarily contract both the external anal sphincter and the puborectalis muscles in response to rectal filling. This is achieved by improving the contraction strength of the pelvic floor musculature, increasing patient perception of rectal sensation, or by a combination of these parameters.[5] Two commonly used techniques are routinely employed. In the first, a balloon is positioned in the rectum and the patient is taught to contract the external anal sphincter in response to rectal distention.[1] The other technique is that of electromyographic (EMG) biofeedback in which a surface EMG sensor is placed in the anal

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canal or adjacent to the anus to provide readings of EMG activity of the pelvic floor muscles.[7] In a further innovation of this technique, the use of standard EMG biofeedback has been combined with active stimulation of the anal sphincter. This technique has been shown to augment the effectiveness of biofeedback.[8]

Biofeedback has not been widely reported in patients with fecal incontinence because of the diverse etiologies. Also, the improvements demonstrated in fecal incontinence using biofeedback techniques have only been seen with subjective assessment parameters. In this study we have retrospectively assessed the efficacy of EMG biofeedback in a population of patients with fecal incontinence due to different etiologies, documenting the response to treatment using both subjective and objective validated scoring systems and anorectal manometry.

METHODS

Patient Cohort

We assessed patients in our colorectal clinic with a presumptive diagnosis of fecal incontinence between June 1999 and June 2002. Patients selected for this study had had fecal incontinence for more than 6 months and were not amenable to surgical intervention. They were highly motivated and had at least one pudendal nerve intact. We excluded patients who had bilateral pudendal nerve neuropathy and those considered unable to undergo training because of limited comprehension or intellectual capacity. In total, 28 consecutive patients were included in the study. Patients underwent a full medical and clinical assessment to determine the underlying etiology of their incontinence. Patients were evaluated with the use of both subjective and objective scoring systems. As part of their diagnostic work-up for fecal incontinence, patients underwent anorectal manometry, endoanal ultrasonography, and pudendal nerve terminal motor latency testing. Patients were then referred for biofeedback therapy.

Electromyographic Biofeedback Therapy

A single specialist pelvic floor physiotherapist (R.M.C) administered the biofeedback program. She obtained an initial history and performed an examination to determine the pelvic floor muscle strength of all patients using the modified Oxford scale. The modified Oxford scoring system measures muscle strength by bimanual palpation during the maximal voluntary contraction of the pelvic floor muscles by bimanual palpation. The score ranges from 0 to 5. A score of 0 indicates no discernible muscle contraction. A score of 5 indicates strong resistance against the examining finger. Perineometry is considered a highly effective measurement of pelvic floor contraction strength. Isherwood et al. showed good correlation between the Oxford score and the perineometer in the assessment of pelvic floor strength. The Oxford scale measures muscle strength by bimanual palpation during maximal voluntary contraction of the pelvic floor.[9,10] All patients were given an explanation of the relevant anatomy and muscle function of the pelvic floor with emphasis on the external and sphincter complex. They were all instructed in selective pelvic floor muscle recruitment and exercise with particular emphasis on the anal muscles with visual aids and verbal explanation. Patients with an Oxford score of $\geqslant 3$ began a muscle-strengthening exercise program using EMG biofeedback.

This technique involves placement of an intra-anal surface EMG sensor, which detects the activity of the anal sphincter muscles. This electrical activity is converted to a signal that the patient can both hear and view on a monitor. The biofeedback unit used was the Myomed 398 (Enraf Nonius, The Netherlands) with a surface EMG sensor and stimulator probe (NEEN Healthcare, Norfolk, England). If patients could not tolerate an anal EMG sensor, a vaginal EMG surface probe was used (NEEN Healthcare). Patients were treated in the supine, sitting, and standing positions as their anal muscle strength improved. The patients were reviewed at 1- to 4-week intervals to assess progress and monitor their treatment. If the modified Oxford score was poor (grade 3), then muscle stimulation was also included in the program. Muscle stimulation at 35 Hz with a pulse duration of 250 μsec, as recommended by Laycock and Jerwood,[9] was applied using the intra-anal electrode and a muscle stimulator with a repeating cycle of a ramp-up time of 2 seconds, hold for 8 seconds, and ramp-down time of 1 second with a 10-second rest interval.

Patients who had moderate Oxford scores (grade 0 to 1) used a home muscle stimulator on a daily basis for 4 to 6 weeks. All patients started with 5 minutes' duration of treatment and progressed up to 15 minutes. Patients who had a score of 2 were treated once weekly for 3 to 4 weeks. Patients who progressed to a score of 3 were transferred to biofeedback. The remaining patients with grade 2 muscle strength also began biofeedback to optimize their limited muscle function. Patients were requested to perform a customized pelvic floor exercise program three times a day at home for the duration of therapy.

Quality-of-Life Assessment

Patients completed a standard quality-of-life (QOL) scoring card (Carol Andrejasich, Eli Lilly)

that assessed the impact of fecal incontinence on their life-styles. This subjective scoring system ranges from 0 to 120—the higher the score, the better the quality of life. Patients scored their QOL before and after the biofeedback program. The formal scoring of our patients' QOL was introduced as part of our standard evaluation protocol shortly after the initial patients were treated. As a result, not all of the patients in our study had pre- and postbiofeedback QOL scores available for analysis.

Incontinence Assessment

Patients were assessed with the use of validated Vaizey and Wexner scoring cards. The Vaizey scale is a 24-point measure, of fecal incontinence. Patients with a score of zero are considered to have normal continence.[11] Fifteen-minute deferral of evacuation after initial sensation forms part of the Vaizey score and is considered to be a significant index of a patient's continence. The Wexner scale is a 20-point assessment of fecal incontinence.[12] Patients with a score of zero are considered to have normal continence.

Anorectal Manometry

Anorectal manometry was performed in all patients who presented with fecal incontinence, using a four-channel water perfusion catheter (MUI Scientific four-channel catheter model 9012P2301; Medtronic Synectics Anorectal Manometry Analysis Module version 2.0 using the Polygram for Windows function testing software; Synectics Medical, Stockholm, Sweden). The procedure was performed in a standard fashion, using the station pull-through technique.[13]

Endoanal Ultrasound

Endoanal ultrasound imaging was performed using the Bruel and Kjaer (B & K) Medical 2002 Panther ultrasound machine with a radial endoscopic probe and a 10 MHz (model 6004) transducer. The internal and external anal sphincters were assessed in a standard fashion.

Pudendal Nerve Terminal Motor Latency

Pudendal nerve terminal motor latency (PNTML) was measured using a St. Mark's Hospital electrode (Dantec, Skovlunde, Denmark). A stimulus of 50 volts for 0.1 msec was delivered at 1 pulse/sec, and the shortest reproducible latency was recorded on a Myomed 398 (Enraf Nonius). The PNTML was considered prolonged if it was greater than 2 ± 0.2 msec.[14]

Follow-up

All patients were reassessed by means of subjective and objective scoring systems and anorectal manometry at 6 to 18 months after EMG biofeedback therapy.

Statistical Analysis

All data were stored on an IBM-compatible Microsoft Excel spreadsheet. Statistical analysis was performed using the SPSS for Windows statistical software package.

RESULTS

Patients

Twenty-eight women were referred for EMG biofeedback therapy. The mean age of the study group was 49.2 years (range 31 to 70 years). The mean duration of symptoms prior to therapy was 43 months (range 7 to 120 months). Thirteen patients had a postobstetric injury (parity > 5 in 4; multiple forceps delivery in 5; and third-degree tear in 4), 11 had idiopathic fecal incontinence, and four had postsurgical trauma (anterior resection in 3; rectovaginal fistula repair in 1). There was no significant difference in the duration of the symptoms between these patients; however, those with idiopathic fecal incontinence had a significantly higher mean age (Table 1). Four patients were lost to follow-up. These patients never began the treatment and their data were not included. In total, 24 patients completed the therapy. The mean number of sessions was 6.4 and the median follow-up was 18 months (range 5 to 30 months). In total, 21 patients are currently 1 year post therapy.

Quality-of-Life Data

QOL was assessed in 13 of the 24 patients. The mean QOL score before biofeedback was 62 (range 38 to 100) and after biofeedback therapy it was

Table 1. Patient cohort

	No. of patients	Age (yr)	Mean Duration of symptoms (mo)
Postobstetric injury	13	46.6 (range 31–68)	44.4 (range 8–120)
Idiopathic	11	43.6 (range 39–58)	43.2 (range 10–96)
Postsurgical intervention	4	59 (range 54–70)*	39.6 (range 12–96)

*P value considered significant.

77 (range 53 to 106). This represented a significant improvement in the patient's QOL ($P < 0.01$; Mann-Whitney test).

Vaizey and Wexner Score

The Vaizey and Wexner scores were assessed in 24 patients. The mean Vaizey score before biofeedback therapy was 13.7 (range 9 to 18) and after biofeedback was 9.5 (range 3 to 16). This represented a significant improvement in patient continence ($P < 0.001$; Mann-Whitney test) (Fig. 1). In total, 80% of patients had significant improvement in their Vaizey scores. Two patients were able to defer their bowel motion by 15 minutes before biofeedback; after therapy, 14 patients could defer their bowel motion for 15 minutes (Fig. 2). This also represented a significant improvement ($P < 0.001$) (Table 2). The mean Wexner score before biofeedback was 12.3 (range 7 to 16) and after biofeedback was 8.7 (range 2 to 17). This again represented a significant improvement in the patients' continence levels ($P < 0.001$; Mann-Whitney test) (Fig. 3).

Anorectal Manometry

Twenty-four patients underwent anorectal manometry before EMG biofeedback therapy. Eighteen patients underwent follow-up anorectal manometry after completion of therapy. Of the six patients who were not reassessed; four refused reassessment and two declined on medical grounds. There was a significant improvement in the resting pressure, duration of the squeeze, and amplitude of the squeeze after EMG biofeedback therapy (see Table 2). There was no significant improvement in the squeeze pressure after EMG biofeedback.

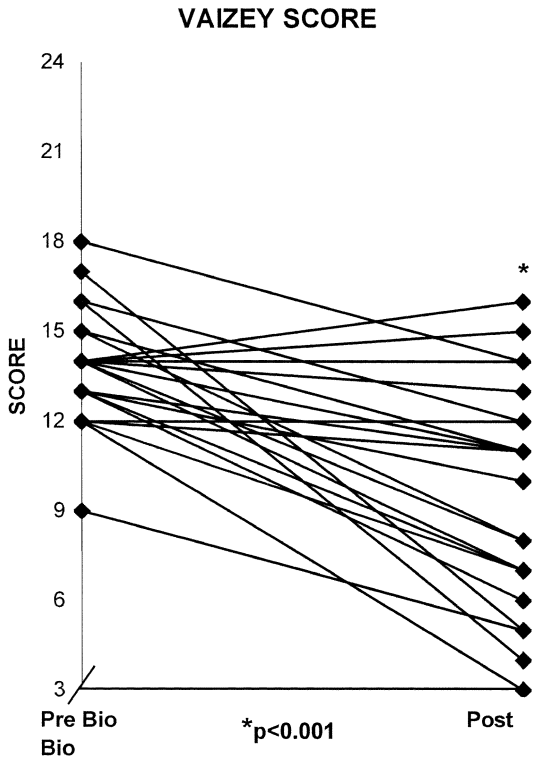

Fig. 1. Vaizey scores before and after EMG biofeedback.

DISCUSSION

Fecal incontinence is often a socially debilitating condition for which there are a number of therapeutic options. These range from simple dietary manipulation and chemical agents to surgical reconstruction and external sphincter stimulation. Surgery remains the optimum treatment in those patients with documented sphincter defects who are deemed suitable. However, when surgery is not an effective therapeutic option, other treatment modalities are needed and biofeedback training in selected patients has been shown to achieve results comparable to those of surgery.[15]

Biofeedback therapy combines an increase in perceived rectal sensation with augmentation of pelvic floor muscle contraction. The training protocols employed may be categorized into coordination, sensory, and strength training strategies. EMG biofeedback, as used in our study, improves strength and coordination. Our understanding of the exact role, technique, and benefit of biofeedback, however, is as of yet not completely clear. Several groups have published their results with a variety of biofeedback treatment modalities. Improvements in rectal sensory perception and patient-perceived continence using a variety of incontinence scoring systems have been reported.[7,8,16,17] Critics of the treatment argue that the improvement in continence is a result of the supportive interaction of the biofeedback physiotherapist with the patient. These investigators identify decreased anxiety, increased confidence, and lack of published long-term follow-up data showing any benefit in these patients as support for their criticism. Furthermore, until now biofeedback therapy has not been shown to have an impact on any objective anorectal manometric measurements.[5]

In this initial pilot study, we have assessed our experience in our own unit with this technique. The study was not restricted to patients with obstetric injuries alone, and our population was representative of mixed etiologies. Patients were assessed using the validated Vaizey and Wexner incontinence scoring systems along with a QOL questionnaire and anorectal manometry. Our results have confirmed the

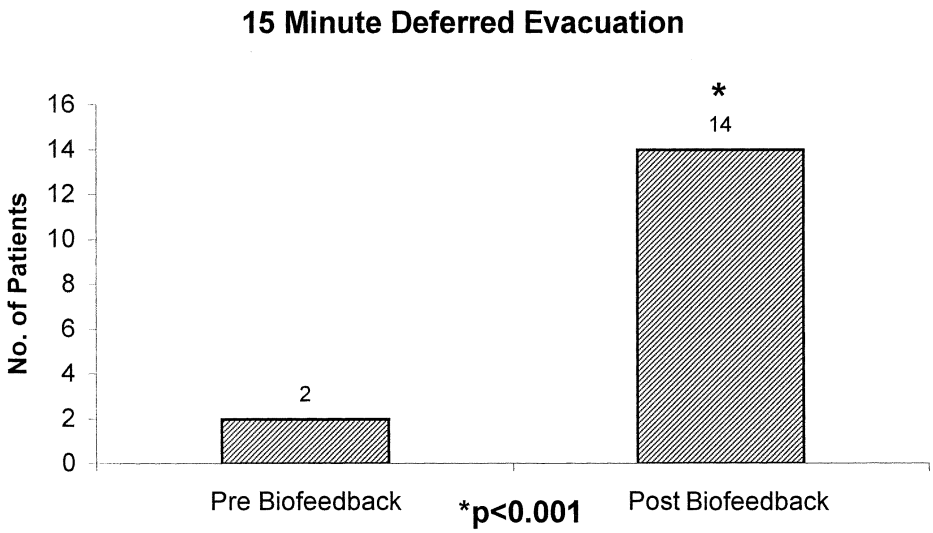

Fig. 2. Fifteen-minute deferred evacuation before and after EMG biofeedback.

subjective improvements seen in other studies using this technique.[5] Our patients recorded an increase in their QOL scores, which represented a significant improvement in their overall QOL status. Similar subjective results have been reported in the literature using a variety of QOL scores.[18,19] We also showed significant objective improvements in the level of continence following biofeedback therapy using the Vaizey and Wexner scoring systems. We further showed objective improvements after biofeedback in the anorectal manometry data. Patients significantly increased their maximum resting pressures, along with the maximum squeeze duration and peak amplitude of squeeze pressure, although there was no significant improvement in the maximum squeeze pressure, a finding that is consistent with reports in the literature.[16,17]

This represents the first objective documentation of manometric improvement after biofeedback in a diverse population of patients with fecal incontinence. Initial attempts to demonstrate objective manometric changes secondary to biofeedback therapy have proved difficult. Factors that tend to negatively affect the efficacy of biofeedback therapy include evidence of any underlying neurologic impairment and wide-spectrum patient selection.[20,21] Our patients, despite having diverse etiologies, were well motivated and had at least unilateral preservation of pudendal nerve function. The recent study by Fynes et al.[8] with the use of augmented (stimulated) EMG biofeedback focused on patients with fecal incontinence after obstetric trauma. They demonstrated significant improvements in maximum resting pressure, maximum squeeze pressure, and squeeze increment.

Table 2. Results before and after EMG biofeedback

	No. of patients	Before biofeedback	After biofeedback	*P* value
Scoring system				
Vaisey score	24	13.7 (range 9–18)	9.5 (range 3–16)	<0.01*
Wexner score	24	12.3 (range 7–16)	8.8 (range 2–17)	<0.01*
Quality of life	13	61.6 (range 38–100)	78.9 (range 55–106)	<0.01*
15 min deferred evacuation	24	8.3%	54.1%	<0.01*
Manometry				
Mean Max RP (mm Hg)	18	20.3 (range 10.2–30.5)	26.65 (range 10–43.6)	<0.01*
Mean Max SP (mm Hg)	18	32.95 (range 14.7–88.3)	34.12 (range 14.5–79.1)	0.3
Duration of squeeze (sec)	18	13.31 (range 4.8–19.9)	16.16 (range 9.9–19.9)	<0.01*
Peak amplitude (mm)	18	50.44 (range 15–124)	62.88 (range 30–106)	0.05*

Mean Max RP = mean maximum resting pressure; Mean Max SP = Mean maximum squeeze pressure.
**P* value considered significant.

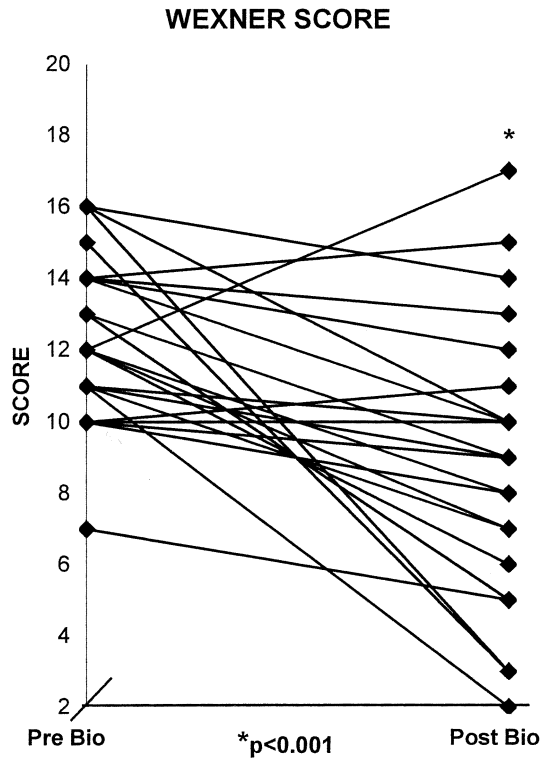

Fig. 3. Wexner scores before and after EMG biofeedback.

In our study, the selection of well-motivated, neurologically intact patients may partially account for some of the subjective improvements seen. This, however, should not confound the objective manometric findings demonstrated in our patients after treatment. We believe that the standardization of our treatment protocols, having a dedicated biofeedback physiotherapist, and initially actively stimulating those patients with an Oxford score of <3 before EMG biofeedback was formally begun may account for our own encouraging results.

The objective assessment of biofeedback has suffered from a lack of consensus on treatment protocols and variability in the recording of outcome parameters. There have been many different scoring systems used for assessing fecal incontinence and biofeedback therapy. Many of these were designed by an individual's own unit and others have employed visual analogue scales. The use of systems such as the Likert scale, the Rockwood system, and the Pescatori and American Medical System scales have been similarly employed over the past few years.[11,12,22–26] Differences in these systems create difficulties in achieving a meaningful comparison between various studies. We are the first group to use both the Vaizey and Wexner incontinence scoring systems to evaluate our results. These scoring systems are now both widely used in clinical practice; they are well validated and are specific for the assessment of fecal incontinence. They indicate the severity of fecal incontinence. We did not highlight the number of episodes of incontinence because they are in-built into the scores. Our results have confirmed that an improvement in these incontinence scores can be achieved in the short term after biofeedback therapy. This is further emphasized by the significant improvement in the "deferred 15-minute evacuation time" recorded in our patients, and this measurement alone must translate into a particular benefit for this group of patients. We would advocate that the combined use of these scoring systems should be used when reporting results of biofeedback therapy in the future. We found it difficult to show a correlation between our QOL indexes and our objective scoring systems because this subjective element was only built into our incontinence protocol after it was initiated. However, as part of the Vaizey and Wexner scoring systems there is one question that deals with QOL, and there was a significant improvement in these scores.

In our treatment paradigm, the physiotherapist used the modified Oxford scale to assess muscle strength and stratify the therapeutic approach, but because this assessment has a subjective component we chose to assess sphincter strength by means of anorectal manometry. It would be interesting in future studies to compare the modified Oxford score after treatment with anorectal manometric findings. There was good correlation between improvements in anorectal manometry and Vaizey and Wexner scores.

The short-term efficacy of biofeedback therapy has been shown to be comparable to that of surgery.[27] Initial improvements in fecal incontinence after biofeedback therapy are reported as approaching 70% of patients treated.[7] This figure is largely applicable to those assessed 12 to 24 months after treatment.[1] There is no doubt that biofeedback therapy is an extremely effective treatment for selected patients in the short term. The permanence of these results, however, has not been satisfactorily addressed in the literature. Enck et al.[28] assessed patients after a 5-year follow-up period and found no significant decrease in the functional improvements gained. Ryn et al.[19] had a median follow-up period of 44 months for their 37 patients. They reported a decrease in the success rate from 60% to 41% over this time period. A similar finding was reported by Glia et al.[1] with an initial success rate of 54% decreasing to 41% with time. Other investigators, however,[16,29] have failed to confirm the benefit of these improvements over time. In our own study the median follow-up period was 18 months, and the results must therefore only be interpreted as further evidence of the initial successful

impact of this promising treatment modality. Further long-term prospective studies are therefore needed to conclusively determine whether there is indeed any lasting benefit with biofeedback therapy.

The development of innovative approaches in the treatment of patients with fecal incontinence has progressed rapidly in recent years. These include stimulated graciloplasty, artificial anal sphincters, and sacral nerve stimulation. Graciloplasty was first used in the treatment of end-stage fecal incontinence in 1946, and the addition of electric stimulation was introduced almost 15 years ago. Rongen et al.[30] reported good long-term results with success in more than 70% of patients treated, but there were frequent complications. Dynamic graciloplasty is mostly indicated in patients with end-stage fecal incontinence but its role remains undefined.[31] Initial reports of artificial anal sphincters have shown a high failure rate (50%), but more recently Christianson et al.[30–34] and Michot et al.[33] reported success rates of nearly 80%. This is a similar response to that achieved in our study in which 80% of patients showed improvement in their Vaizey scores. However, all of these surgical techniques are invasive, and few patients in our study could meet the inclusion criteria. Sacral nerve stimulation is a promising technique for the treatment of fecal incontinence but requires further evaluation.[34]

The benefits of EMG biofeedback therapy are clear in the short term; however, we believe that EMG biofeedback is also still evolving as a definitive treatment option. The improvements in rectal sensation,[1] rectal squeeze, and resting pressures documented by our own group and others have demonstrated the clear benefit of EMG biofeedback therapy in selected patients. Its application as an adjunctive therapy to these exciting innovative therapies, however, has yet to be addressed but may be the future direction of fecal incontinence amelioration.

CONCLUSION

Despite the retrospective nature of this study, our data support the efficacy of EMG biofeedback in addressing fecal incontinence when critically assessed using validated scoring systems. The study also lends itself to the increasing body of evidence of the manometrically recordable improvements in anal sphincter muscle strength and contraction in patients after biofeedback therapy. Our successful results are indeed reflective of the short-term follow-up period studied; however, they do indicate that this treatment may be applicable to a wider number of patients than has heretofore been recorded. We would therefore suggest that EMG biofeedback should be considered a valuable part of the integrated treatment package for patients with documented fecal incontinence that is not amenable to surgical correction.

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Invited Discussion—Expert Commentator

James W. Fleshman, M.D. (St. Louis, MO): I congratulate the authors on an excellent presentation and thank them for the opportunity to review the manuscript prior to this meeting. Biofeedback has been around as treatment for incontinence for many years starting with Schuester's balloons 30 years ago. The results have been mixed and hard to interpret for many of the reasons exhibited by this study. The authors have described a mixed group of patients with mechanical, neurogenic/idiopathic, traumatic incontinence, all being described as "not amenable to surgical treatment." They have all been evaluated fully by anal physiology, but not ultrasonography of the sphincter, to determine mechanical defects. An unspecified number of patients underwent muscle stimulation before receiving biofeedback and without repeating the anal physiology studies before the biofeedback. The three groups—obstetric injury, idiopathic, and traumatic—are very small. The manuscript does not describe the statistical adjustment used for comparing such small groups.

I have the following questions for the authors:

1. What does not amenable to surgical therapy really mean?
2. How many patients had muscle stimulation prior to biofeedback?
3. Could there be a placebo effect on the patients? We do not have a control group of patients with sham biofeedback or no treatment.
4. Could there have been an effect on function by changing diet, giving enemas before treatment, or just talking to the patients?
5. How has anal stimulation affected the measured parameters?

An improvement rate of 92%, an increase in 15-minute fecal deferment up from 8% to 54%, and an objective increase in squeeze pressure amplitude, and duration, are all very impressive results. Unfortunately, we do not know which patients from these three different groups would most likely benefit because they are all lumped together and we are not given specific results.

The Rockwood/ASCRS Fecal Incontinence Quality of Life score of FIQOL and the ASCRS Fecal Incontinence Severity Index or FISI are the only scoring systems developed by a panel of experts and validated in two directions (patient and physician) by multiple independent institutions. These scores are now being used to evaluate therapies and outcomes of colorectal procedures. I would advocate that all investigators in volved in the study of anorectal physiology and related disease states use these scoring systems to bring consistency to our reporting and discussions.

Discussion

Dr. M. Stelzner (Seattle, WA): You showed us that you have seen improvements both in the subjective and the manometry parameters you measured. I wondered if you had looked at correlations between the two and if manometric improvement always reflected an improvement of subjective scores. Did you see outliers or were you able to identify a subgroup where correlations were different from the other patients?

Dr. P. Beddy: Yes, we did look at that correlation. There is good correlation between the Vaisey and Wexner scores and the anorectal manometry parameters. However, there was not good correlation between the subjective assessment (quality of life) and the objective data. This may be rectified in future studies by using subjective scoring systems specifically designed for fecal incontinence. The Rockwood scoring system is one such

scoring system for fecal incontinence, and it is now employed in our unit as part of a prospective evaluation of patients attending our anorectal physiology unit. We stress, however, that the most important part of our results is the objective improvements that we have clearly demonstrated.

Dr. A. Wald (Pittsburgh, PA): It is of concern that there are no controls. There is a study that was published in abstract form last year from the St. Mark's Hospital group. It was a controlled study of biofeedback in a similar population. These investigators found that the use of nonbiofeedback produced an increase in resting and squeeze pressures. I wondered why that would be the case, and second, how EMG biofeedback would increase resting sphincter pressures, particularly, in the internal anal sphincter?

Dr. Beddy: These are very interesting points. Our hypothesis for the improvement in the resting pressure is twofold. As I stated, the resting pressure is reflective of internal anal sphincter function. This also utilizes some of the slow twitch fibers of the external sphincter, and we think that the improvement in these slow twitch fibers may account for the improvement in resting pressure. We also used electrical stimulation with biofeedback, and we speculate that this initial electrical stimulation may, in fact, recruit muscle groups in the internal anal sphincter, hence resulting in improvement in the resting pressure. Three years ago, Feynes et al. published a very interesting paper published in *Diseases of the Colon and Rectum*. They specifically looked at this parameter and also showed improvement in the resting pressure.

In answer to your first question, our study was retrospective and did not have a control group. I am unfamiliar with the abstract you mentioned; however, in designing long-term prospective studies of biofeedback therapy, the inclusion of a control arm is desirable but often proves difficult.

Dynamic Magnetic Resonance Imaging of the Pelvic Floor in Patients With Idiopathic Combined Fecal and Urinary Incontinence

Emmanuel I. Eguare, M.D., Paul Neary, M.D., James Crosbie, M.D., Sean M. Johnston, M.D., Peter Beddy, M.D., Bernadette McGovern, R.G.N., William C. Torreggiani, M.D., Kevin C. Conlon, M.D., Frank B.V. Keane, M.D.

The etiologies of combined fecal and urinary incontinence may be interrelated but remain poorly understood. A potential variable in this process is global pelvic floor dysfunction. The aim of this study was to prospectively assess the use of phased-array, body coil dynamic MRI in identifying pelvic floor abnormalities in patients with combined incontinence symptoms. Symptomatic patients were compared to asymptomatic control subjects and were selected from those referred to the pelvic physiology laboratory with complaints of combined urinary and fecal incontinence. All patients underwent standard urodynamic studies and anorectal physiologic assessment. Colonoscopy and endoanal ultrasonography were also performed. A standardized protocol was used for dynamic MRI, and the parameters were measured using workstation software (callipers, compass, and densitometer). In the incontinent group there was a significant difference, when compared to control subjects, in the angle of the levator ani muscle arch of the levator plate complex (3.0 ± 5 degrees vs. 14 ± 10 degrees; $P = 0.004$), the width of the levator hiatus (58.3 ± 8 mm vs. 46.5 ± 8 mm; $P = 0.001$), the area and tissue density of the levator ani muscle (19.5 ± 1 mm^2 vs. 26.9 ± 1 mm^2; $P = 0.001$, and 157.3 ± 47 pixels vs. 126.1 ± 23 pixels; $P = 0.025$, respectively), and in the length of the external anal sphincter (20.0 ± 5 mm vs. 26.6 ± 13 mm; $P = 0.03$). Body coil dynamic MRI is a noninvasive and well-tolerated imaging modality. Our data show that it can identify changes in pelvic muscle morphology in patients with disorders of incontinence, and this may help in planning better management strategies. (J GASTROINTEST SURG 2004;8:73–82)

KEY WORDS: Magnetic resonance imaging, combined fecal and urinary incontinence, levator ani muscle

Combined fecal and urinary incontinence occurs more frequently than is generally assumed with a prevalence estimated to be approximately 5% in males and 10% in females.[1] In the hospital setting, the prevalence of fecal incontinence in female patients attending urology and urogynecology clinics varies from 15% to 30%.[2] Stress urinary incontinence is a symptom that results from damage to the muscles, nerves, and connective tissue of the pelvic floor leading to disruption of urethral support and vesical neck function. Similarly, symptoms of fecal incontinence arise when there is damage to the muscles and the endopelvic fascial support system of the anorectal complex. A combination or overlap of these defects results in the manifestation of combined symptoms of fecal and urinary incontinence.[3,4]

Most patients referred for assessment in the pelvic physiology laboratory, from either a colorectal, gastrointestinal, gynecologic, or urologic clinic, are referred with solitary symptoms of fecal or urinary incontinence or with symptoms arising from organ prolapse (rectocele, enterocele, cystocele, or uterine prolapse). Detailed assessment of these patients shows that a large proportion of them have synchronous symptoms in adjacent compartments that were overlooked or unreported. The failure to identify these

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associated symptoms may be related, in part, to specialist focus but may also be attributed to the lack of access to an investigative modality that is capable of assessing the pelvic floor globally. This, in turn, may lead to inappropriate patient selection for therapeutic procedures and less than optimal results in the amelioration of all pelvic symptoms.

To understand the morphology and function of the pelvic floor, routine investigations were carried out including endoscopic procedures such as colonoscopy, cystoscopy, hysteroscopy, physiologic testing of urodynamics, anorectal manometry, electromyography, pudendal nerve function, and imaging, which included endoanal ultrasonography and radiologic contrast studies such as evacuation proctography, cystography, and cystocolpoproctography. Although all of these investigations are useful in detecting specific abnormalities in particular compartments, they offer little or no information about the surrounding support structures, and are often intrusive and interfere with normal physiology and function.[5–7]

Magnetic resonance imaging (MRI) avoids these disadvantages and can provide a high-resolution global assessment of the pelvis, its constituent organs, and the musculofascial support structures. Endoanal MRI using an EndoCoil (C.R. Bard, Inc., Murray Hill, NJ) is also used to evaluate the pelvis, but it has similar disadvantages to endoanal ultrasound imaging in that it props up the anal canal, causes anatomic distortion, and interferes with normal physiologic function.

The objective of our study was to evaluate the results of dynamic, phased-array, pelvic MRI in the assessment of patients with idiopathic combined fecal and urinary incontinence, using the widely available closed magnet system.

MATERIAL AND METHODS

All patients requiring assessment of disorders of fecal or urinary incontinence at the Adelaide and Meath Hospital, Dublin, are referred to the Pelvic Physiology Laboratory, which has the facilities to assess both urodynamics and anorectal physiology. Our study population consisted of a group of patients who had a confirmed diagnosis of idiopathic combined fecal and urinary incontinence, and these patients were compared to a group of healthy asymptomatic age-matched control subjects.

To exclude known causes of fecal and urinary incontinence, after a complete history and clinical examination, all of the patients with combined incontinence underwent colonoscopy, cystoscopy, endoanal ultrasonography, anorectal manometry, urodynamic studies, and pudendal nerve terminal motor latency testing. The severity of each patient's fecal and urinary incontinence was evaluated using the Vaizey, Wexner, and urinary incontinence scoring systems.[8,9] We defined idiopathic combined fecal and urinary incontinence as incontinence in which no physiologic or anatomic cause could be identified by means of the standard tests mentioned earlier.

Anorectal manometry was performed in all patients using an eight-channel, water-perfusion catheter (MUI Scientific, Mississauga, ON, Canada). The station pull-through technique as previously described was adopted in this study.[10] The parameters measured were mean maximal resting pressures, mean incremental squeeze pressures, rectal volume compliance, rectal sensation, and rectoanal inhibitory reflex. The pudendal nerve terminal motor latency test was performed in the standard fashion to assess the integrity of the pudendal nerves (St. Marks pudendal stimulating electrode; Dantec Medical, Skovlunde, Denmark). The normal reference value used was 2.0 ± 0.2 msec, and latency periods greater than 2.5 msec were regarded as abnormal.[10] An experienced colorectal surgeon (F.B.V.K.) performed the endoanal ultrasound imaging in the standard fashion (2002 Panther; Brüel & Kjaer Medical Systems, Inc., Herlev, Denmark) to rule out sphincter defects in symptomatic patients. Urodynamic study was performed in the standard fashion.[11]

Phased-array dynamic MRI was performed in all patients and healthy volunteers after detailed informed consent was obtained (1.5 T Magnetom Symphony; Siemens Corp., Erlangen, Germany). The phased-array scan uses a combination of circularly polarized (CP)-spine array coil, which includes a 12-coil design with six CP pairs of preamplifiers integrated into the patient table, and CP body array, which consists of a four-coil design with two CP pairs of integrated preamplifiers that connect and operate in an integrated fashion with the CP-spine array.

Initial localizer images were obtained in the coronal, sagittal, and axial planes using T2-weighted turbo spin-echo sequences. Because we wanted to simulate natural physiologic conditions, no special bowel preparation or rectal contrast medium was administered. The examination was performed with the patient in the supine position lying head first on the scanner with the legs kept slightly apart. The dynamics of the pelvic floor and its constituent structures were assessed with axial T1-weighted spin-echo, coronal, and sagittal T1- and T2-weighted turbo spin-echo sequences (with a repetition time of 5130 msec, echo time of 109 msec, and 2 to 3 mm slice thickness). Images were obtained in the coronal, sagittal, and axial planes, and in the static and straining

phases (Figs. 1 and 2). Because of the problem of artifacts created by movement and the inability of patients to sustain prolonged breath-holding during the straining mode, the straining phase was performed with the fast-gradient echo sequence. With the patient in the supine position, images were obtained in the resting phase, and the patient was then asked to strain with breath-holding for the acquisition of the straining-phase images. The image acquisition time was approximately 25 minutes. Twenty-five individual parameters were measured using built-in software (callipers, compass, and densitometer) of the workstation. The parameters measured were validated by comparison with previously published data (Beets-Tan et al.,[5] Morren et al.,[12] Kruyt et al.,[13] Comiter et al.,[14] Fielding et al.,[15] Yang et al.,[16] Healy et al.,[17] and Rociu et al.[18]).

Levator ani muscle length was measured by drawing the straightest line through the long axis of the muscle (from its pelvic ring insertion—arcus tendineus levator ani—to the commencement of the puborectalis) on both sides in the midcoronal image.[4,12] The levator ani muscle thickness was measured at the region of maximum thickness of the muscle in the midcoronal plane. The levator ani angle was measured (in the coronal plane) by joining a straight line connecting both ends of the levator ani muscle with another line from the apex of the levator arch; the angle of intersection of the two lines forms the levator angle. The levator ani muscle area and tissue density were measured with the specialized image analysis software of the workstation. The midcoronal image was selected in each case, and the pixel values for pure black and white colors were standardized. The levator ani muscle area on both sides was calculated by tracing the perimeter of the levator ani muscle on both sides in the midcoronal section, and the mean cross-sectional levator ani muscle area was derived. The minimum, maximum, and mean pixel values (levator ani muscle tissue density) were automatically calculated (Fig. 3). Great care was taken to trace the muscle perimeter. The levator tissue density was measured using a built-in tissue densitometer in the workstation. To eliminate the differences likely to arise from equipment setup, penetrance of images, and variation in body mass, each patient was used as her

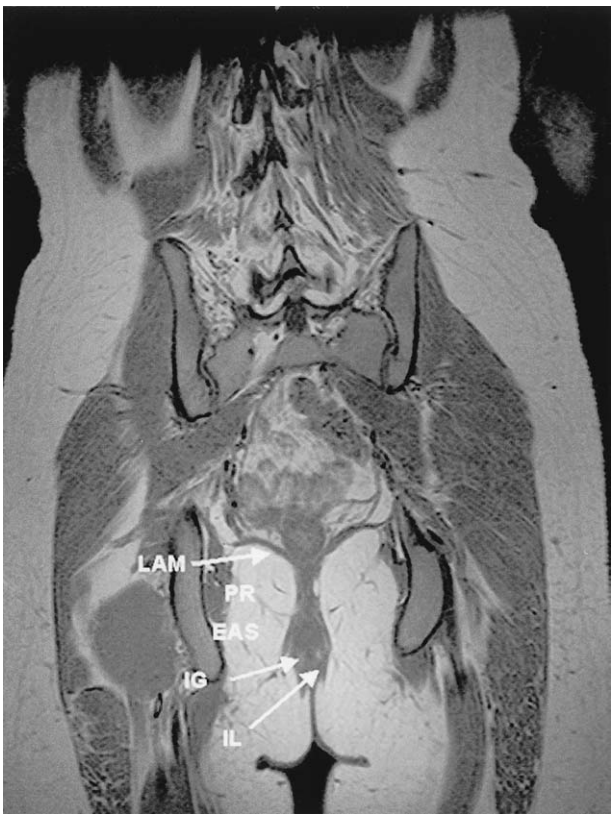

Fig. 1. Midcoronal phased-array magnetic resonance image of the pelvis showing the levator ani "levator dome" (*LAM*), puborectalis (*PR*), external anal sphincter (*EAS*), inner layer of external anal sphincter (*IL*), and intersphincteric groove (*IG*).

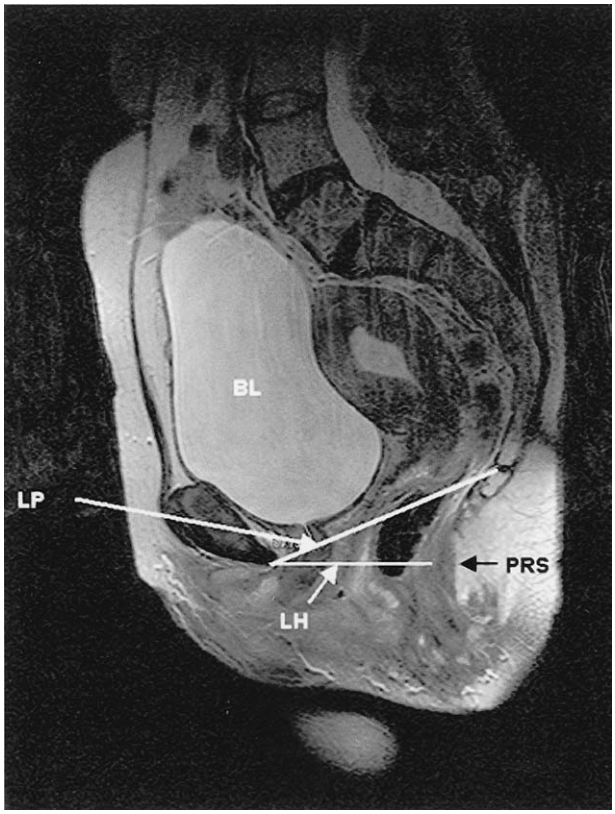

Fig. 2. Midsagittal phased-array MRI of the pelvis showing components of the levator and anal sphincter complex, levator plate "pubococcygeal line" (*LP*), levator hiatus (*LH*), and puborectalis sling (*PRS*). BL = urinary bladder.

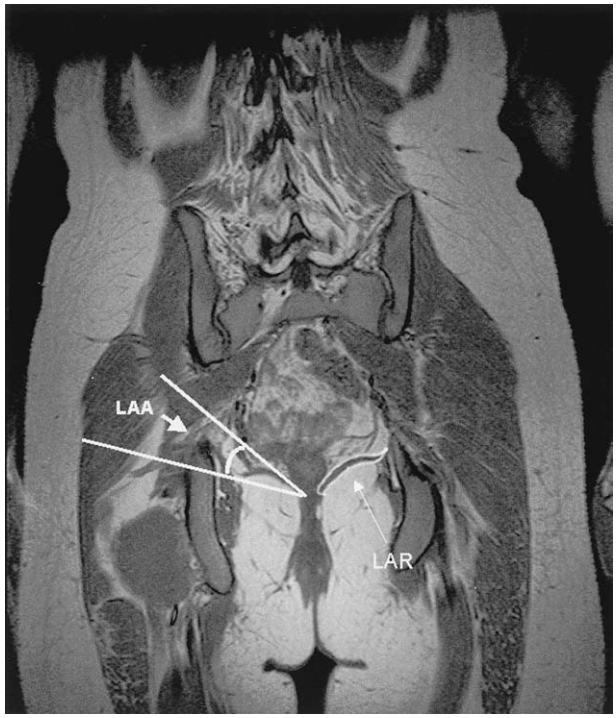

Fig. 3. Midcoronal phased-array MRI of the pelvis showing the levator ani angle (*LAA*) and levator ani area (*LAR*).

own control by measuring the tissue density of an equivalent area of the ipsilateral gluteus maximus. This was done on both sides (using the midcoronal segment image), and the difference in the levator-gluteal mean density was calculated. The tissue density was measured in pixels. The higher the pixel values, the lower the tissue density. The percentage fat content for each levator ani muscle complex was calculated using the formula described by Williams et al.[19]:

$$\textit{Percentage fat in LAM} = \frac{(X - X_m) \times 100}{(X_f - X_m)}$$

Where X is the mean pixel density for the levator ani muscle, X_f is the mean pixel density for fat, and X_m is the mean pixel density for muscle.

The pubococcygeal line, levator hiatus, and anorectal angle descent were measured as described by Comiter et al.[14] and Fielding et al.[15] The anorectal angle was measured in the standard way, as described by Healy et al.[17] and Matsuoka et al.[20] The anal sphincter complex was analyzed as described by Beets-Tan et al.[5] and Morren et al.[12] The investigator performing the MRI was blinded to both the clinical history and the results of physiologic studies of the patients. The study was approved by the research and ethics committee of the hospital.

Statistical Analysis

Statistical analysis was performed using the SPSS 10.0 for Windows software package (SPSS, Inc., Chicago, IL). All results are presented as means ± standard deviation (SD) unless otherwise stated. The independent t test for equality of means was applied. A value of $P < 0.05$ indicates a statistically significant difference.

RESULTS

Of the 1501 patients referred to the pelvic physiology laboratory over a 2-year period with complaints of pelvic floor dysfunction, 85 presented with combined fecal and urinary incontinence. A diagnosis of idiopathic combined fecal and urinary incontinence was made in 18 female patients (21.2%) after they fulfilled the established criteria (12 urge incontinence and 6 mixed stress and urge incontinence). A control group of 14 asymptomatic females with no history of fecal or urinary incontinence, constipation, gastrointestinal disease, or anorectal or urinary surgery was also recruited. Both groups were matched for age and parity; with a mean age of 46.2 ± 14.4 years and 37.1 ± 12.9 years ($P = 0.07$) for the symptomatic and control groups, respectively. The parity was 2.6 ± 1.7 and 1.7 ± 1.2 ($P = 0.8$) for the symptomatic and control groups, respectively. The mean Vaisey score for the incontinent patient group was 11.6 ± 3. The mean Wexner score for fecal incontinence was 9.7 ± 3. Results of colonoscopy and cystoscopy were normal in all incontinent patients.

All symptomatic patients had a physiologic evaluation of the pelvic floor. The mean values for the anal high-pressure zone length, resting pressure, squeeze pressure, and maximum tolerated volume were 2.25 ± 1 cm, 63 ± 32 mm Hg, 45 ± 20 mm Hg, and 57 ± 69ml, respectively. The mean pudendal nerve terminal motor latency test score for the combined fecal and urinary incontinence group was within normal limits (1.96 msec and 2.2 msec on the right and left pudendal nerves, respectively). In this study a morphologically intact anal sphincter was determined in each patient by means of endoanal ultrasound imaging, and this was confirmed with axial MRI of the anal sphincter. The body mass index did not show a correlation with age, parity, or parameters for thickness or length of muscles of the pelvic floor on MRI. The mean of the urinary incontinence quality-of-life score was 75.2/110 ± 20. Urodynamic studies showed that these patients had a mean flow rate of 8 ± 5 ml/sec (normal range = 10 to 18 ml/sec) and a mean voiding volume of 147 ± 100 ml (normal range = 300

to 450 ml). All of the 32 participants tolerated the phased-array dynamic MRI procedure satisfactorily.

Levator Plate Complex

Analysis of the levator plate complex in the midcoronal image (Fig. 4) demonstrated a significant decrease in the angle of the arch of the levator ani muscle in the combined incontinence group compared to the control group (3.0 ± 5 degrees vs. 14 ± 10 degrees; $P = 0.004$). The levator ani angle was completely lost in 12 (67%) and significantly decreased in six (33%) patients in the incontinent group. The width of the levator hiatus showed a significant increase in the symptomatic group compared to the control group (58.3 ± 8 mm vs. 46.5 ± 8 mm; $P = 0.001$). Although the levator ani muscle length and thickness did not differ significantly in the two groups (45 ± 6 mm vs. 44 ± 5 mm; $P = 0.6$, and 4.5 ± 1 mm vs. 5.0 ± 1mm; $P = 0.2$, respectively), there was a significant reduction in the area of the levator ani muscle of the symptomatic group compared to the control group (19.5 ± 1 mm^2 vs. 26.9 ± 1 mm^2; $P = 0.001$). There was also a significant reduction in the tissue density of the levator ani muscle of the symptomatic group vs. the control group (157.3 ± 47 pixels vs. 126.1 ± 23 pixels; $P = 0.025$) (Table 1).

Anal Sphincter Complex

There were significant differences in measurements of the external anal sphincter length between the symptomatic and control groups (20.0 mm ± 5 vs. 26.6 mm ± 13; $P = 0.03$). The length of the inner layer of external anal sphincter[21] was also significantly decreased (3.1 ± 3 mm vs. 7.0 ± 2 mm; $P = 0.001$). Similarly, there was a significant decrease in the measurement of the intersphincteric groove (2.4 ± 2 mm vs. 3.9 ± 1 mm; $P = 0.003$), which was measured using a reported protocol.[9] There was, however, no statistically significant difference in the thickness of the external anal sphincter muscles (3.7 ± 1 mm vs. 7.7 ± 11 mm; $P = 0.3$) (see Table 2). There was complete obliteration of the inner layer of the external anal sphincter in eight patients.

Urethrovesical Complex and Perineal Descent

The anterior urethrovesical angle was significantly wider in the incontinent group compared to the control group (91 ± 23 degrees vs. 73.5 ± 10 degrees

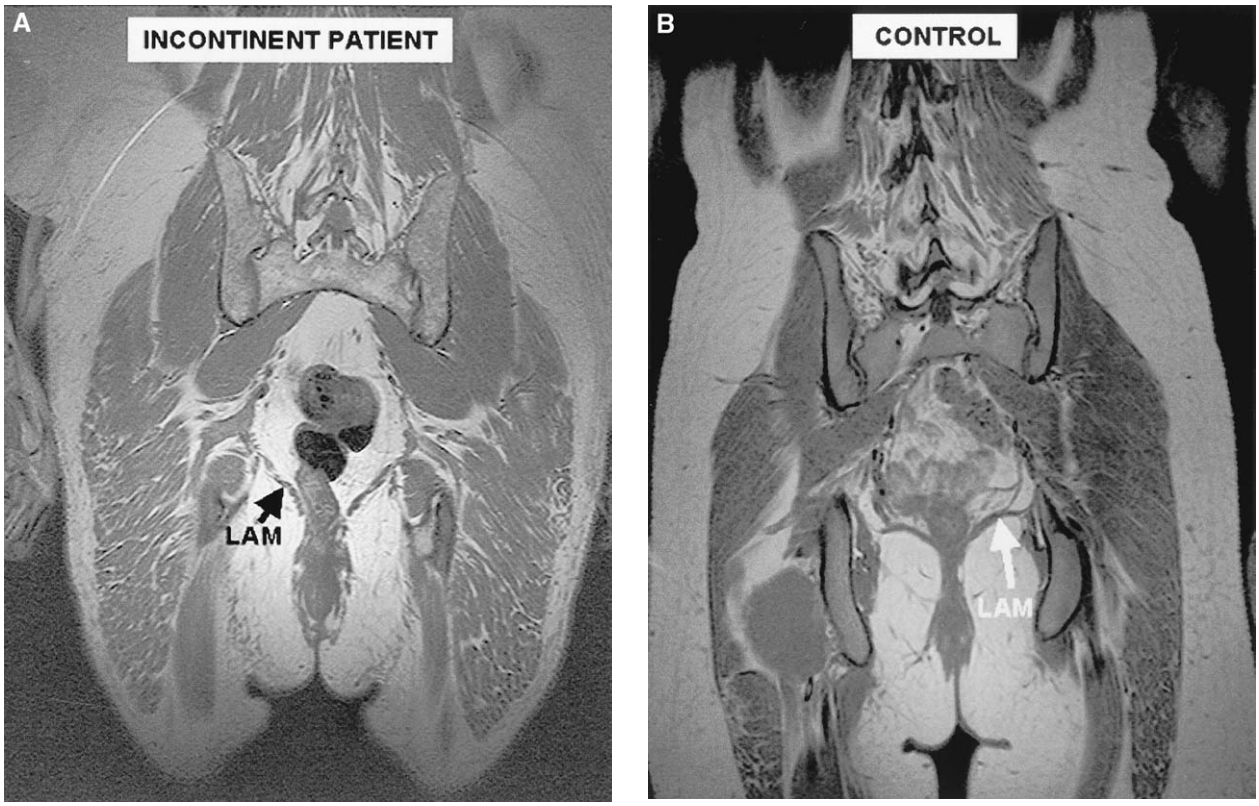

Fig. 4. Comparative midcoronal phased-array MRIs of a symptomatic patient (**A**) and a healthy control subject (**B**). LAM = levator ani muscle.

Table 1. Parameters of the levator plate complex

Parameter	Study group (N = 18)	Control group (N = 14)	*P* value
Urethrovesical angle (degrees)	91 ± 23	73.5 ± 10	0.004
Levator hiatus (mm)	58.3 ± 8	46.5 ± 8	0.001
Levator angle (degrees)	3.0 ± 5	14 ± 10	0.004
Levator density (pixels)	157.3 ± 47	126.1 ± 23	0.025
Levator area (mm^2)	19.5 ± 1	26.9 ± 1	0.001
Puborectalis thickness (mm)	9.5 ± 3	12.5 ± 3	0.016

Significance: $P < 0.05$.

[mean ± SD], respectively; $P = 0.004$. The degree of perineal descent (Fig. 5) was also significantly greater in the symptomatic group compared to the control group (8.7 ± 6 mm vs. 3.8 ± 3 [mean ± SD]; $P = 0.005$.

DISCUSSION

Since the introduction of MRI as an imaging modality, there has been a growing interest in its use as an investigative tool in the evaluation of patients with pelvic floor dysfunction. This interest is reinforced by the fact that MRI, apart from offering the capability of global visualization, with enhanced tissue resolution of the pelvic organs and their musculofascial support structures, is also free of ionizing irradiation. The invasiveness of fluoroscopic procedures and the high doses of ionizing irradiation they deliver render many of them unacceptable for many patients. For instance, with evacuation proctography, the somatic dose of ionizing irradiation ranges from 100 to 200 Gy (in men and women), and the gonadal dose ranges from 40 Gy in men to 90 Gy in women. Defecating proctography (also referred to as defecography or evacuation proctography) has been used in the assessment of defecation disorders for nearly 50 years, but its clinical value remains controversial. Although some investigators have defined a role for it in the assessment of fecal incontinence, its main indication is in the evaluation of constipation due to obstructive defecation.[22,23] Rentsch et al.[24] commented that although defecating proctography has increased our knowledge of evacuation disorders, the causes of combined pelvic floor disorders in females and complex disorders of the posterior compartment in males remains unclear in some patients. Frequently, multiple opacification techniques of the different compartments and additional neurologic and physiologic measurements are necessary to identify the predominant disorder.[25,26] The result of the advent of MRI is the broadening of the spectrum of patients that can be investigated for pelvic floor dysfunction.

Employing previously validated techniques used in the measurement of pelvic floor parameters, we have demonstrated new and significant findings in the structure and quality of the levator ani muscle and anal sphincter muscle complexes.[9,24,27–29] The length and thickness of the levator ani muscle were not significantly different when comparing control and incontinence subjects, but the levator ani muscle area was significantly reduced in the combined incontinence group. Thus measurement of the muscle area gave a better representation of the muscle volume than the length or thickness, which were measured at points of maximum values. This was in agreement with the findings of Williams et al.[19] in which the measurements of the length and width of the anal sphincter at specified levels did not correlate with the cross-sectional area because of the vast heterogeneity of sphincter morphology.

The levator ani muscle tissue density was significantly diminished in the combined incontinence

Table 2. Parameters of the anal sphincter complex

Parameter	Study group (N = 18)	Control group (N = 14)	*P* values
External anal sphincter length (mm)	20.0 ± 5	26.6 ± 13	0.03
Inner layer of external anal sphincter length (mm)	3.1 ± 3	7 ± 2	0.001
Intersphincteric groove (mm)	2.4 ± 2	3.9 ± 1	0.003
Superficial transverse perineal thickness (mm)	5.3 ± 2	7.5 ± 2	0.009
Perineal descent (mm)	8.7 ± 6	3.8 ± 3	0.005
Puborectalis thickness (mm)	9.5 ± 3	12.5 ± 3	0.016

Significance: $P < 0.05$

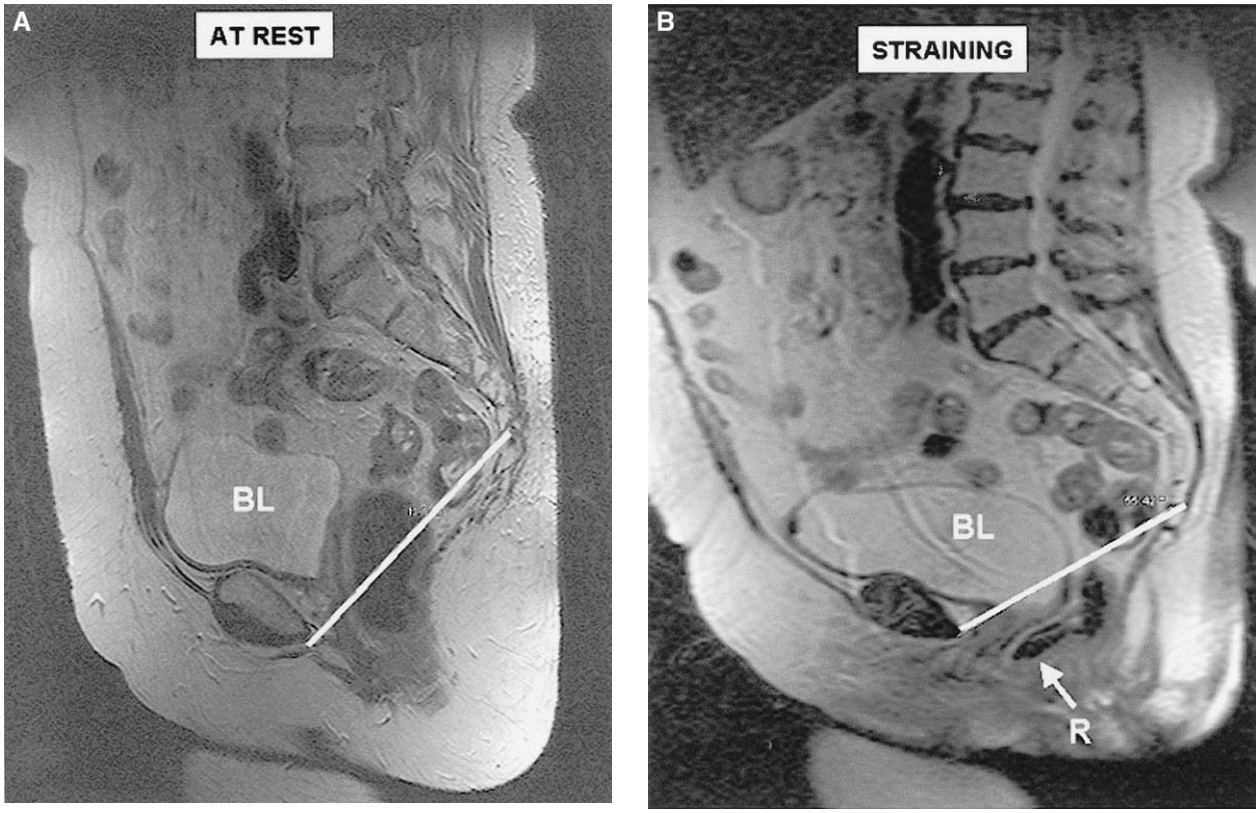

Fig. 5. Sagittal images of a patient illustrating perineal descent and organ prolapse. **A**, At rest; **B**, Straining.

group compared to the control group. Neither the mean tissue density nor the percentage fat content correlated with age. This suggests that age is not a causal factor in the alteration of levator ani muscle tissue morphology. Previous studies looking at the anal sphincter have identified changes in the anal sphincter that correlated with increasing age,[30–32] although this correlation was not confirmed by Williams et al.[19]

These findings suggest that apart from anal sphincter problems, patients with idiopathic fecal and urinary incontinence also have structural defects (above the puborectalis area) in the levator ani muscle; this may explain why some of these patients may have relatively normal results of anorectal neurophysiologic and urodynamic studies, and still suffer from significant symptoms of fecal and urinary incontinence. The levator ani angle, which is an index of the degree of arching of the levator ani muscle, was significantly reduced in the symptomatic group. This we believe would alter the dynamics of the pelvic floor, especially in the upright position or during phases of increased intra-abdominal pressure, when considerable stress is brought to bear predominantly on the levator plate.

The most striking finding of our study was the identification of the arch of the levator ani (the "levator dome"), and this is the first report of this distinct anatomic entity as an MRI finding. Our contention is that in normal subjects with the arch of the levator ani muscle intact, pressure from above, while trying to depress the arch, is deflected to closing the levator hiatus, thus reinforcing continence. Conversely, in patients with idiopathic fecal incontinence the arch of levator ani muscle is either lost completely or significantly diminished, causing the muscle to assume a "funnel" or "bowl" configuration. The result of this is that when pressure is applied on the levator plate from above, the levator hiatus opens and causes incontinence, particularly if this is coupled with an already compromised anal sphincter. This hypothesis has been corroborated by results of a study conducted by Shafik et al.[33] on the intrinsic myoelectric activity of the levator ani muscle. In this study it was determined that the levator ani muscle had resting myoelectric activity, and this could be attributed to histologic evidence of the presence of smooth muscle bundles interlaced with the striated muscle bundles. These smooth muscle fibers appear to generate an increased tone, reflected in an elevation of electrical

activity of the levator ani muscle, in response to an increase in intra-abdominal pressure and visceral weight, thus reinforcing continence. Shafik et al.[33] further suggested that increased levator ani muscle myoelectric activity and contraction appears to protect the infralevator pelvic structures that compromise the rectal neck (anal canal), urethra, and vagina from the effect of increased intra-abdominal pressure, thus preventing leakage. In conditions such as chronic straining at defecation or in acute straining during labor, the brunt of the increased intra-abdominal pressure falls on the levator ani muscle, which may ultimately subluxate or sag, directly exposing the infralevator structures and leading to the levator dysfunction syndrome.[34,35]

The parameters (such as perineal descent, organ descent, cystocele, and rectocele) measured on phased-array MRI performed with the patient in the supine position were probably lower than those obtained in the upright position with the patient sitting in an open MRI unit. This was previously confirmed by Kelvin et al.,[36] who showed that the supine values were underestimated by approximately 10% to 15% compared with the upright position, and we know that gravitational influence is known to exacerbate pelvic floor weakness.

Identifying structural alterations in the levator plate complex as part of a preoperative evaluation in the selection of patients for incontinence surgery may be very important. Although in the short term, the success rate of surgical intervention for fecal incontinence is as high as 70% to 80%, in the medium and long term the outcome is disappointing, with less than 2% remaining fully continent.[37–40] Surgical procedures such as anterior levatoplasty or postanal repairs do not restore or sustain the levator ani muscle and its intrinsic myoelectric properties. The loss of the levator ani muscle arch and its function may respond better to activation by physiotherapy, biofeedback, or electrical stimulation.[35] Neuromodulation by sacral nerve stimulation might be the way forward in the management of these patients.[38–44] Sacral nerve stimulation improves anal resting and squeeze pressures, rectoanal inhibitory reflex, and volumetric compliance, as well as significantly increasing defecation deferral time, and anal canal length. Rosen et al.[39] confirmed the positive stimulation response during acute-phase testing by the typical contraction features of the pelvic floor and the anal sphincter; they were also able to demonstrate a significant increase in sphincter pressures and anal canal length.[39] Shafik et al.[33] have also shown that skeletal muscle may have some adaptive response to increased use of chronic electric stimulation and that the presence of smooth muscle fibers represents a process of structural-functional adaptation in the levator ani muscle.[33,45,46] The initial results of sacral nerve stimulation are very encouraging with recovery from incontinence of up to 70% to 80% in treated cases.[46] Future prospective studies are planned to rescan patients after neuromodulation therapy to determine what structural changes are effected and how these might correlate with symptomatic improvement.

CONCLUSION

We have demonstrated for the first time distinct anatomic abnormalities in the levator plate complex in patients with idiopathic combined fecal and urinary incontinence. Our findings suggest that pelvic floor MRI may play an important role in selecting patients who will benefit from these new, minimally invasive treatment modalities. Findings on MRI may also suggest novel surgical remedies as well as provide better selection criteria for established surgical procedures in order to achieve better outcomes. Further studies to standardize reference values are therefore necessary to more clearly define the clinical role of MRI.

We thank Mr. Tommy Walsh, Mr. Shane Duggan, and Ms. Breda Kearns (Department of Clinical Photography and Illustration) for the photographs, and Ms. Debbie Prior and the entire staff of the Department of Radiology for the high-quality magnetic resonance images.

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Discussion

Dr. A. Wald (Pittsburgh, PA): These are very elegant studies that you have done. They remind us of the development of defecography and dynamic proctography. The issues that might be raised are the interobserver variability in reading and how can these be reproduced. Which of these parameters are of critical importance in determining indications for surgery and determining outcome after surgery?

Dr. E. Eguare: In establishing measurements we had two persons read these MRIs individually, and they were blinded from the presenting complaints of the patients. The measurements of the two investigators showed a strong correlation and confirmed that these readings can be reproduced.

In looking at the parameters that we considered, what we tried to highlight in this area was to find some new targets toward which we could direct our clinical remedy. In these patients where we cannot identify sphincter defects, surgery is really not an option. So we think that identifying the levator ani muscle abnormality will tend to suggest that neuromodulation in the form of sacral nerve stimulation and biofeedback might be the way forward in the treatment of these patients.

Invited Discussion—Expert Commentator

James W. Fleshman, M.D. (St. Louis, MO): The use of dynamic MRI to study the pelvic floor is rapidly becoming an alternative to the standard transrectal ultrasound and anal physiology tests over the past decade. Those of us with an interest in anal physiology understand that many of the parameters that we rely on to confirm or diagnose abnormal pelvic floor function are artificial at best and only a reflection of true function. The measurement of angles, descent, distances, and openings on contrast defecography has not been adequate to explain function and dysfunction in a discriminatory manner. The group from Dublin has compared a pure group of patients (combined idiopathic urinary and fecal incontinence), which is small because of its rarity, with a group of normal individuals. This is an excellent prospective, case-controlled, descriptive study. I have the following questions for the authors:

1. How do you explain the loss of the inner layer of the external anal sphincter in incontinent patients? I do not consider there to be a double layer of the external anal sphincter.
2. How does the finding of loss of the levator arch in "idiopathic anal incontinence" compare to pelvic floor descent in patients with rectal prolapse? How does the loss of the levator arch compare to nonrelaxing puborectalis muscle in oulet obstruction constipation?

The theory of loss of the levator arch as a cause of incontinence is enticing because it partially explains why sacral nerve stimulation works in patients with neurogenic/idiopathic incontinence and corrects constipation in patients with urinary incontinence and outlet obstruction constipation. Returning the pelvic floor to its intended diaphragm function and position may correct more than just incontinence, for example, pelvic pain syndrome. The authors have a great opportunity to study and confirm the changes and function after intervention using their technique. Some of the parameters that they have measured may be actual predictors of functional abnormalities. However, they must be compared in a multivariate analysis in specific functional disorders and must be shown to change or return to normal when the functional disorders are corrected either by surgical or biofeedback, and/or by physical therapy.

Surgical Treatment of Gallbladder Cancer

C. Burcin Taner, M.D., David M. Nagorney, M.D., John H. Donohue, M.D.

Gallbladder cancer is usually a fatal illness because early stages of this carcinoma cause no specific signs or symptoms. Although the best chance of cure for gallbladder cancer remains incidental discovery, radical resection of the gallbladder, with the adjacent liver, adherent structures, plus a regional lymphadenectomy, has been suggested to improve survival. We retrospectively analyzed all patients with gallbladder cancer who were treated surgically at Mayo Clinic (Rochester) between 1984 and 2000. There were 131 patients for whom complete survival information was available. Patients who underwent a radical cholecystectomy had a significantly longer median survival (24 months) than patients who had a simple cholecystectomy (6 months) or noncurative treatment (4 months) ($P < 0.0001$). The radical cholecystectomy group had significantly longer survival than the simple cholecystectomy group for all American Joint Committee on Cancer (AJCC) stages except stage I. Of the different variables tested in a univariate analysis (sex, surgical treatment modality, AJCC stage, tumor grade, jaundice, hyperbilirubinemia, and adjuvant therapy), all variables except sex, tumor grade, and adjuvant therapy were statistically significant predictors for the survival of patients with gallbladder cancer. AJCC stage and surgical treatment modality were the only significant predictors in a multivariate analysis. Our results support radical surgical resection for the treatment of gallbladder cancer to improve patient survival.
(J Gastrointest Surg 2004;8:83–89)

Key words: Gallbladder cancer, radical cholecystectomy

Gallbladder cancer is the most common cancer of the biliary tract and the sixth most common cancer of the gastrointestinal tract.[1] Despite improved diagnostic capabilities, better perioperative care, and more aggressive surgical approaches, overall survival remains low because of aggressive tumor biology. Failure to improve patient outcome is primarily due to late recognition of gallbladder cancer. The best chance of cure for gallbladder cancer remains incidental discovery of an early cancer during cholecystectomy for symptomatic gallstones. Although no randomized controlled trials have identified the surgical treatment of choice for patients with gallbladder cancer, multiple reports of cholecystectomy, liver resection, hepatoduodenal ligament lymphadenectomy, and resection of adjacent organ involvement for patients with invasive gallbladder carcinoma have accrued accumulating independent support.[2–6] We have reviewed our recent experience with gallbladder cancer to evaluate the effect of aggressive surgical treatment on patient outcome. Because a substantial proportion of gallbladder tumors are discovered incidentally during elective laparoscopic or open cholecystectomy, we also studied the effect of a prior operation on survival. We also evaluated the effect of several laboratory, presentation, and demographic variables on patient survival.

PATIENTS AND METHODS

We retrospectively reviewed the records of all patients with carcinoma of the gallbladder treated surgically at Mayo Clinic (Rochester) between 1984 and 2000. Patient demographics, symptoms and signs, laboratory data, pathologic findings, operative management, hospital morbidity and mortality, and the type of adjuvant therapy were recorded. A total of 144 patients underwent operative treatment for gallbladder cancer at our institution during this time period. Only 131 patients had complete survival data. This cohort forms our study population. Follow-up

Presented at the Forty-Fourth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Florida, May 18–21, 2003 (oral presentation).
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included data present in the patient records or telephone calls. All postoperative survivors were followed for a minimum of 5 years or until they died.

Radical cholecystectomy was defined as cholecystectomy with at least subsegmental resection of hepatic segments IVB and V adjacent to the gallbladder fossa and a regional lymphadenectomy. The lymphadenectomy usually included all lymph nodes in the hepatoduodenal ligament plus periduodenal, peripancreatic, hepatic artery, and celiac lymph nodes. Pathology reports were reviewed to confirm the diagnosis and stage of the tumor. The cancers were classified by stage using the criteria established by the American Joint Committee on Cancer (AJCC).[7]

Survival was estimated using the Kaplan-Meier product limit method.[8] The assessment of discrete variable effects on survival was calculated using the log-rank test and the Cox proportional hazards model.[9]

RESULTS

Patient Population

A total of 144 patients underwent surgical treatment of gallbladder cancer at our institution between 1984 and 2000. Excluding the 13 patients lost to follow-up, 131 patients form our study group. Women outnumbered men by a ratio of 2.5:1 (95 to 36). The median age was 66 years for women (range 37 to 93 years) and 64 years for men (range 32 to 88 years). Thirty-nine percent of the patients had a recorded family history of cancer. Twelve patients had a total of 15 previous malignancies (2 melanomas, 2 basal cell cancers of the skin, 2 breast cancers, 2 cervical cancers, 2 prostate cancers, 1 lung cancer, 1 bladder cancer, 1 rectal cancer, 1 lymphoma, and 1 pancreatic cancer).

Presentation

Abdominal pain was the most common symptom (73%), followed by nausea and vomiting (43%), jaundice (37%), anorexia (35%), and weight loss (35%). Among patients with documentation of weight loss available (n = 30), the mean weight loss was 6.2 kg. Only 8% of the patients presented with a palpable abdominal mass on physical examination.

Serum liver function tests were the most common abnormal laboratory result. Serum total and direct bilirubin, alkaline phosphatase, aspartate, and alanine transferase levels were abnormal in 55% of patients. The total bilirubin level was elevated in 44% of the patients who underwent simple cholecystectomy (median 6.7 μmol/L), 23% of the patients who had radical cholecystectomy (median 10.5 μmol/L), and 44% of the patients who had noncurative surgical procedures (median 11.5 μmol/L). Hypoalbuminemia (⩽35 g/L) was detected in 25% of patients (median 31 g/L).

Abdominal ultrasonography, carried out in 41% of the patients, was the most frequently performed preoperative imaging test. Computerized tomography (CT) of the abdomen alone was performed in 24% of patients. Twenty percent of the patients had both ultrasound and CT scans. The ultrasound scan was interpreted as normal in 5% and abnormal in 95% of patients (cholelithiasis, thickened gallbladder wall, or a combination of findings). A mass in the gallbladder suspicious for carcinoma was detected in only 20% of patients using preoperative ultrasonongrapy.

Operative Treatment

Forty-five patients (34%) underwent simple cholecystectomy, whereas 60 patients (45%) underwent radical resection with curative intent. In 17 patients radical cholecystectomy was extended by partial gastrectomy, duodenectomy, or colectomy because of adherence to or invasion into these organs. Moreover, three major liver resections (more than 2 liver segments) were undertaken to address the extent of hepatic involvement. Major bile duct resection was performed in three patients. The remaining 26 patients (20%) had noncurative surgical procedures for either diagnosis or palliation. None of the patients who underwent radical cholecystectomy or simple cholecystectomy had grossly positive resection margins. Thirty-six patients had undergone simple cholecystectomy elsewhere and were referred to the Mayo Clinic for further management. Of these patients, 24 had previously undergone laparoscopic cholecystectomy and 12 had undergone open cholecystectomy.

The operative mortality rate was 2% (3 patients). One patient who had undergone radical cholecystectomy died on postoperative day 11 from a presumed cardiac event. Another patient who underwent gastrojejunostomy and tube gastrostomy for unresectable cancer died on postoperative day 16 of a pulmonary embolism. One patient who had undergone surgical exploration of the abdomen for unresectable cancer died on postoperative day 13 (5 days after discharge from the hospital); the cause of death was unknown.

Thirteen percent of all patients had postoperative complications. Eight patients (13%) developed complications following radical cholecystectomy and eight patients (17%) developed complications following simple cholecystectomy ($P = 0.89$). Bile leaks (6 patients), wound infections (4 patients), and a dynamic ileus (4 patients) were the most common postoperative complications. One patient had a retained

common bile duct stone after simple cholecystectomy, which was managed with endoscopic removal. Other postoperative problems included myocardial infarction, subhepatic hematoma, duodenal stump leak, episode of cholangitis, and subhepatic abscess (1 of each). None of the patients required a second operation because of their complications.

Additional Treatment

Forty-eight patients received adjuvant therapy. Thirty-eight received both radiotherapy (range 1975 to 5580 cGy; mean = 4843 cGy) and chemotherapy (5-fluorouracil, leucovorin, irinotecan, doxorubicin, and mitomycin-C). Nine patients received only chemotherapy (5-fluorouracil, doxorubicin, 6-thioguanine), and one patient received only radiotherapy. Eighty-three patients had no adjuvant therapy.

Pathologic Findings

Primary Cancer. The tumor size was recorded in 87 patients. The mean diameter (± standard deviation [SD]) was 4.4 ± 2.8 cm. Eighty-two patients (63%) had adjacent organ adherence by their gallbladder cancer. Direct extension to the liver occurred in 49 patients (37%). Tumor grade was documented in 127 patients. The distribution was as follows: grade 1, 1%; grade 2, 25%; grade 3, 52%; and grade 4, 17%.

Tumor Metastasis. Regional lymph node status was documented pathologically in 81 patients (62%). Regional lymph nodes were uninvolved in 35 patients (43%), hepatoduodenal ligament lymph nodes (N1 according to the AJCC staging system [5th ed]) were positive in 27 patients (33%), and more distant regional nodes (N2 according to the AJCC staging system [5th ed]) were positive in 19 patients (24%). Data for distal organ involvement were recorded in 113 patients. Thirty-four (30%) had distal metastases noted.

Tumor Staging. Pathologic staging of patients was performed in accordance with the the fifth edition of the AJCC classification system.[7] Overall stage distribution was as follows: stage I = 6 (5%), stage II = 15 (12%), stage III = 40 (31%), stage IVA = 19 (15%), and stage IVB = 49 (38%). The distribution of pathologic staging by surgical procedure is shown in Table 1. Of the 26 patients who underwent noncurative, palliative treatment, 25 (96%) had stage IVA or IVB tumors.

Patient Survival

At the time of the last follow-up, 89% of the patients had died. Overall median survival was 11 months. Forty-eight percent of the patients were alive 1 year postoperatively, but only 13% of patients survived 5 years or more (Fig. 1) after their operation.

Table 1. American Joint Committee on Cancer (5th edition) stage of gallbladder cancer by treatment

AJCC stage	Palliative	Radical	Simple
I	0 (0%)	1 (17%)	5 (83%)
II	0 (0%)	13 (87%)	2 (13%)
III	1 (2%)	24 (60%)	15 (38%)
IVA	8 (42%)	7 (37%)	4 (21%)
IVB	16 (33%)	14 (28%)	19 (39%)

The effect of AJCC stage, type of surgical treatment (simple vs. radical vs. noncurative), patient sex, tumor grade, adjuvant therapy, previous operation (open cholecystectomy vs. laparoscopic cholecystectomy vs. none), total bilirubin elevation (>1.0), clinical jaundice, and individual components of tumor (T), node (N), and metastasis (M) stage on patient survival were evaluated using univariate analysis (Table 2). Of these variables, AJCC stage ($P < 0.0001$) (Fig. 2), surgical treatment ($P < 0.0001$), elevated total bilirubin ($P = 0.0039$), and clinical jaundice ($P = 0.0001$) correlated significantly with patient survival.

Radical cholecystectomy was associated with significantly longer survival than either simple cholecystectomy or noncurative therapy. The hazard ratio for death associated with radical vs. simple cholecystectomy was 0.42 (95% confidence interval = 0.27 to 0.64). The hazard ratio of radical surgery compared to noncurative therapy was 0.23 (95% confidence interval = 0.12 to 0.45). The hazard ratio for patient death for the simple cholecystectomy group did not differ significantly from that in the noncurative

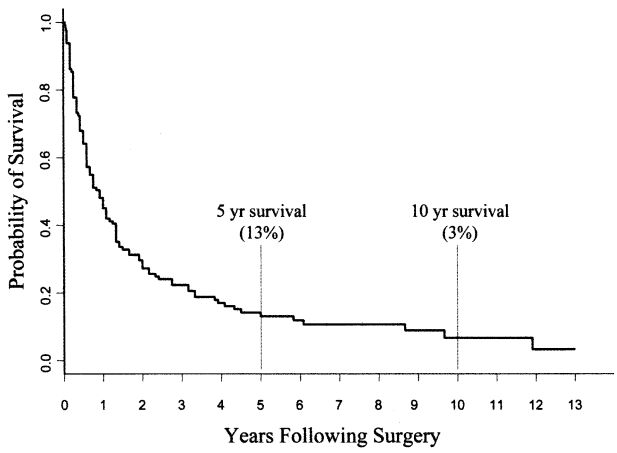

Fig. 1. Survival for all patients.

Table 2. Mean survival after curative surgery by American Joint Committee on Cancer stage

AJCC stage	Radical (mo)	Simple (N) (mo)	*P* value (log-rank test)
I	49 (n = 1)	33 (n = 5)	0.95
II	33 (n = 13)	10 (n = 2)	0.0097
III	27.5 (n = 24)	7 (n = 15)	0.04
IVA	9 (n = 7)	3.5 (n = 4)	0.0004
IVB	21.5 (n = 14)	5 (n = 19)	<0.0001

therapy group (noncurative vs. simple = 1.83; 95% confidence interval = 0.88 to 3.81) (Fig. 3).

Patients with a previous cholecystectomy who underwent subsequent radical resection showed a tendency for longer survival ($P = 0.08$). The overall survival rate for patients who had a laparoscopic cholecystectomy was greater than that for patients with either no previous cholecystectomy or open cholecystectomy (Fig. 4). Patient sex ($P = 0.81$), tumor grade ($P = 0.13$), and adjuvant therapy ($P = 0.06$) did not correlate significantly with patient survival, although there were trends toward improved outcome with more differentiated carcinomas and the use of adjuvant therapy.

Survival for each AJCC stage after complete gross resection was analyzed by means of the log-rank test (Table 3). Radical cholecystectomy was associated with greater survival than simple cholecystectomy for each tumor stage. These differences were statistically significant for all stages except stage I, where the data were insufficient for statistical comparison. Overall survival at 1 and 5 years for patients with radical cholecystectomy were 80% and 17%, respectively,

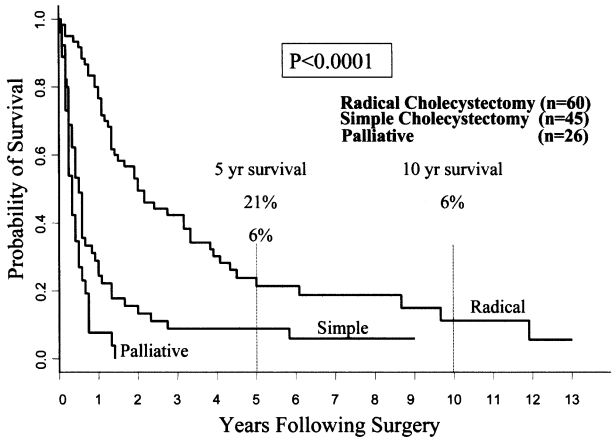

Fig. 3. Patient survival by type of operation.

compared to 29% and 7% for simple cholecystectomy. Extension of radical cholecystectomy to treat gross direct invasion of adjacent structures was associated with a survival of 9% at 5 years. It is important to note that although long-term survival in patients having en bloc resection was infrequent, 5-year survival was achieved in two patients.

The Cox proportional hazards method was used to determine the multivariate relationship of AJCC stage, type of surgical treatment, sex, tumor grade, adjuvant therapy, previous surgery, total bilirubin elevation, and presence of jaundice to patient survival. AJCC stage and radical cholecystectomy ($P = 0.002$) were the only variables that were significantly related to patient outcome. During the preparation of this manuscript, the sixth edition of the AJCC staging system was published.[10] We also analyzed our data

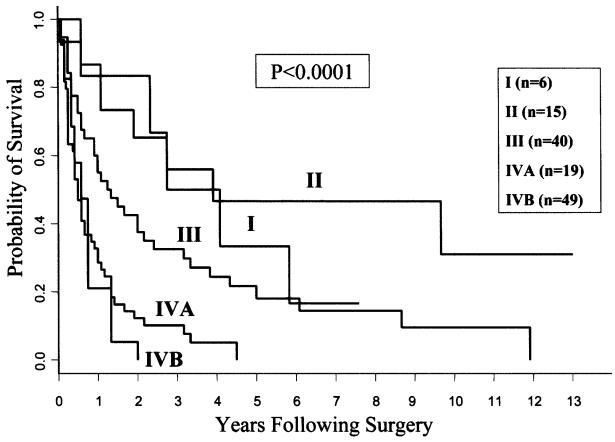

Fig. 2. Patient survival according to American Joint Committee on Cancer stage (5th edition).

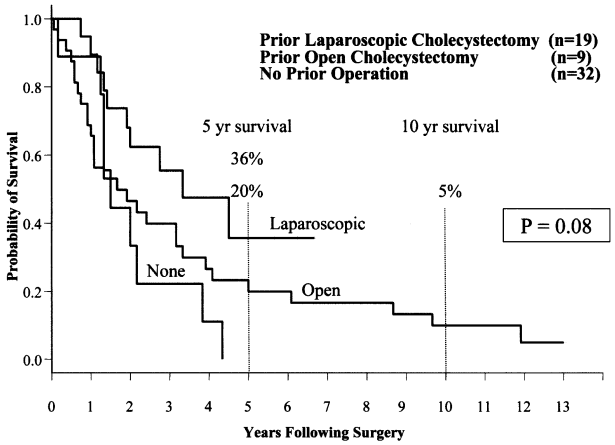

Fig. 4. Patient survival after curative surgery in patients with prior cholecystectomy.

Table 3. Univariate analysis of variables on patient survival

Variable	*P* value
AJCC stage	<0.0001
Tumor	<0.0001
Node	<0.0001
Metastasis	<0.0001
Surgival treatment modality	<0.0001
Total bilirubin	0.0039
Jaundice	0.0001

according to the new stage definitions and determined stage to be a significant variable for patient survival ($P < 0.0001$). There was no statistically significant difference between the median survival in stages IA to IIB ($P < 0.151$), although there was a trend toward longer survival in earlier stages. Stage III was associated with a significantly worse survival than stages IA to IIB ($P < 0.0001$). Stage IV had significantly worse survival than stage III ($P < 0.0001$). When treatment modalities were compared, radical resection resulted in significantly longer patient survival in stages IIB and above ($P < 0.0001$).

DISCUSSION

Our findings in patients treated surgically for gallbladder cancer provide additional data identifying clinicopathologic factors associated with survival and outcomes supporting radical cholecystectomy for prolonged survival. Our data support the findings of others who have examined similar relationships between clinicopathologic and treatment factors and survival[2–6] including our own.[11] Although our findings provide further support for an aggressive surgical approach, overall outcome dictates pursuit of effective adjuvant therapies to further enhance survival.

Several clinicopathologic factors were significantly related to patient survival. Overall tumor stage, each TNM component, jaundice, and serum bilirubin elevation were significantly correlated with survival. Advancing tumor stage and jaundice adversely affected patient survival. Similar findings have been reported by others.[4,12] Interestingly, the type of prior biliary tract operation correlated with variable patient survival contrary to some earlier reports.[13–16] Although we found no other clinicopathologic factors that correlated with survival, others have shown a survival effect from tumor grade and adjuvant therapy.[17–19] We cannot explain our failure to correlate patient survival and these factors based on demographic variables. Factors related to patient treatment selection are a more plausible explanation. It is of great importance that few of these factors can be used preoperatively for patient selection. Although recognition of jaundice almost always implies malignant bile duct obstruction, and large tumors or enlarged lymph nodes may affect the extent of hepatectomy or regional lymphadenectomy, such findings do not preclude a radical, potentially curative resection. With the advent of effective adjuvant therapy for gallbladder cancer, preoperative use of such therapy for locally advanced gallbladder cancer may be used to reduce the extent of resection required to achieve an R0 resection.

The only interventional factor that correlated with survival in our study was performance of a radical operation. For all tumor stages beyond stage I, radical cholecystectomy was associated with longer survival than simple cholecystectomy. These findings are similar to those in several other reports.[2–5,11,12] The overall patient survival after an R0 resection using a radical cholecystectomy was also greater than R0 resection with simple cholecystectomy, again confirming prior publications.[20] This finding is not unexpected because of the probable understaging of patients with gallbladder cancer treated by simple cholecsytectomy— that is, unrecognized local-regional disease. Although morbidity rates for radical surgical management of gallbladder cancer may exceed those for simple cholecystectomy, we do not consider the operative risk of a radical cholecystectomy excessive, inasmuch as the morbidity rates after these two treatment modalities were similar in our cohort. Given the frequency of lymph node metastases with invasive gallbladder carcinoma and the frequency of occult direct extension into the liver, at least radical cholecystectomy should be employed for T2 or more extensive gallbladder carcinomas unless distant metastases are identified.

Our data do not fully address the impact of radical surgical procedures because most surgical procedures were limited to a radical cholecystectomy consisting of subsegmental liver resection and regional lymphadenectomy. The extent of the primary cancer often dictates resection of adjacent involved structures.

We have not routinely employed bile duct resection in patients with prior cholecystectomy because of the reputed intraoperative difficulty of differentiating postoperative inflammation from residual cancer at the junction of the cystic duct with the common hepatic duct.[12] We have resected the cystic duct stump to exclude residual cancer in most patients with prior cholecystectomy. Typically, a long cystic duct stump remains after laparoscopic cholecystectomy and does

not necessitate a bile duct resection for differentiation of residual malignancy. Although this approach may risk a second intraoperative tumor transection, the outcomes seen herein have not been adversely affected. Additional data are needed to determine the preferable operative approach to the cystic duct stump and the adjacent bile duct.

The extent of hepatic resection also remains disputable. We employed major hepatic resections or major hepatic resection with bile duct resection in only six patients. With bisegmental or nonanatomic subsegmental resection of the gallbladder fossa, the hepatic margins of resection are asymmetrical—that is, the extent of tumor-free margins near the fundus almost always exceeds those near the infundibulum (region adjacent to the cystic duct area) where the liver parenchyma interposed between the gallbladder and the main right or right sectoral bile ducts is thin. Although routine right lobar resection unquestionably addresses this concern, the long-term survival statistics after bisegmentectomy (segments IVB to V), or even subsegmental resections, suggest that this approach is not routinely necessary. Further assessment of T stage in relation to the hepatic margins and survival might clarify this selection issue.

Finally, our regional lymphadenectomy addressed all of the hepatoduodenal ligament lymph nodes but only a portion of the more distant regional lymph nodes. We have not employed pancreaticoduodenectomy as suggested by others for a more complete lymphadenectomy.[21,22] Although we currently believe that the risk of pancreaticoduodenectomy to improve the extent of lymphadenectomy exceeds the overall survival benefit, we have no data to substantiate that position. We believe that the current data warrant further assessment of this issue.

CONCLUSION

Gallbladder cancer continues to be a challenge for surgeons because of overall poor patient survival. Radical cholecystectomy results in longer patient survival in gallbladder cancer as an initial surgical procedure or after incidental discovery of cancer during a prior cholecystectomy. Current adjuvant treatment modalities do not significantly change the course of the disease, emphasizing the need for more effective adjuvant treatment protocols.

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Discussion

Dr. H. Chen (Madison, WI): I noted that you had a significant number of patients who were referred to you after their initial laparoscopic or open cholecystectomy. I was wondering what procedure you recommend for someone who has had a laparoscopic cholecystectomy and now presents with a gallbladder cancer at your institution? Specifically, I was wondering what you do with the port sites.

Dr. B. Taner: Our departmental policy is excision of the port sites, and if the cancer is resectable, these patients undergo a radical resection.

Invited Discussion—Expert Commentator

David Fromm, M.D. (Detroit, MI): The report by Dr. Taner and associates is one among others suggesting a benefit for radical cholecystectomy in the treatment of carcinoma of the gallbladder. This study is remarkable for the number of cases of this relatively rare cancer. Several studies emphasize the importance of R0 resection, but how radical does one have to be to achieve this? Improvement in survival has been noted following resections that include lymph node metastases limited to the hepatoduodenal ligament, but metastases to nodes beyond this region are considered by some to be a contraindication to resection. There is increasing agreement that T2 and T3 tumors should include some form of incontinuity resection of the liver with regional lymph node dissection. There is also an increasing tendency to perform an extended right hepatectomy and common bile duct resection when there is involvement of the gallbladder neck or cystic duct, but not all advocate lymph node dissection if there is no gross involvement in this situation. The incidence of resectability progressively decreases as the depth of invasion increases, so that somewhat less than half of the patients in several reports involving T3 tumors are resectable, and the figure approaches 20% or less for T4 tumors. A major problem is that survival after radical resection for stages III and IV usually is in terms of months and not years.

Fluorodeoxyglucose PET Imaging in the Evaluation of Gallbladder Carcinoma and Cholangiocarcinoma

Christopher D. Anderson, M.D., Michael H. Rice, M.D., C. Wright Pinson, M.D., William C. Chapman, M.D., Ravi S. Chari, M.D., Dominique Delbeke, M.D., Ph.D.

Our goal was to evaluate fluorodeoxyglucose (FDG) positron emission tomography (PET) in staging patients with biliary tract cancers. Fifty consecutive patients who underwent FDG-PET for suspected cholangiocarcinoma (n = 36) or gallbladder carcinoma (n = 14) were reviewed. Patients with cholangiocarcinoma were divided into two groups: group 1 had nodular type (mass > 1 cm) (n = 22) and group 2 had infiltrating type (n = 14) cholangiocarcinoma. Thirty-one of 36 patients evaluated for cholangiocarcinoma had cholangiocarcinoma and five did not. Sensitivity was 85% for nodular morphology but only 18% for infiltrating morphology. Sensitivity for metastases was 65% but false negative for carcinomatosis in three of three patients. One false positive result occurred in a patient with primary sclerosing cholangitis who had acute cholangitis. Seven (58%) of 12 patients had FDG uptake along the tract of a biliary stent. FDG-PET led to a change in surgical management in 30% (11 of 36) of patients evaluated for cholangiocarcinoma because of detection of unsuspected metastases. Eleven of 14 patients with gallbladder carcinoma were newly diagnosed by cholecystectomy or another type of exploratory procedure, whereas three patients were undergoing follow-up. Nine had residual gallbladder carcinoma at the time of PET. Sensitivity for gallbladder carcinoma was 78%. Sensitivity for extrahepatic metastases was 50% in eight patients; six of them had carcinomatosis. These data suggest that PET is accurate in predicting the presence of nodular cholangiocarcinoma (mass > 1cm) but was not helpful for the infiltrating type. PET was also helpful for detecting residual gallbladder carcinoma following cholecystectomy, but was not helpful in patients with carcinomatosis. Although FDG-PET led to a change in management in 30% of patients with cholangiocarcinoma, it must be interpreted with caution in patients with primary sclerosing cholangitis and with stents in place, as well as in those with known granulomatous disease. (J GASTROINTEST SURG 2004;8:90–97)

KEY WORDS: Positron emission tomography, PET, cholangiocarcinoma, gallbladder carcinoma, biliary tract tumors

Biliary tract tumors, cholangiocarcinoma, and gallbladder carcinoma present a diagnostic and surgical challenge. Both of these malignancies are often unresectable at the time of diagnosis. Approximately 60% to 70% of cholangiocarcinoma occur at the hepatic duct bifurcation, and the remainder occur in the distal common bile duct (20% to 30%) or in the liver (5% to 15%).[1] Cholangiocarcinoma can be divided into three morphologic categories: infiltrating lesions, exophytic lesions, and polypoid intraluminal masses. Gallbladder carcinoma is more common than cholangiocarcinoma. It is discovered in approximately 1% of cholecystectomy specimens and is the fifth most common gastrointestinal malignancy.[2,3] Early in their course, these tumors are insidious, unsuspected clinically, and are usually discovered in the surgical specimen after cholecystectomy. They later spread to the liver and throughout the abdomen via peritoneal implants. Distant metastases occur in the lungs, pleura and diaphragm, and bone.

The response rates for systemic chemotherapy and/or radiation therapy regimens for both of these

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malignancies are limited, and thus surgical resection offers the best chance for curative therapy.[1,4] Operative procedures for cholangiocarcinoma and gallbladder carcinoma are associated with significant morbidity; therefore accurate noninvasive preoperative staging of these patients is critical. Ideally, preoperative imaging studies should be able to detect distant metastases, bilateral or multifocal intrahepatic metastases, and the presence of vascular invasion. Morphologic imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) are the best imaging modalities to evaluate the local extent of disease as well as the relationship with vascular structures. Limitations of anatomic imaging with CT or MRI for detection of tumor recurrence or metastases are well know and are often related to size criteria for determination of lymph node involvement. In addition, CT cannot reliably differentiate residual or recurrent tumor from scarring after therapy.

For these reasons a focus has been given to the development of functional imaging for preoperative evaluation. Positron emission tomography (PET) using the glucose analog fluorodeoxyglucose (FDG) is a rapidly evolving functional imaging modality that has proved useful for preoperative staging of a number of tumor types.[5] It is well established that a variety of malignant tumors avidly accumulate FDG. This is due, in part, to increased numbers of glucose transporter proteins and increased intracellular enzyme levels of hexokinase and phosphofructokinase, among others, which promote glycolysis.[6–9] FDG-PET imaging can be used to exploit the metabolic differences between benign and malignant cells for imaging purposes.[10,11] Thus FDG-PET imaging is well established for the differentiation of benign from malignant lesions, staging malignant lesions, detection of malignancy recurrence, and monitoring therapy for various malignancies.[5] At the present time, the major indication for FDG-PET imaging is staging and restaging malignant tumors leading to detection of unsuspected metastases in 25% to 30% of the patients and major changes in therapy. In two thirds of these cases, unnecessary surgery is avoided, decreasing the number of invasive procedures and increasing the cost-effectiveness of health care.[12,13]

However, FDG-PET has varying sensitivity for primary hepatobiliary versus metastatic tumors based on tumor expression of certain metabolic enzymes. For example, the sensitivity of FDG-PET for hepatocellular carcinoma is low (approximately 50% to 70%) because of varying degrees of activity of the enzyme glucose-6-phosphatase in these tumors.[14–18] The activity of glucose-6-phosphatase is low in cholangiocarcinoma and gallbladder carcinoma, and therefore the sensitivity of FDG-PET should be high for detecting these tumors.[14,15]

It is also important to consider how the many morphologic types of cholangiocarcinoma influence their detection with FDG-PET imaging. Lesion detection with imaging modalities is dependent on the resolution of the modality and the degree of contrast enhancement (FDG uptake in the case of PET) compared to background. Lesions with a size of less than twice the resolution of the imaging system suffer from partial volume-averaging artifact. The intrinsic resolution of state-of-the-art PET scanners is in the 4 to 5 mm range, meaning that lesions less than 8 mm will suffer from partial volume-averaging artifact and may not be detectable even if the degree of FDG uptake is higher than in the normal liver. Cholangiocarcinoma of the infiltrating morphology may not have the cellular density required to form a lesion larger than 8 mm, and therefore may not be detected by PET.

Although there are several reports in the literature regarding evaluation of cholangiocarcinoma by means of FDG-PET imaging,[16,19–23] only case reports of gallbladder carcinoma imaged with FDG-PET can be found.[24,25] One group of investigators has recommended PET only for the detection of distant metastases from gallbladder carcinoma.[26] Better preoperative staging of gallbladder carcinoma with PET before radical cholecystectomy would be useful in preventing unnecessary operations; however, there are inherent difficulties with PET imaging of these patients. The majority of these patients are evaluated early after incidental finding of gallbladder carcinoma in cholecystectomy specimens. FDG uptake in the operative bed because of early postoperative inflammatory changes makes the interpretation difficult and affects the accuracy for detecting residual tumor in the gallbladder fossa. Therefore the purpose of this study was to assess the value of FDG-PET for evaluation of patients with gallbladder carcinoma and cholangiocarcinoma including morphologically different tumors.

MATERIAL AND METHODS

Patient Population

Fifty consecutive patients who underwent whole-body FDG-PET imaging for suspected cholangiocarcinoma or gallbladder carcinoma during a 7-year period were included in the analysis. These patients all underwent whole-body FDG-PET imaging. Data collected included sex, age, clinical history and follow-up, CT or MRI results, pathology, and data regarding FDG-PET imaging. Thirty-six patients

with cholangiocarcinoma and 14 with gallbladder carcinoma were reviewed. All patients with cholangiocarcinoma or gallbladder carcinoma had their diagnoses confirmed by histopathologic or cytopathologic examination. The absence of carcinoma was established by normal pathologic findings and clinical follow-up for at least 1 year. The FDG-PET images were evaluated for evidence of primary tumor and metastatic disease. Patients with cholangiocarcinoma were divided into two groups: group 1 (nodular type) was defined as patients with evidence of a lesion (>1 cm) detected by CT, MRI, or pathologic examination (n = 22) and group 2 (infiltrating type) was defined as patients without evidence of a mass (n = 14).

FDG-PET Imaging

The FDG images were acquired with one of two dedicated PET machines (Siemens ECAT 933; CTI, Knoxville, TN, or GE Advance; General Electric Medical Systems, Milwaukee, WI). Patients were required to fast for at least 4 hours before PET imaging. One hour after the intravenous administration of 10 mCi of FDG, emission images were acquired over the neck, chest, abdomen, and pelvis. Transmission images were acquired to correct for attenuation. The FDG-PET images were interpreted independently by two nuclear medicine physicians/radiologists with extensive experience in FDG-PET imaging.

RESULTS

Patients

The final diagnoses of patients included in the study are summarized in Fig. 1. The 36 patients evaluated for suspected cholangiocarcinoma ranged in age from 38 to 84 years (mean 63 years); there were 20 males and 16 females. Thirty-one patients were referred for initial evaluation and five for suspected recurrence. Thirty-one of the 36 patients had cholangiocarcinoma and four of these were recurrences. The remaining five had primary sclerosing cholangitis without evidence of malignancy. Seventeen patients had metastases. Eleven patients had hepatic metastases, and three of these patients had associated carcinomatosis. The six other patients had extra-abdominal metastases. Seven total patients had primary sclerosing cholangitis and two of these had cholangiocarcinoma. Twelve patients had biliary stents in place at the time of PET imaging.

Fourteen patients were evaluated after the diagnosis of gallbladder carcinoma; these patients ranged in age from 54 to 77 years (mean 65 years); there were eight males and six females. Three of the 14 patients had previously undergone liver resection; in these three patients, PET was performed because of suspicion of recurrence. Nine of the 14 patients had residual gallbladder carcinoma (local extension/recurrence, or metastatic disease) at the time of PET imaging. Absence of residual malignancy (gallbladder carcinoma) was confirmed by liver resection specimens in four patients. A fifth patient underwent FDG-PET imaging 3 years after radical cholecystectomy and remained disease free. Of the 11 patients undergoing initial evaluation, nine were diagnosed from cholecystectomy specimens, and two were diagnosed from biopsies taken during surgical exploration for a separate diagnosis. Nine patients with residual gallbladder carcinoma at the time of PET imaging had local (gallbladder fossa) extension and seven of nine had hepatic metastases. In addition, three of these patients had distant extra-abdominal metastases. Six patients had carcinomatosis, and two had seeding at a laparoscopic port site.

Cholangiocarcinoma

The results of FDG-PET imaging are summarized in Table 1. Overall, sensitivity and specificity for cholangiocarcinoma were 61% (19 of 31) and 80% (4 of 5), respectively. However, when only patients with the nodular morphology were included in the analysis, the sensitivity rose to 85% (17 of 20) whereas it was only 18% (2 of 11) for patients with infiltrating cholangiocarcinoma. Fig. 2 shows an example of nodular cholangiocarcinoma detected using FDG-PET imaging. Overall, there were 12 false negative results, but only three false negative results in patients with lesions larger than 1 cm. FDG-PET results were true positive (n = 4) and true negative (n = 1) in the five follow-up patients.

Seven patients had underlying primary sclerosing cholangitis (4 true negative, 2 true positive, 1 false positive). The false positive result occurred in a patient with an episode of acute cholangitis. The overall sensitivity and specificity in patients with primary sclerosing cholangitis was 100% (2 of 2) and 80% (4 of 5), respectively. Seven of 12 patients (58%) with biliary stents in place had FDG uptake along the tract of the stent presenting diagnostic challenges, but all were was interpreted correctly as inflammation when the images were interpreted in correlation with the CT scan (Fig. 3).

The overall sensitivity for metastatic disease in patients with cholangiocarcinoma was 65% (11 of 17). However, these metastases were unsuspected based on other imaging modalities and led to a major change in therapy in all 11 patients. PET imaging was false negative for metastatic disease in three of

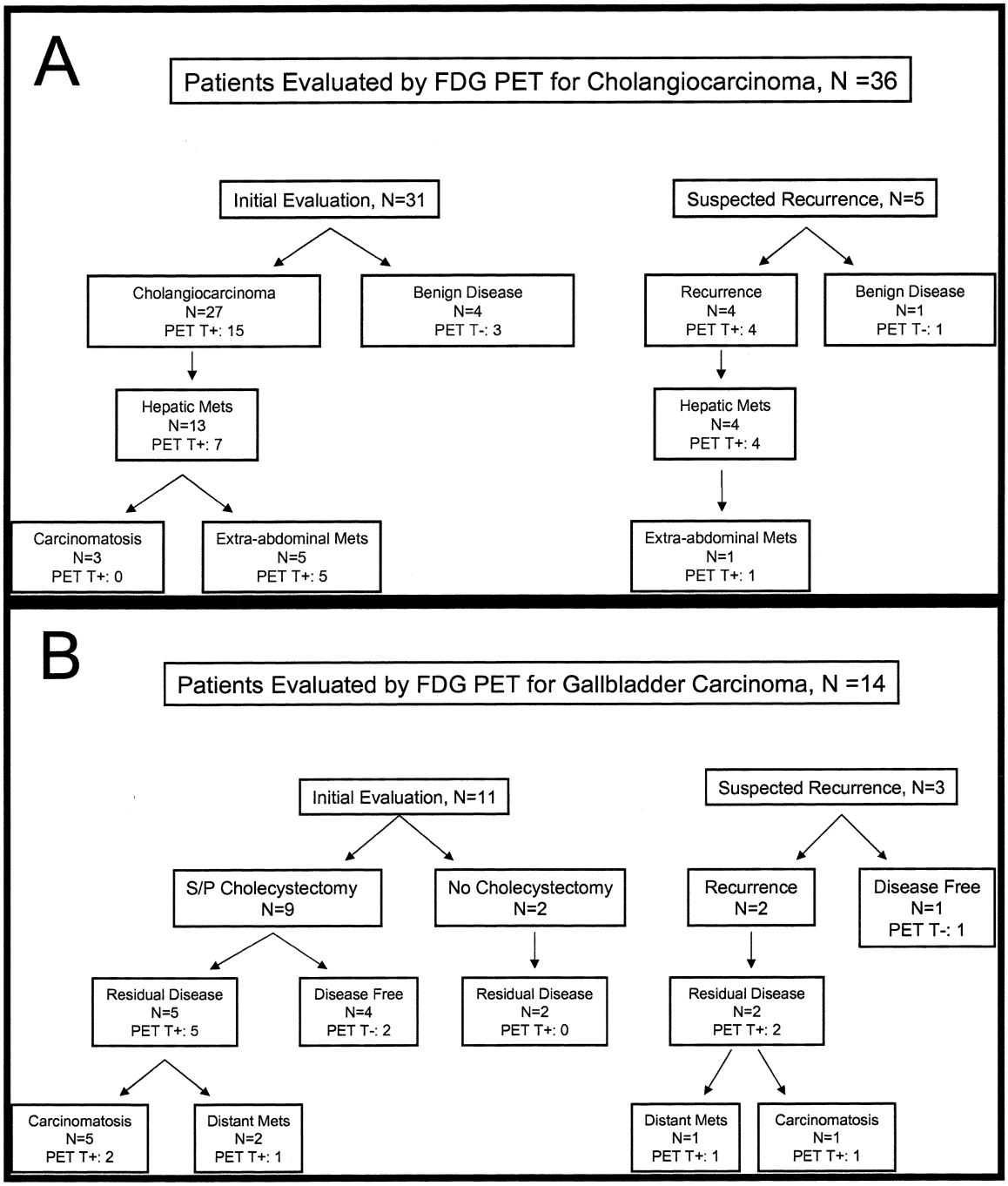

Fig. 1. Flow charts demonstrating the breakdown of final diagnoses of 50 patients evaluated by FDG-PET for biliary tract cancers. **A**, Thirty-six patients referred for suspected cholangiocarcinoma. **B**, Fourteen patients referred for evaluation of gallbladder carcinoma. T+ = true positive results; T− = true negative results.

three patients with carcinomatosis. In each of these three cases, small (<1cm) intraperitoneal metastases were found at exploration. All six patients with extra-abdominal (pulmonary and mediastinal) metastases were detected by PET imaging and there was one false positive small apical pulmonary granuloma.

The findings of FDG-PET imaging led to a change in management in 30% (11 of 36) of patients

Table 1. FDG-PET imaging performance for detection of 1) cholongio- (CC) in 50 patients and 2) gallbladder carcinoma (GC) in 14 patients

	GC	Nodular CC (Mass >1 cm)	Infiltrating CC (No mass)	Total CC
Total patients	14	22	14	36
True positive	7	17	2	19
False negative	2	3	9	12
True negative	4	1	3	4
False positive	1	1	0	1
Sensitivity	78% (7/9)	85% (17/20)	18% (2/11)	61% (19/31)
Specificity	80% (4/5)	50% (1/2)	100% (3/3)	80% (4/5)
PPV	88% (7/8)	94% (17/18)	100% (2/2)	95% (19/20)
NPV	67% (4/6)	25% (1/4)	25% (3/12)	25% (4/16)

GC = gallbladder cancer; CC = cholangiocarcinoma; PPV = positive predictive value; NPV = negative predictive value.

evaluated for cholangiocarcinoma because of detection of unsuspected metastases.

Gallbladder Carcinoma

Residual gallbladder carcinoma was defined as primary tumor, local gallbladder fossa invasion, or hepatic metastases. Sensitivity for residual gallbladder carcinoma was 78% (7 of 9) and specificity was 80% (4 of 5). Table 1 summarizes the PET findings in patients with gallbladder carcinoma. There was one false positive result in a patient who underwent FDG-PET imaging within 1 month of his cholecystectomy. The absence of disease was verified by liver resection. The two patients who had false negative findings had bulky intra-abdominal metastases and carcinomatosis at the time of surgical exploration for potential resection for reasons that were unclear.

Sensitivity for extrahepatic metastases (distant metastases or carcinomatosis) was 56% (5 of 9) in patients with gallbladder carcinoma. Two of three patients with distant metastases and one of two patients with laparoscopic port-site recurrences were detected by FDG-PET imaging. PET detected intra-abdominal metastases in only three of six patients with confirmed carcinomatosis. For the three patients with gallbladder carcinoma being followed after liver resection, FDG-PET imaging was accurate (no false positive or false negative results). PET imaging

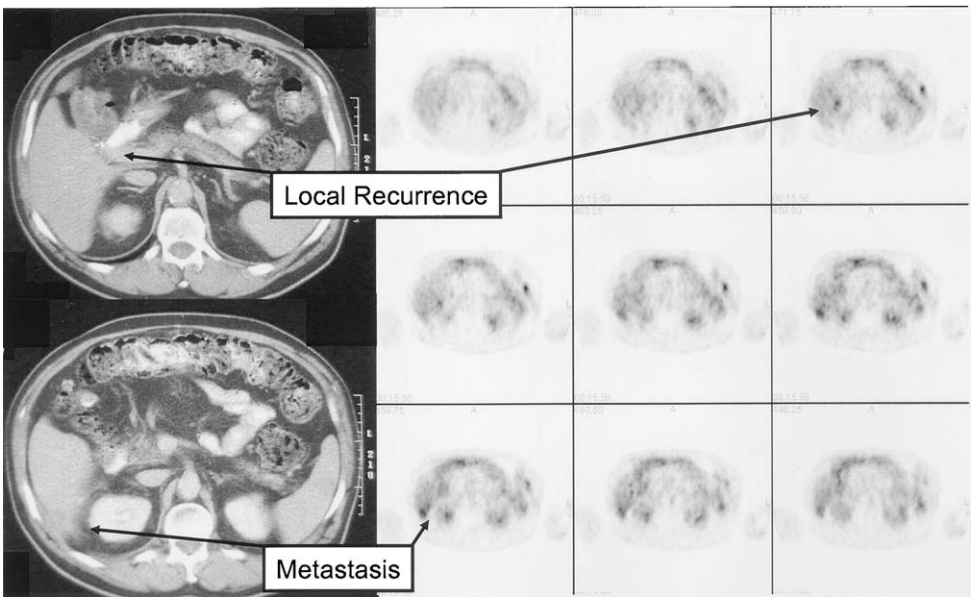

Fig. 2. Example of FDG-PET imaging in a patient undergoing surveillance after resection of cholangiocarcinoma. The PET images demonstrate two focal areas of FDG uptake corresponding to a local hilar recurrence and a hepatic metastasis retrospectively seen on CT.

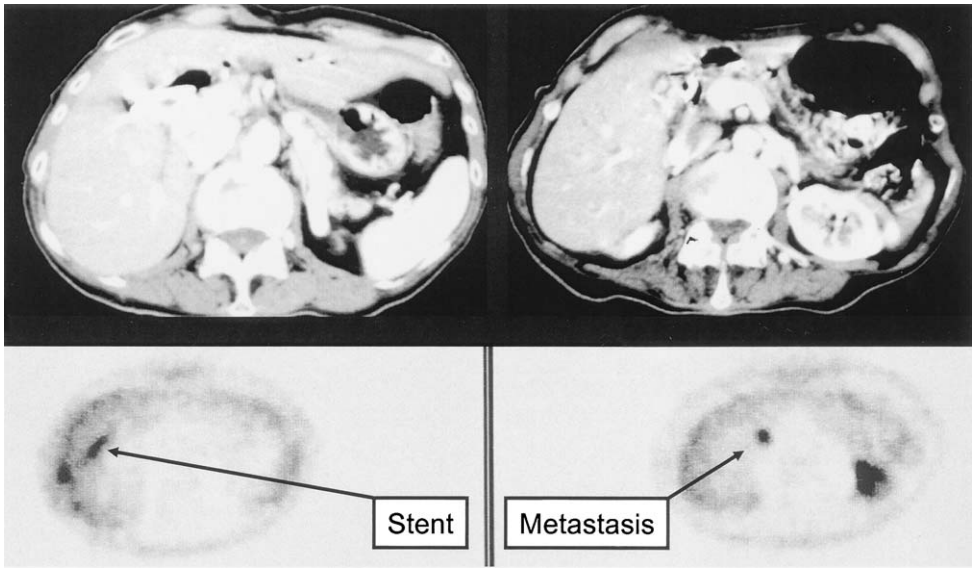

Fig. 3. PET imaging demonstrating FDG uptake corresponding to the course of a biliary stent on the CT image indicating a benign etiology from inflammation. The true intrahepatic metastasis may be distinguished from the biliary stent when FDG-PET and CT images are interpreted in conjunction with each other.

showed a laparoscopic port-site recurrence in one patient, multiple intrahepatic metastases in a second, and no disease in the third. Fig. 4 illustrates a patient with a locally recurrent gallbladder carcinoma detected by means of FDG-PET imaging.

DISCUSSION

Metabolic imaging, when used in the appropriate setting, allows a significant reduction in the utilization of costly invasive procedures for diagnosing and staging disease in patients with suspicious lesions. Currently FDG-PET imaging is approved for Medicare reimbursement for staging and restaging patients with lymphoma, melanoma, non–small cell lung, colorectal, head and neck, esophageal, and breast carcinoma.

Several other groups have investigated the use of FDG-PET in cholangiocarcinoma. It has been reported that FDG-PET has a 90% or greater sensitivity for primary cholangiocarcinoma.[16,20–22] These studies did not stratify patients based on tumor morphology, but almost all patients reported had tumors 1 cm or greater in size. Kluge et al.[22] demonstrated that PET may be useful for the detection of distant metastatic disease, but other investigators questioned its usefulness in detecting regional lymph node metastases.[23] In our study the overall sensitivity for detecting the primary tumor in cholangiocarcinoma was

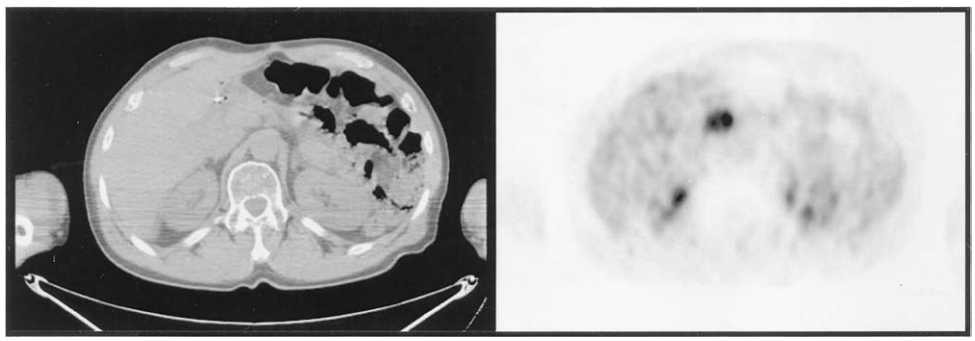

Fig. 4. FDG-PET imaging from a patient undergoing surveillance after treatment for gallbladder carcinoma. The FDG-PET images demonstrate FDG uptake corresponding to the gallbladder fossa on the corresponding CT image and indicating local recurrence.

61% (19 of 31); however, when only nodular-type lesions were evaluated, the sensitivity of FDG-PET imaging for detecting these lesions rose to 85% (17 of 20) with a positive predictive value of 94% (17 of 18). The three false negative results in the nodular group were large masses (>3cm) in the porta hepatis, two of which were unresectable because of extensive local invasion. It is unclear why these large tumors failed to accumulate FDG, but a change in tumor biology with advanced-stage gallbladder carcinoma is possible. Most of the false negative findings were in the group of patients with cholangiocarcinoma of the infiltrating-type morphology, probably secondary to the poor cellular density. This illustrates the intrinsic limitation of PET resolution in lesions less that 8 to 10 mm in diameter.

FDG-PET must be interpreted carefully in patients with primary sclerosing cholangitis, biliary stents, known granulomatous disease, or other benign inflammatory conditions that may accumulate FDG. Other investigators have reported that FDG-PET imaging may be useful in the diagnosis and management of small cholangiocarcinomas in patients with sclerosing cholangitis.[20,22] In our study, FDG-PET imaging was accurate in six of seven patients with primary sclerosing cholangitis; foci of acute cholangitis in the setting of primary sclerosing cholangitis were falsely interpreted as malignant disease in one patient. A similar true positive rate has been reported by Kluge et al.[22] Inflammatory changes along biliary stents often caused FDG uptake that was challenging to interpret. However, correlation of FDG-PET and CT images allowed accurate interpretation.

As has been demonstrated for other types of tumors such as metastatic colorectal carcinoma,[27] FDG-PET detected unsuspected metastases in 30% (11 of 36) of patients evaluated for suspected cholangiocarcinoma. These findings were biopsy proved and led to cancellation of potential resections in 10 patients. PET also detected several patients with locoregional (perihilar) lymph node involvement. Among those with false negative results for metastatic disease were patients with carcinomatosis. This has been demonstrated in other types of tumors and occurs when the peritoneal lesions are smaller than PET resolution.[28]

Because most patients with gallbladder carcinoma are diagnosed incidentally after cholecystectomy, it is uncommon that a patient with a primary gallbladder carcinoma will undergo FDG-PET imaging for diagnosis. Therefore the usefulness of FDG-PET in patients with gallbladder carcinoma is for initial staging or restaging when recurrence is suspected. In this study, the ability of FDG-PET imaging to detect both residual disease (local extension, hepatic metastases) and distant metastases was evaluated. The overall sensitivity for residual gallbladder carcinoma was 78% (7 of 9), and the specificity was 80% (4 of 5). The one false positive result was in a patient with a T2 gallbladder carcinoma detected in a cholecystectomy specimen; liver resection showed no residual tumor. This illustrates the difficulties in detecting local residual gallbladder carcinoma with PET imaging in the early postoperative period.

For detecting metastatic gallbladder carcinoma in our patients, FDG-PET had a sensitivity of only 56% (5 of 9). However, the overwhelming shortcoming of FDG-PET imaging in this regard is its inability to detect carcinomatosis, as discussed above. Despite the limitation in detecting small peritoneal metastases, FDG-PET was accurate in the three patients undergoing routine surveillance, two true positive with recurrence and one true negative. However, this small number of patients is inadequate to draw any conclusion about the role of FDG-PET imaging for routine surveillance in the setting of gallbladder carcinoma. In addition, the ability of FDG-PET to influence primary therapy is unclear. In this series, PET prevented unnecessary surgery in only one case; it failed to do so in five others.

One limitation of this study is that the FDG images were obtained with two models of PET tomographs, one of them now being outdated. The technology of dedicated PET tomographs has significantly improved over the past decade. State-of-the-art PET tomographs have superior resolution to the older tomograph used in the early part of this study. Technical developments are still evolving with better iterative reconstruction algorithms to improve the quality of images and better systems to correct of attenuation, providing better image quality and resolution. The recent development of integrated PET-CT systems provides CT and FDG-PET images obtained in a single setting allowing for co-registration of images. The fusion images provided by these systems allow the most accurate interpretation of both CT and FDG-PET studies. These technical improvements of PET imaging will undoubtedly improve the accuracy of image interpretation allowing fewer false negative results for the smaller infiltrating morphology tumors. In addition, the continued evolution of PET-CT will offer the advantage of improved anatomic localization and definition of lesions which may decrease false positive results from stents or other benign inflammatory lesions.

CONCLUSION

In this series of patients, the sensitivity of FDG-PET imaging was 85% for the detection of primary

cholangiocarcinoma of the nodular morphology (mass > 1cm), and 78% for the detection of residual gallbladder carcinoma following cholecystectomy. Because FDG-PET imaging is a whole body technique, it allows detection of unsuspected distant metastases that may lead to major changes in the surgical management of these patients. In this review, 30% of patients evaluated for suspected cholangiocarcinoma had their therapy plans altered because of detection of unsuspected metastases on FDG-PET imaging. In addition, evaluation of cholangiocarcinoma in the setting of sclerosing cholangitis is possible with FDG-PET imaging. However, FDG-PET imaging seems to have a high false negative rate for cholangiocarcinoma of the infiltrating type (mass < 1cm) and for the detection of carcinomatosis. Foci of inflammation along biliary stents or from acute cholangitis may accumulate FDG, as well as early postoperative changes after cholecystectomy and interfere with the accurate interpretation of FDG imaging for residual or recurrent tumor.

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Specific Gene Expression and Therapy for Pancreatic Cancer Using the Cytosine Deaminase Gene Directed by the Rat Insulin Promoter

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Suicide gene therapy has been shown to be an effective means of destroying pancreatic cancer cells, but cell-specific delivery of the gene is required to limit host toxicity. The objective of this study is to determine whether the rat insulin promoter (RIP) will permit cell-specific gene delivery and subsequent cell death in human pancreatic cancer cells. The RIP DNA was amplified using polymerase chain reaction (PCR), and the purified fragment was inserted into pCR-Blunt II-TOPO plasmid at the SpeI site, which contains the coding sequence of yeast cytosine deaminase (CD). Transfection assays were carried out using both RIP-lacZ and RIP-CD DNA constructs in two human pancreatic cancer cell lines, PANC-1 and MIA PaCa-2. Reporter assays using X-gal staining were performed, and the in vitro cytotoxicity was examined in RIP-CD–transfected cells treated with 5-flucytosine for 5 days. The expression levels of CD protein in the transfected cells were determined 2 days after transfection by Western blot analysis. The expression levels of insulin promoter factor (IPF-1/PDX-1) in these human pancreatic cell lines, as well as in freshly isolated human pancreatic cancer specimens, were determined using in situ immunohistochemistry analysis. After transfection with RIP-lacZ, only PANC-1 cells, but not MIA PaCa-2 cells, were positive for RIP-lacZ expression, indicating that RIP-directed reporter gene expression occurred only in PANC-1 cells. After transfection with RIP-CD and treatment with 5-flucytosine, PANC-1 cells had a significantly increased cell death rate compared with that of MIA PaCa-2 cells, suggesting that RIP-directed suicide gene expression occurred only in PANC-1 cells. Western blot analysis demonstrated that only PANC-1 cells were able to express the CD protein and that significantly increased levels of PDX-1 were found in PANC-1 but not in Mia PaCa-2 cells. In situ immunohistochemical analysis of both cell lines showed that PDX-1 was only expressed in the nuclei of PANC-1 cells and not in MIA PaCa-2 cells. Furthermore, two freshly isolated human pancreatic cancer specimens had significantly increased levels of PDX-1. The RIP is activated in PANC-1 cells, but not in Mia PaCa-2 cells, and the mechanism of activation is via PDX-1. Pancreatic cancer–specific cytotoxicity can be achieved with the use of RIP-CD and 5-flucytosine treatment in vitro. Significantly increased levels of PDX-1 have been found in human pancreatic cancer specimens. These results suggest that RIP could be used for cell-specific suicide gene therapy to target human pancreatic tumors. (J GASTROINTEST SURG 2004;8:98–108)

KEY WORDS: Pancreatic cancer, gene therapy, transcription factor, tissue specific, suicide gene

Pancreatic cancer is one of the leading causes of cancer-related death in the United States with an incidence of approximately 26,000 cases annually.[1] Currently there are no effective treatments for the overwhelming majority of patients with this disease; thus pancreatic cancer is one of few diseases with an incidence rate equal to its mortality rate.[2] The prognosis for patients with nonresectable pancreatic cancer is extremely poor because no effective alternative therapy has been established.[3] Therefore the need for novel therapeutic strategies is manifested by the dismal clinical outcome of this disease.

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Cytosine deaminase (CD) is a suicide gene that has been shown to be cytotoxic to pancreatic cancer cells. CD is an enzyme found in many bacteria and fungi but not in mammalian cells.[4,5] CD normally catalyzes the deamination of cytosine to uracil, which is necessary for DNA synthesis during the cell cycle. CD is also able to deaminate 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which is a highly toxic agent used in the adjuvant treatment of pancreatic cancer.[4,6] In earlier studies, the *E. coli* CD gene was transferred into murine pancreatic cancer cells utilizing an adenoviral vector. Cell growth was inhibited when the adenoviral vector was directly injected into the tumors followed by systemic delivery of the prodrug 5-FC.[7,8] Another study used overexpression of yeast CD combined with 5-FC resulting in cytotoxicity of pancreatic cancer cells.[9] However, results from several clinical trials have demonstrated a limited response to this suicide gene therapy/prodrug strategy, perhaps because of low levels of gene expression and low specificity.[4,10–12]

Tissue-specific gene expression can be achieved by using promoter elements of certain genes only transcribed by tumor cells. The pancreatic duodenal homeobox-1 homeodomain transcription factor (PDX-1), which activates the insulin promoter, is an essential regulator of pancreatic endocrine cell development and adult islet β-cell function.[13,14] Recently, expression of PDX-1 has been found in several human pancreatic cancer cell lines.[15] Our group has shown that the rat insulin promoter (RIP), which drives the suicidal gene thymidine kinase, results in cytotoxicity of PDX-1–positive insulinoma cells.[16] To further explore the potential of RIP-directed gene therapy for pancreatic cancer, we have generated an RIP-directed yeast cytosine deaminase (RIP-CD) DNA construct and transfected it into human pancreatic cancer cell lines to determine the cytotoxicity of the RIP-CD prodrug in pancreatic cancer cell lines.

MATERIAL AND METHODS

Cell Lines and Cytotoxicity Test

Human pancreatic cancer cell lines PANC-1 and MIA PaCa-2 were obtained from American Type Culture Collection (ATCC, Bethesda, MD). PANC-1 cells were maintained in Dulbecco's minimum essential medium (Invitrogen, Carlsbad, CA) containing 3.7 g/L of sodium bicarbonate and 10% fetal bovine serum. Antibiotics consisting of 10,000 units/ml of penicillin and 10,000 μg/ml of streptomycin also were added. MIA PaCa-2 cells were cultured in Dulbecco's minimum essential medium as described earlier and further supplemented with 2.5% horse serum. Cells were maintained in a 37C incubator with CO_2.

To determine the drug sensitivity of 5-FC and 5-FU in nontransfected cell lines, cells were plated in 96-well plates at a concentration of 5×10^3 cells/well. After 24 hours, the medium was replace with fresh medium, and cells were exposed to various concentrations of 5-FC or 5-FU (InvivoGen, San Diego, CA). Fresh medium containing the two drugs was changed daily for 5 days. After the fifth day, cell proliferation and survival rates were determined using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) (MTS) method according to the manufacturer's instruction (Promega, Madison, WI).

Plasmid Construction

The plasmid pTOPO-CD containing the CD gene from yeast was provided by Dr. M. Fujimori (Department of Surgery, Shinshu University School of Medicine, Matsumoto, Japan). Plasmid DNA was linearized using restriction enzyme *Spe* I and repaired with T4 DNA polymerase to yield blunt ends. A portion of the RIP DNA containing 508 bp of 5′-untranslated region, originally cloned in the pBlue-Script KS^+ vector, was provided by Dr. Ming-Jer Tsai (Department of Cell Biology, Baylor College of Medicine, Houston, TX). The RIP promoter DNA was amplified using the polymerase chain reaction (PCR) technique. Primers for DNA amplification were: 5′CTTGAATTCTGCTTTCCTTCTAC CT CT-3′ and 5′-TGGAGAGTACATACCTGCTTG-CTGATGGTTTCC-3′. PCR amplifications were carried out for 5 minutes at 94C, followed by 30 one-minute cycles at 94C, 60C, and 72C. A final 6-minute cycle at 72C was performed. The amplified fragment was also repaired using T4 polymerase to yield blunt ends. The repaired fragment was then inserted into the linearized vector pTOPO-CD. Limited sequencing was carried out to confirm the correct orientation and ligation.

Transient Transfection and Cytotoxicity Assays

Cells were plated into six well dishes at 200×10^3 cells per well or into 100 mm dishes at 1×10^6 cells per plate. Cells were then allowed to grow in logarithmic phase (60% to 80% confluent) for 24 hours prior to transfection. Cells were then transfected with 2 μg of DNA per well or 5 to 10 μg of DNA per 100 mm plate using lipofectamine plus reagent (Invitrogen). After 24 hours, the medium was refreshed and cells were either treated with drugs or stained for reporter genes.

Cells were then transiently transfected with pTOPO-CD or pRIP-CD, and the cytotoxicity by

5-FC treatment was determined. The day before transfection, 1×10^6 cells were plated into a 100 mm dish. Cells were then transfected with 5 μg of each plasmid. Approximately 24 hours after transfection, cells were collected using a solution containing 0.05% trypsin and 0.5% EDTA. Cells were washed and then 5×10^3 cells were plated again into 96-well plates. 5-FC was then added into the medium at various concentrations, 0, 50, 100, and 500 μg/ml, and was returned to the incubator at 37C. Culture medium was refreshed daily for 5 days, and cell survival rate was determined using the MTS method. All experiments were repeated six to nine times.

Western Blot Analysis

PANC-1 and MIA PaCa-2 cells (1×10^6/dish) were plated and transfected as described earlier. After 48 hours, cells were collected and washed three times with ice-cold, phosphate-buffered saline solution. Cell pellets were resuspended in a cell lysis buffer containing 20 mmol/L HEPES (pH 7.9), 420 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L EDTA, and 2.5% glycerol. Proteinase inhibitors were also added to the lysis solution including 1 mmol/L DTT, 1 mmol/L PMSF, 3.3 μl/ml aprotinin, 5 μg/ml leupeptin, and 5 μg/ml pepstatin. Cells were incubated in the solution at 4C for 30 minutes with occasional vortexing and then centrifuged at 13,000 rpm for 30 minutes at 4C. The supernatant was transferred to a fresh tube, and protein concentrations were determined using Bradford reagent (Sigma, St. Louis, MO). Purified bacterial cytosine deaminase was obtained from Biogenesis (Poole, England) and used as controls. Two micrograms of purified CD protein and 50 μg of cell extracts were separated through a 14% SDS-polyacrylamide gel. After blotting onto polyvinylidene fluoride (PVDF) membrane, the membrane was blocked with 5% milk in Tris-bufferd saline solution containing 0.1% Tween 20, followed by incubation with the sheep anti-yCD serum diluted 1/200 (Biogenesis). The secondary horseradish peroxidase–labeled rabbit antisheep IgG (H + L) (Bio-Rad Laboratories, Hercules, CA) was diluted 1:500, and the incubation was carried out for 1 hour. The CD protein was visualized by enhanced chemiluminescence (ECL) detection (Amersham Phamacia Biotech Corp., Piscataway, NJ). The density of each spot was analyzed using NIH image software and a scanner.

Immunohistochemistry Staining

Fresh human pancreatic cancer specimens were procured with consent at the time of operation. A portion of the specimen was fixed in 10% formalin for 24 hours and embedded in paraffin. Tissue sections were cut and slides were deparaffinized in xylene five times for 5 minutes each. Sections were hydrated gradually through graded alcohol. Slides were placed in a humidified chamber overlaid with diluted antibodies against insulin (Vector Laboratories, Burlingame, CA) and PDX-1 overnight at 4C. After washing with phosphate-buffered saline solution, sections were incubated with FITC-conjugated antirabbit IgG and Cy3-conjugated antimouse IgG for 1 hour at room temperature. Slides were washed with phosphate-buffered saline solution as before and cover slides were overlaid. Sequential observation was performed under the appropriate filter system with a fluorescence microscope, and photography was carried out.

PANC-1 and MIA PaCa-2 cells were collected as before and fixed in a 4% paraformaldehyde solution at 4C for 4 hours. Cells were pelleted and embedded in paraffin. Multiple sections were obtained, and in situ immunohistochemistry analysis was carried out using antibodies against PDX-1 and HMGI proteins (Santa Cruz Biotechnology, Inc., San Diego, CA).

Statistical Analysis

Titration and cytotoxicity tests were performed in 96-well plates. Each treatment was repeated in six wells, and six to nine separate experiments were carried out. All values are expressed as mean ± standard error (SE) of the mean. Mean values were compared by analysis of variance (ANOVA) with the Mann-Whitey U test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

5-FC and 5-FU Sensitivity and Cytotoxicity In Vitro

To determine the sensitivity of PANC-1 and MIA PaCa-2 cells to 5-FU and 5-FC, cells were placed into 96-well culture plates. Cells were treated with various concentrations of 5-FU or 5-FC, and the cytotoxicity of these drugs was determined by measuring surviving cells using the MTS method. As shown in Fig. 1, *A*, the viability of both cell types remained unchanged regardless of the dosages of 5-FC used in the assay, confirming that 5-FC is nontoxic to these cell lines. When the cell lines were treated with 5-FU, a significant decrease in cell viability was found to be proportional to the increasing amount of 5-FU used, suggesting that 5-FU is toxic to these cell lines (see Fig. 1, *B*).

Transient transfection assays with RIP-CD transgene were carried out in both cell lines as described in Material and Methods. The control plasmid pTOPO-CD, driven by a cytomegalovirus promoter,

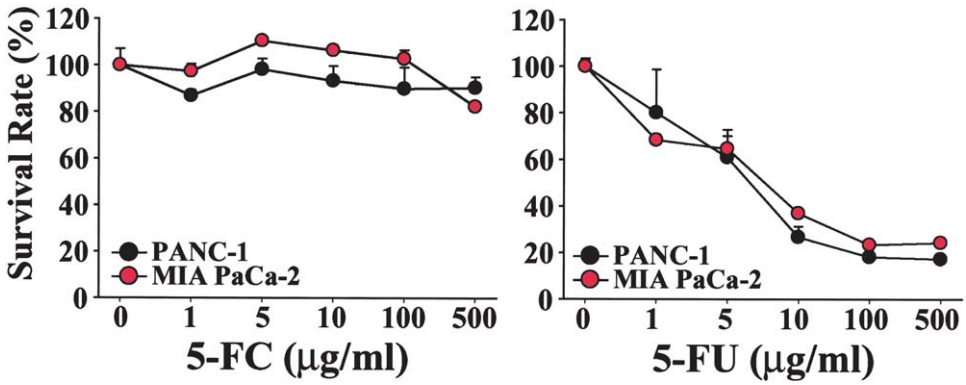

Fig. 1. Drug sensitivity of 5-FU and 5-FC in nontransfected cells. PANC-1 (●) and MIA-PaCa-2 (●) cancer cells (5×10^3 cells) were plated in 96-well plate. After 24 hours, cells were mixed in 5-FU or 5-FC containing media at various concentrations. The medium including drug was refreshed every other day. After 5 days, a viable cell number was assessed using an MTS assay. Each data point represents the mean of six samples. Six independent experiments were carried out.

was used to ensure that each cell line was adequately transfected. After transfection, cells were transferred into 96-well plates and incubated with various concentrations of 5-FC for 5 days. The cytotoxicity result of PANC-1 cells transfected with RIP-CD or pTOPO-CD and treated with 5-FC is shown in Fig. 2, *A*. The result of MIA PaCa-2 cells is shown in Fig. 2, *B*. RIP-CD transfection of PANC-1 cells treated with 5-FC resulted in similar degrees of cytotoxicity compared with that of the control plasmid pTOPO-CD. As seen in Fig. 2, *B*, the survival rate of MIA PaCa-2 cells transfected by RIP-CD was not affected by 5-FC treatment. However, when cells transfected with the control pTOPO-CD plasmid were treated with 5-FC, a significantly decreased survival rate was found.

RIP-CD Expression in PANC-1 cells

To determine whether the cell death induced by the 5-FC treatment in PANC-1 cells is mediated

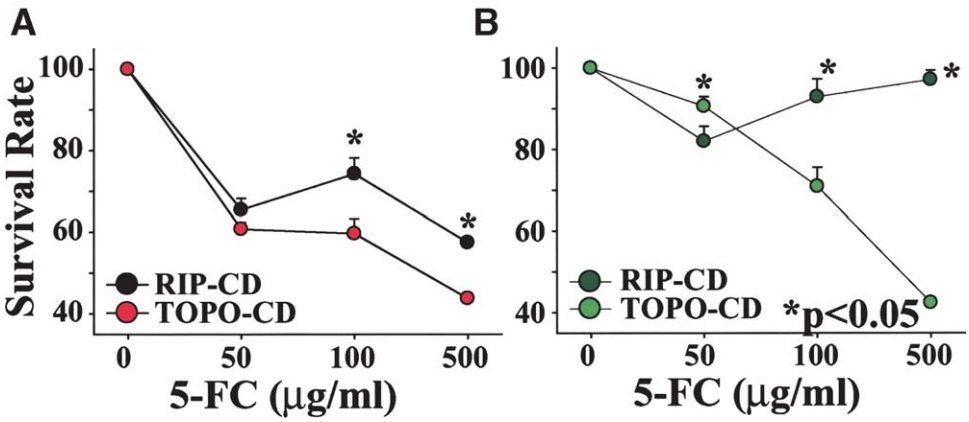

Fig. 2. Determination of cell viability as measured by MTS assay. PANC-1 and MIA PaCa-2 cells were transfected by RIP-CD or TOPO-CD and plated at 5×10^3 cells/well in 96-flat-well plates after 24 hours. Twenty-four hours later, 5-FC at various concentrations (0, 50, 100, and 500 μg/ml) were added. The medium, including drug, was changed every 2 days. After 5 days, viable cell number was assessed using an MTS assay. Shown is a representative experiment. Each data point represents a mean of six to nine times' determinations. The data expressed are relative to the proliferation of each cell at zero drug concentration. Data are shown as mean ± SE. **A**, Dose-response curve of transfection of RIP-CD and TOPO-CD gene in PANC-1 cells. ● = PANC-1/RIP-CD; ● = PANC-1/TOPO-CD. **B**, Dose-response curve of transfection into MIA PaCa-2/RIP-CD cells. ● = MIA PaCa-2 cell transfected with RIP-CD; ● = MIA PaCa-2 cells transfected with TOPO-CD. Significance of difference from the control value, $^*P < 0.05$.

by the increased expression of CD driven by RIP, Western blot analysis was used to detect the expression levels of CD in these transfected cells. As shown in Fig. 3, MIA PaCa-2 cell lysate that was transfected with pTOPO-CD plasmid produced the highest levels of CD protein, whereas pTOPO-CD–transfected PANC-1 cells also showed significantly increased levels of CD. Transfection of RIP-CD resulted in CD protein expression in PANC-1 cells, but not in MIA PaCa-2 cells (see Fig. 3, *A*). The relative expression levels of CD protein from PANC-1 and MIA PaCa-2 cells transfected with two different DNAs are shown in Fig. 3, *B*.

To determine that RIP-directed gene expression occurred only in PANC-1 cells, and not in MIA PaCa-2 cells, the reporter plasmid LacZ was used in a control experiment. Cells were plated onto a 24-well culture plate and transfected with either respiratory syncytial virus-LacZ (RSV-LacZ) or RIP-LacZ, respectively. After 48 hours, X-gal was used as a substrate to determine reporter gene expression levels. As shown in Fig. 4, transient transfection of RSV-LacZ into three human pancreatic cancer lines resulted in blue-stained cells indicating LacZ expression with a transfection efficiency of less than 10%. When RIP-LacZ was used in the transfection assay, blue-stained cells were only found in PANC-1 and CAPAN-1 cells, but not in MIA PaCa-2 cells, suggesting that RIP-directed reporter gene expression was prohibited in MIA PaCa-2 cells.

PDX-1 Expression in PANC-1 and MIA PaCa-2 cells

To determine PDX-1 expression levels in both PANC-1 and MIA PaCa-2 cells, we used in situ immunostaining methods in our study. Untreated cells were fixed and embedded. Antibodies against PDX-1 abd HMGI proteins were incubated with cell sections. FITC- and Cy3–conjugated secondary antibodies were then applied. As shown in Fig. 5, PDX-1 protein was found in both the nuclei and cytoplasm of PANC-1 cells, but not in MIA PaCa-2 cells. HMGI protein was also found in PANC-1 cells, but only in the nuclei, not in the cytoplasm. HMGI protein was not found in MIA PaCa-2 cells.

PDX-1 Expression in Human Pancreas Cancer Specimens

Because expression of PDX-1 protein in pancreatic cancer cell lines appears to be associated with RIP-directed gene activation in these cells, we were interested in determining PDX-1 expression levels in freshly isolated human pancreatic cancer specimens. As shown in Fig. 6, increased levels of PDX-1 were found in all of the pancreatic cancer specimens, including two liver metastases specimens.

DISCUSSION

Pancreatic cancer is a highly lethal disease that frequently presents at advanced stages and lacks effective systemic therapies. More innovative treatment

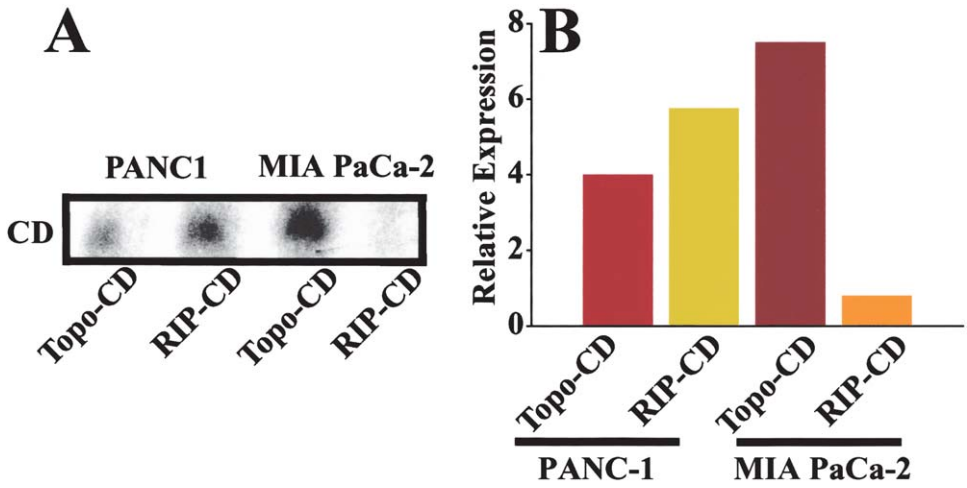

Fig. 3. A, Western blot analysis of CD protein levels. Total protein was extracted from the CD-transfected cells at 48 hours after transfection, including PANC-1/TOPO-CD, PANC-1/RIP-CD, MIA PaCa-2/TOPO-CD, and MIA PaCa-2/RIP-CD cells. Cell extracts (50 g) were resolved on a 14% SDS-PAGE gel, transferred to PVDF membrane, and incubated with sheep anti-yCD polyclonal antibody. *Arrow* indicates the presence of yCD (Mr 20,000). **B**, Relative density of the expression of CD protein. The data are expressed as percentage of control (optical density of no transfected cells).

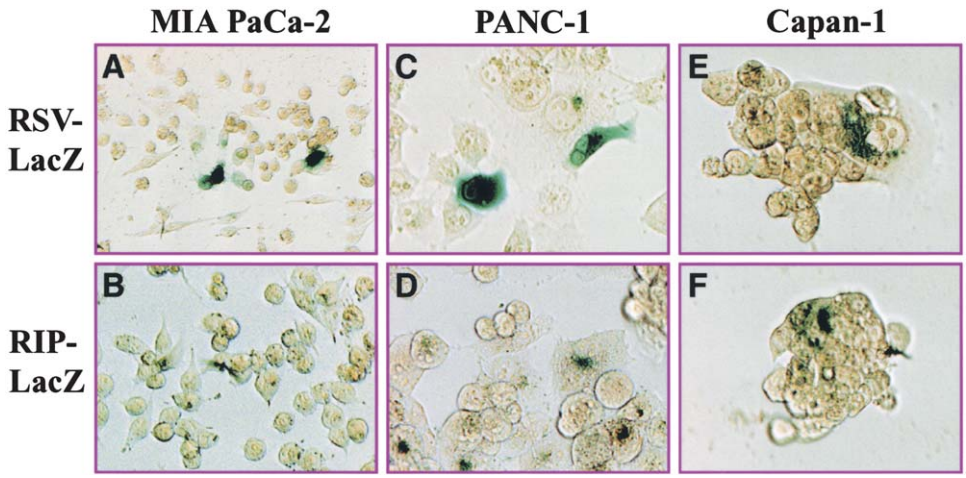

Fig. 4. Reporter gene expression. PANC-1, CAPAN-1, and MIA PaCa-2 cells were placed into 24-well cultures 24 hours prior to transfection. RSV-LacZ was transfected into MIA PaCa-2 (**A**) PANC-1 (**C**), and CAPAN-1 (**E**) cells. The LacZ protein levels were determined 48 hours later according to the manufacturer's instruction and compared with RIP-LacZ–transfected MIA PaCa-2 (**B**), PANC-1 (**D**), and CAPAN-1 (**F**) cells, respectively.

strategies are needed to improve the outcome of patients with this disease. Gene therapy allows direct treatment of tumor cells, either by modifying the malignant cells to alter their phenotype or by delivering a cytotoxic gene to the tumor. With a ubiquitous promoter, direct injection of the gene construct is possible; however, this method of gene therapy is not likely to result in a clinically effective treatment for pancreatic cancer because of the presence of metastases, which often have occurred by the time of diagnosis. A pancreatic cancer–specific promoter driving a suicide gene/prodrug would theoretically

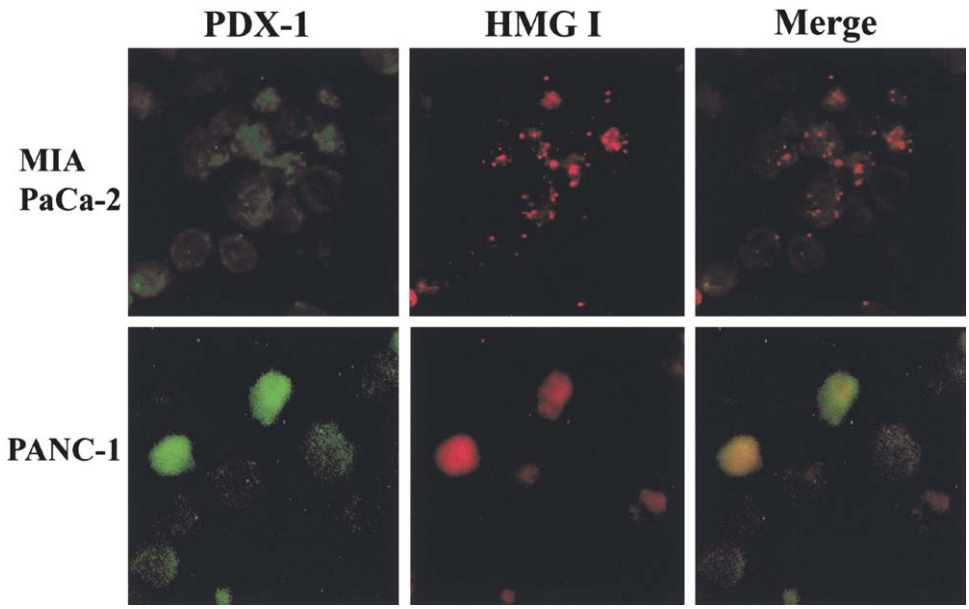

Fig. 5. PDX-1 protein expression in cell lines. Both PANC-1 and MIA PaCa-2 cells were fixed and embedded in paraffin. FITC-conjugated antirabbit IgG (H + L) and rabbit anti–PDX-1 antibodies were used to determine the location and intensity of PDX-1 proteins. Cy3–conjugated antigoat IgG (H + L) and goat antimouse HMGI protein antibodies were used to visualize HMGI protein. *Top panel* shows the result from MIA PaCa-2 cells. *Lower panel* demonstrates results from PANC-1 cells.

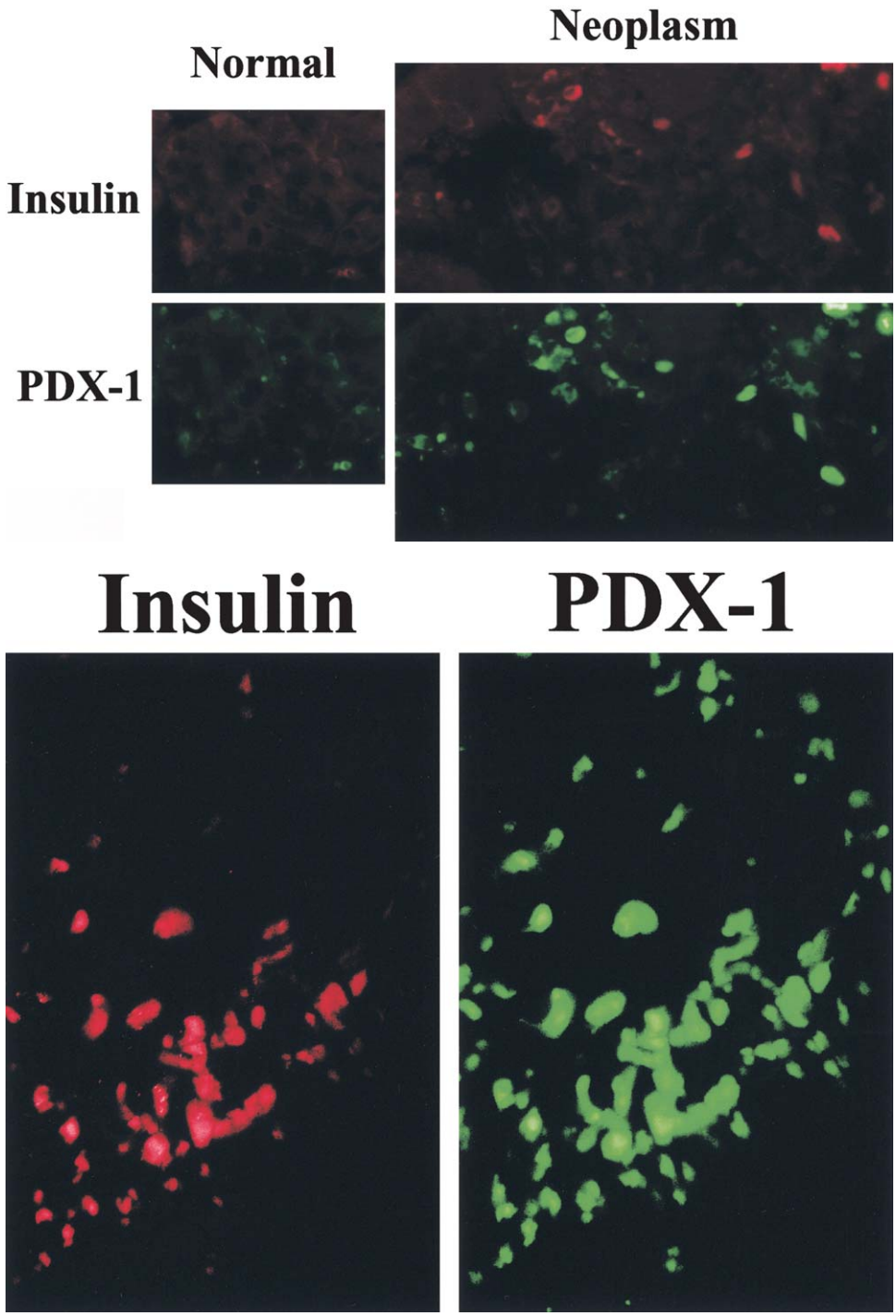

Fig. 6. PDX-1 protein expression in human specimens. Both normal pancreatic tissue and neoplasm sections were obtained with proper consent. Cy3 conjugated anti-insulin and rabbit anti–PDX-1 antibodies were used. FITC-conjugated antirabbit IgG was applied to visualize PDX-1 proteins. Dual fluorescent microscopic observation was carried out. **A**, Comparison of insulin and PDX-1 expression in normal and neoplasm sections. **B**, Enlarged view of neoplasm portion of human pancreatic cancer tissue.

result in more effective therapy for pancreatic cancer while limiting toxicity. In the present study, a pancreatic cancer cell–specific suicide gene therapy was studied in vitro.

Viruses, bacteria, and fungi often use unique metabolic pathways that are not used by mammalian cells, and contain genes for enzymes that perform metabolic conversions that mammalian cells do not

perform. Such agents are lethal or inhibitory for the infecting microbe but do not harm the host cell because it lacks the enzyme system needed to activate the drug. The efficacy of "suicide gene therapy" for cancer relies on the principle that a transfer of these microbial enzymatic genes to tumor cells can confer the same distinctive metabolic sensitivity to these agents known as prodrugs.[17] If such genetically altered cells are produced in or placed in a host, treatment of the host with the prodrug will produce toxicity confined to the altered cell and/or its microenvironment without generation of significant systemic toxicity.[18]

CD is an enzyme that is found in many bacteria and fungi but not in mammalian cells.[19–21] CD catalyzes the deamination of cytosine to uracil, and thus the conversion of 5-FC to 5-FU, which is highly toxic.[22] 5-FU is a chemotherapeutic agent that is commonly used in the treatment or palliation of various gastrointestinal tumors including pancreatic cancer. However, the efficacy of 5-FU is limited by unfavorable side effects, especially when a large dose is given systemically. Selective expression of CD within targeted tumors and combined with 5-FC administration would theoretically result in locally high concentrations of 5-FU while minimizing systemic 5-FU toxicity. However, a major limitation of the use of conventional bacterial CD is that it is inefficient in the conversion of 5-FC to 5-FU. It has been demonstrated that yeast CD has been more efficient at deaminating 5-FC to yield 5-FU; therefore in this study we used the yeast CD expression DNA as the suicide gene.[19,23] In this study, transfection of the yeast CD driven by a ubiquitous promoter with subsequent treatment with the prodrug 5-FC resulted in significant cytotoxicity of both PANC-1 and MIA PaCa-2 cell lines. These data suggest that yeast CD/5-FC is an effective suicide gene/prodrug combination for these human pancreas cancer cell lines, confirming results seen in other studies.[22]

To achieve tumor-specific suicide gene therapy, the RIP was used in this study as a pancreatic cancer–specific activator of CD. Tumor-specific therapy depends on the use of a tumor cell–specific promoter that can effectively activate the cytotoxic gene in the targeted tumor. To achieve this, activation of the cytotoxic gene construct requires the presence of promoter-activating transcription factors within the tumor, as well as the use of an effective gene delivery system. The insulin promoter activating transcriptional factor PDX-1 has been reported to be present in a number of human pancreatic cancer cell lines.[24] We have used PDX-1 to effectively drive another suicide gene, thymidine kinase, in both pancreatic cancer cell lines and insulinomas.[16,17] The mechanism of action of RIP in pancreatic cancer cell lines appears to be the unexpected presence of the transcription factor PDX-1, which is known to activate the insulin promoter. Although PDX-1 is responsible for both early embryonic pancreatic development and for insulin promoter activation in the mature β cell where it is expressed in low levels, it is normally not found outside the islet once the pancreas has matured.[13,25–27] In the mature pancreas, PDX-1 is present in β and δ cells, in a limited number of mucosal cells in the proximal duodenum, and at very low levels in pancreatic acinar cells.[28–30] The presence of high levels of PDX-1 in selected pancreatic cancer cell lines enables RIP-driven suicide gene therapy as seen in this study with RIP-CD. The presence of PDX-1 in human pancreatic cancer specimens is exciting in that it suggests that RIP could be used to target human pancreas cancer in vivo.

The cytotoxic effect of suicide genes driven by RIP to normal islet cells is of potential concern, however, because CD-mediated cytotoxicity occurs only in proliferating cells. Because mature islets of Langerhans are no longer proliferating, the impact of RIP-CD on these cells should be minimal. Our previous data showed that when TK-GCV (thymidine kinase and ganciclovir prodrug combination) was used to treat mice with insulinomas, these mice had a normal life span and had normal basal glucose levels.[16]

The current major limitation of suicide gene therapy is the delivery system. Although viruses are highly-effective systems of gene-delivery, they are known to be toxic to the host. We chose to use a nontoxic lipofectin gene delivery system to determine whether this delivery system would be effective. The transfection efficiency for PANC-1 cells using the RIP-LacZ gene and the RSV-LacZ gene in vitro was approximately 10% in this study, which is consistent with the known efficiency of this delivery system. Despite this low transfection efficiency, an approximate 50% decrease in cell survival was observed in PANC-1 cells transfected with both the RIP-CD and the TOPO-CD genes or in MIA PaCa-2 cells transfected with the control TOPO-CD gene. We believe this can be accounted for by the bystander effect resulting from the transfer of CD protein into untransfected cells. However, different mechanisms for this phenomenon have been proposed.[17,31] The bystander effect allows for cells that were not originally transfected with a CD gene to become susceptible to the cytotoxicity of 5-FC, thereby increasing the number of cells being killed. The difference in CD-protein expression levels between PANC-1 and MIA PaCa-2 cells both transfected with RIP-CD was 1.9:1, whereas the increase in cytotoxicity od PANC-1 cells was 142 times higher than that of MIA PaCa-2 cells.

In conclusion, our study has demonstrated that the CD gene, under the control of the RIP promoter and followed by 5-FC administration, can efficiently inhibit the growth of PDX-1–positive pancreatic cancer cells in vitro. The presence of PDX-1 in human pancreatic cancer specimens suggests that this strategy may have clinical relevance.

We thank Dr. C. Wright (Vanderbilt, TN) for providing the PDX-1 antibody and Dr. M. Fujimori (Shinshu University School of Medicine, Matsumoto, Japan) for providing the original pTOPO-CD plasmid used in this study. We also thank Ms. Susie Lee and Katie Elsbury for their constant assistance throughout this project.

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Discussion

Dr. L. Buscail (Toulouse, France): Do you think that besides PDX-1 some other mechanism may be applied in the absence of targeting with your insulin promoter in several cell lines such as methylation? Could your RIP contain CpG islands that might be methylated in vitro?

Dr. F. Brunicardi: The segment of the rat insulin promoter we are using has several transactivation sites, which are activated by a number of factors, including PDX-1, BETA2, E47, and other epidermal growth factor family members. We do have promoter data that were not discussed during this presentation. It is interesting to note that in addition to activating the RIP promoter. PDX-1 can also promote cell proliferation and differentiation. We have not studied RIP and CpG yet, but we do know that human insulin promoter has a large region that contains the CpG sequence.

We have conducted studies where we have mutated the RIP at specific sites, and it no longer works in human pancreatic cancer cell lines and we know the site of activation.

Prof. J. Neoptolemos (Liverpool, UK): Gene-directed enzyme prodrug therapy using a selective tumor promoter is at least 10 years old. So I am looking for something novel in your work, and you are proposing that the RIP is a selective expresser in human pancreatic cancer. I have struggled to see if you have shown anything selective about this in either human pancreatic ductal cancer relative to other pancreas cell types or other tissues.

Dr. Brunicardi: Although this concept is 10 years old, there have been no studies of gene therapy published to date that specifically target human pancreatic cancer. We have found a promoter that works effectively and specifically in human pancreatic cancer, and this is the novel finding in our study. Certainly there have been no human trials done with any tissue-specific promoters.

Prof. Neoptolemos: Sorry, but what you are saying is just not true at all. There are a number of promoters that are selected for human pancreatic cancer, some of which are actually in clinical trials at the moment, and you have not shown that your promoter is selected for human pancreatic ductal cancer tissue. You showed expression in one or possibly more cell lines, and you have not shown that it is selective in these cell lines as opposed to other pancrease cell types or other types of tissue.

Dr. Brunicardi: Once again, thank you for your comments. We have accumulated 4 years of data, both in vivo and in vitro, using the RIP and other suicide genes such as the thymidine kinase (TK). We have performed studies of tissue specificity in the RIP promoter, but these data were not presented today. A literature search performed last week did not yield any studies that have been published using tissue-specific promoters for pancreatic cancer, so we believe this is a novel finding and might help other researchers interested in targeting pancreas cancer.

Dr. S. Ashley (Boston, MA): It seems to me that this therapy might be most effective in a tumor in which the insulin promoter is believed to be activated. Is there an islet cell cancer line, particularly insulinoma, that you could look at? You would think that the insulin promoter might be particularly useful in such a tumor.

Dr. Brunicardi: That is a good question. The islet cells do express the RIP-driven transgene in mice, both in vivo and in vitro. However, when the mice receive RIP-TK followed by ganciclovir, there are no changes in blood glucose over a period of 8 months. Although the islets are transfected, we believe they are not destroyed since the islets are nonproliferating, whereas the pancreatic cancers and insulinomas are proliferating and are vulnerable to RIP-TK and the prodrug. It is exciting that we have a technique to deliver a gene into islets in vivo using the RIP.

Dr. P. Muscarella (Columbus, OH): I think this is an excellent study, and I think that molecular-targeted therapy for pancreatic cancer is really something that we need to start looking at a lot more. How do you envision delivery for the vector in humans and in what clinical setting would you use gene therapy for pancreatic cancer?

Dr. Brunicardi: That is an excellent question because it is essential that we translate these findings into a clinical trial. We would like to use a liposomal delivery system. The liposomes are nontoxic; however, they have a much lower transfection efficiency than the viruses, which is cause for concern because of their toxicity.

We have designed a clinical trial where we would give RIP-TK gene therapy to patients with resectable pancreatic cancers 1 week before their operations. They would be given the prodrug for 1 week followed by surgical resection. Specimens would be examined for the presence of the transgene, and patients would be followed for complications and length of survival to see if there were any adverse or positive effects of the gene therapy.

Invited Discussion—Expert Commentary

Sean J. Mulvihill, M.D. (Salt Lake City, UT): This elegant paper explores the concept of "suicide gene therapy" in pancreatic cancer. The authors have determined that the rat insulin promoter (RIP) permits cell specific delivery of a gene (cytosine deaminase) causing cell death in human pancreatic cancer cells exposed in vitro to 5-fluorocytosine through its deamination to the toxic metabolite, 5-fluorouracil. This effect was observed in PANC-1 but not Mia PaCa-2 pancreatic cancer cell lines. The difference appears to be due to the expression of the pancreaticoduodenal homeobox-1 homeodomain transcription factor PDX-1 in the PANC-1, but not the Mia PaCa-2 cell line. This potential therapy has a long way to go before being ready for clinical trials, but

it is an exciting area for laboratory investigation. Techniques to improve transfection efficiency, determination of the proportion of pancreatic cancers expressing PDX-1, and examination of potential deleterious effects in vivo to normal cells expressing PDX-1, such as islet cells, are a few areas of fruitful study. I hope Dr. Brunicardi's group will return with an update of this work at a future SSAT meeting.

Clinical Application of Porcine Small Intestinal Submucosa in the Management of Infected or Potentially Contaminated Abdominal Defects

Tomio Ueno, M.D., Ph.D., Lisa Clark Pickett, M.D., Sebastian G. de la Fuente, M.D., D. Curtis Lawson, M.S., Theodore N. Pappas, M.D.

The repair of abdominal wall defects in potentially contaminated or grossly infected fields presents a difficult clinical problem. Polypropylene mesh is relatively contraindicated in these settings because of the potential for chronic infection. The alternatives to polypropylene include polyglactin mesh, which is not associated with chronic infection but is associated with a 100% recurrence of hernia. The ideal prosthetic for this patient group should be resistant to infection and ensure a low rate of hernia recurrence. We studied the use of small intestinal submucosa, which has been reported to be resistant to infection and incorporates into the fascia over 3 to 6 months, in 20 patients with ventral or inguinal hernias (18 ventral, 2 inguinal hernia) in the setting of bacterial contamination. The early postoperative complication rate was 50%. One patient with fasciitis had degradation of the small intestinal submucosa and loss of the bioprosthesis within 7 days. Other early complications included seroma (n = 2), ileus (n = 1), and wound infection (n = 8). No patient experienced chronic infection. Mean follow up was 15.7 months and the rate of recurrence documented by CT or physical examination was 30%. We concluded the following: (1) small intestinal submucosa is an effective alternative bioprosthesis in the management of ventral/inguinal hernia when there is associated bacterial contamination; (2) human vs. pig immune response has not been seen in this patient population; (3) early graft failure due to overwhelming fascial infection was noted in one patient and may be a limitation of this technology in a minority of patients; and (4) early hernia recurrence is relatively low but long-term follow-up has not been completed. (J GASTROINTEST SURG 2004;8:109–112)

KEY WORDS: Small intestinal submucosa, SIS, hernia, contaminated field

Hernias are a common surgical problem that requires operative repair. Recent data suggest that the use of prosthetic material as opposed to primary repair is superior with respect to the recurrence of hernia, regardless of the size of hernia.[1,2] One of the main problems in the use of prosthetic materials, however, is infectious complications. Implantable prostheses, such as expanded polytetrafluoroethylene (PTFE) (Gore-Tex Soft Tissue Patch; W.L. Gore & Associates, Inc., Flagstaff, AZ) or polypropylene mesh, have infection rates that range from 0.5% to 7.7%.[2–5] The traditional surgical treatment for infected mesh is removal and placement of a new prosthetic mesh for abdominal wall reconstruction at a later operation.[6,7]

The repair of abdominal wall defects in potentially contaminated or infected fields presents a difficult clinical problem. Routine use of polypropylene mesh in this setting can result in chronic mesh infection.[6,8,9] Placement of permanent mesh in heavily contaminated fields has been found to result in infection rates as high as 50% to 90%, whereas the use of absorbable polyglactin mesh has an expected 100% incidence of recurrent hernia.[10–13]

Recently a new prosthetic material derived from porcine small intestinal submucosa (SIS) (Surgisis; Cook Surgical, Bloomington, IN) has become available that addresses many of the deficiencies of the previously mentioned prosthetic materials. It has been applied to ventral hernias in experimental studies, is completely absorbed in approximately 3 to 6

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months,[14–16] and has been reported to be resistant to infection.[17–20] Pig versus human immune responses have not been seen with this bioprosthetic material.

The purpose of this study was to evaluate patients who underwent the placement of SIS mesh into potentially contaminated or grossly infected fields for early outcome, potential clinical evidence of abnormal immune responses, and evidence of hernia recurrence after 6 months.

MATERIAL AND METHODS

We studied the use of SIS in 20 patients with ventral or inguinal hernia (18 ventral, 2 inguinal hernia) in the setting of bacterial contamination in a prospective, nonrandomized approach. This group consisted of 12 men and eight women whose mean age was 60.1 years. Indication for SIS use included infected ventral hernia (n = 9) with removal of infected mesh (n = 8), hernia with bowel incarceration (n = 3), hernia repair with other contaminated gastrointestinal surgery (n = 3), with concomitant cholecystitis (n = 2), with incidental enterotomy (n = 1), with fascia necrosis (n = 1), and with a takedown of enterocutaneous fistula (n = 1). Half of these procedures were performed in a potentially contaminated setting and the other half were in a grossly contaminated field (Table 1). All defects were sufficiently large to prohibit primary fascial closure. SIS bioprosthesis size varied from one sheet (7×10 cm) to three sheets depending on the area of fascial defect. Most of the surgeries were performed by two attending surgeons. Surgisis Gold (8-ply) was placed in each case, using an underlay (17 patients) or onlay (3 patients) technique. Surgisis Gold was soaked in normal saline solution for at least 8 minutes prior to use. Skin was closed primarily at the time of the surgery. Grossly contaminated wounds were closed over drains at the discretion of the surgeons (n = 11).

Wound infection was defined as an abnormal use of antibiotics for cellulitis or drainage that required wound opening. On physical examination all recurrences were symptomatic with enlarging fascial defects. Patients without symptoms and no clinical evidence of recurrence on physical examination were considered to not have recurrence and did not have abdominal/pelvic CT scans. CT scanning was used when there was a discrepancy between physical examination and symptoms. Recurrence was defined as a clear fascial separation on CT scan with eventration during Valsalva maneuver. Clinical evidence of abnormal immune responses was evaluated with the development of thrombocytopenia, bleeding disorders, or renal dysfunction.

Table 1. Entry indication, patient characteristics, and results following repair

Entry indication	Age (yr)	Sex	Early postoperative complication	Recurrence
Infected				
Infected mesh	59	M		Negative
Infected mesh	61	M		Negative
Infected mesh	47	F	Wound infection	Positive (18 mo)
Infected mesh	60	M		Negative
Infected mesh	58	M	Ileus, seroma	Positive (7 mo)
Infected mesh	44	M	Wound infection	Negative
Infected mesh	64	M	Wound infection	Negative
Infected mesh	46	F		Positive (6 mo)
Infected wound	70	F		Positive (6 mo)
Fascia necrosis	60	M	Wound infection and degradation	Positive (1 mo)
Takedown of enterocutaneous fistula	61	M		Negative
Potetially contaminated				
Small bowel injury during operation	64	M		Negative
Hernia repair with acute cholecystitis	79	M		Negative
Hernia repair with chronic cholecystitis	61	M	Wound infection	Positive (12 mo)
Incarcerated hernia	58	F		Negative
Incarcerated hernia	90	F		Negative
Incarcerated hernia	35	F	Seroma	Negative
Contaminated operative field	77	F	Wound infection	Negative
Contaminated operative field	45	M	Wound infection	Negative
Contaminated operative field	62	F	Wound infection	Negative

Numbers in paretheses indicate month when recurrence was detected.

The institutional review boards at Duke University Medical Center, Durham VA Medical Center, and Duke Durham Regional Hospital approved all aspects of this research.

RESULTS

The early postoperative complication rate related to hernia repair was 50% (see Table 1). One patient with overwhelming fascial infection had degradation of the SIS and loss of the bioprosthesis within 7 days. The patient died of congestive heart failure on postoperative day 30. Of the 20 total repairs, other early complications included seroma (n = 2), ileus (n = 1), and wound infection (n = 8). All patients with wound infection eventually healed their wounds and no patient developed chronic infection.

No patient experienced clinically apparent abnormal immune responses.

Mean follow-up was 15.7 months and 30% of patients had a documented recurrence demonstrated on CT or physical examination (see Table 1), including the patient who died. These recurrences were noted at 1, 6, 6, 7, 12, and 18 months after the operation. Five out of six patients with a recurrent hernia were operated on for a concomitant grossly infected wound.

DISCUSSION

The repair of abdominal wall defects in potentially contaminated or grossly infected fields presents a difficult clinical problem. Routine use of polypropylene mesh in this setting can result in chronic mesh infection, fistula formation, erosion into abdominal viscera or skin, repair failure, and mesh extrusion,[6,8,9] while the use of absorbable polyglactin mesh has an expected 100% incidence of recurrent hernia.[10–12] A potential alternative to prosthetic material in this clinical setting is the use of component separation for closure of ventral hernia defects. However, this may not be an attractive alternative in the setting of overt sepsis.

SIS is an acellular collagen-based matrix primarily composed by fibrillar collagens (types I, III, and V)[21] that enhance healing yet stimulate a minimal immune response.[22] The use of extracellular matrix (ECM) materials as tissue-engineered scaffolds for the repair of soft tissue structures has received considerable attention in recent years. The ECM consists of a complex mixture of structural and functional proteins and serves an important role in tissue and organ morphogenesis, maintenance of cell and tissue structure and function, and the host response to injury. Common features of ECM-associated tissue remodeling include extensive angiogenesis, recruitment of circulating progenitor cells, rapid scaffold degradation, and constructive remodeling of damaged or missing tissues. The ECM-induced remodeling response is a distinctly different phenomenon from that of scar tissue formation.[23]

Dejardin et al.[16] reported that the experimental defects were filled with a regenerated tissue that grossly and histologically resembled normal fascia in dogs and that there was no evidence of adhesions to the underlying musculature. Clarke et al.[14] also performed a clinical study in dogs and reported that the SIS implants were completely replaced by host tissue at 4 months, as determined by immunohistochemical analysis. Moreover, the resultant repair was well-organized, smooth, dense collagenous connective tissue that was well incorporated into the adjacent fascia and skeletal muscle fiber bundles. On the contrary, defects repaired with polypropylene mesh were characterized by a dense connective tissue capsule around the mesh with intermittent foci of spindle cells invading the interstices of the mesh.[14] Such large fibrotic conglomerates are more susceptible to infection.[24]

An advantage of using an SIS implant is the lack of permanent foreign material at the implant site, which decreases the risk of implant infection. Moreover, SIS has been reported to be resistant to persistent bacterial infection,[17–20] possibly because of early capillary penetration of the SIS (2 to 4 days after implantation) and delivery of body defenses to the local site.[14,20]

Franklin et al.[25] evaluated the utility of SIS in contaminated or potentially contaminated fields during ventral, incisional, or inguinal hernia repairs in humans and reported that there was no recurrent hernia in their early postoperative follow-up period in 25 patients with 15 months of median follow-up. They examined the repaired defect with a laparoscope and described that the areas of SIS repair had only minimal benign adhesions and near complete incorporation of the SIS by the surrounding tissues with abundant ingrowth of collagen material.

Immune responses between the human host and the pig graft were not observed in our study. Specifically there were no cases of immune thrombocytopenia as has been observed in the clinical use of bovine thrombin.[26] None of these patients have been reintroduced or reexposed to pig tissue in this study period so the issue of immune response upon reintroduction cannot be commented on based on these 20 patients.

We observed a recurrent hernia in 30% of our 20 patients including the patient with fasciitis who sloughed his graft and died. All patients but one with

a recurrent hernia had received operation for a concomitant grossly infected wound. The reason for the 30% early recurrence rate that was relatively high compared to the results by Franklin et al.[25] is unclear but may be related to the level of bacterial contamination in these patients. The alternatives for repair of grossly contaminated hernia wounds include primary closure under tension, component separation, polypropylene mesh, polyglactin mesh, or simple closure of skin over the defect. SIS appears favorably over these techniques because it offers a relatively low recurrence rate without chronic infection and minimal risk of early evisceration.

CONCLUSION

SIS is an effective alternative bioprosthesis in the management of ventral/inguinal hernia when there is associated bacterial contamination. We have not observed human versus pig immune response in our patient population. Early graft failure due to overwhelming fascial infection does occur but is rare. Early recurrence is relatively low but long-term follow-up has not been completed.

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Octreotide Improves Reperfusion-Induced Oxidative Injury in Acute Abdominal Hypertension in Rats

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Ischemia/reperfusion injury plays an important role in the pathogenesis of abdominal compartment syndrome, which is characterized by increased intra-abdominal pressure. The aim of this study was to investigate whether octreotide, a synthetic somatostatin analogue, improves the reperfusion injury after decompression of acute abdominal hypertension. This study was carried out in Wistar albino rats. With the rats under anesthesia, an arterial catheter was inserted intraperioneally and with the use of an aneroid manometer connected to the catheter, intra-abdominal pressure was kept at 20 mm Hg (ischemia group) for 1 hour. In the ischemia/reperfusion group, pressure applied for 1 hour was decompressed and a 1-hour reperfusion period was allowed. In another ischemia/reperfusion group, octreotide was administered (50 μg/kg intraperitoneally) immediately before the decompression of intra-abdominal pressure. At the end of the experiment, liver and intestinal tissues were taken and malondialdehyde (an index of lipid peroxidation) and glutathione (a key to antioxidant) levels and myeloperoxidase (an index of tissue neutrophil infiltration) activity were estimated. The results demonstrated that tissue levels of malondialdehyde and myeloperoxidase activity were elevated, whereas glutathione levels were reduced in both the ischemia and ischemia/reperfusion groups. Octreotide treatment reversed these oxidant responses. In conclusion, increased intra-abdominal pressure causes oxidative organ damage and octreotide, by controlling the reperfusion of abdominal organs and inhibiting neutrophil infiltration, could improve the reperfusion-induced oxidative damage. Therefore its therapeutic role as a "reperfusion injury–limiting" agent must be further elucidated in intra-aortic pressure–induced abdominal organ injury. (J GASTROINTEST SURG 2004;8:113–119)

KEY WORDS: Abdominal hypertension, octreotide, myeloperoxidase, lipid peroxidation, glutathione

Intra-abdominal pressure (IAP) may be acutely increased for a variety of reasons after major trauma or abdominal surgery.[1] When IAP reaches a level at which derangement of normal physiologic function ensues, the "abdominal compartment syndrome" is said to exist.[2] The detrimental effects on the cardiac, pulmonary, hepatic, and renal systems with raised IAP and abdominal compartment syndrome are well known and the easiest to detect clinically.[3,4] The ensuing organ dysfunction will often resolve after surgical decompression of the abdomen; however, this may then lead to complications that can cause serious additional morbidity.[5,6]

Because high venous resistance reduces blood flow to intra-abdominal organs, ischemia/reperfusion (I/R) injury plays an important role in the pathogenesis of abdominal compartment syndrome.[7] Lipid peroxidation mediated by oxygen free radicals is believed to be an important cause of destruction and damage to cell membranes, and attention has been focused on the role of reactive oxygen species in mediating the microvascular disturbances that precede organ damage induced by various chemicals and by I/R.[8,9] Besides their direct damaging effects on tissues, free radicals seem to trigger the accumulation of leukocytes in the tissue involved and thus cause tissue injury also indirectly through activated neutrophils. It has been shown that activated neutrophils secrete enzymes (e.g., myeloperoxidase, elastase, proteases) and liberate oxygen radicals.[10,11] Because the source of

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reactive oxygen metabolites could be neutrophils sequestered in systemic organs as a result of the systemic inflammatory reaction to a reperfusion insult, it is possible that agents which inhibit the activation and adherence of neutrophils might exert protective effects against reperfusion injury.[9]

Somatostatin is a tetradecapeptide that was initially isolated from sheep hypothalami and then was found to be distributed throughout virtually every organ including the gut.[12] Somatostatin has been shown to have numerous pharmacologic effects, which are mostly due to the inhibition of a wide array of physiologic functions.[13]

Somatostatin and the longer acting synthetic analogue octreotide are widely used in the treatment of metastatic neuroendocrine tumors,[14] acute pancreatitis,[15] and gastrointestinal and pancreatic fistulas.[16] The impact of somatostatin and octreotide on intestinal microcirculation has been thoroughly investigated under many pathophysiologic conditions.[12] However, their effect on IAP-induced oxidative multiorgan damage has not yet been elucidated. Therefore the experiment reported in the present study has been designed to determine whether IAP-induced oxidative intestinal and hepatic damage, as assessed by increased tissue neutrophil infiltration and lipid peroxidation, can be decreased by octreotide.

MATERIAL AND METHODS

Animals

Wistar albino rats (200 to 250 g) of both sexes were housed in an air-conditioned room with 12-hour light and dark cycles, where the temperature (22 ± 2C) and relative humidity (65% to 70%) were kept constant. All experimental protocols were approved by the Animal Care and Use Committee of Marmara University School of Medicine.

With the rats under ketamine anesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine intraperitoneally), an arterial catheter was inserted intraperioneally, and by means of an aneroid manometer connected to the catheter, IAP was maintained at 20 mm Hg for 1 hour (ischemia group). In the ischemia/reperfusion (I/R) group, elevated IAP applied for 1 hour was decompressed and a 1-hour reperfusion period was allowed. In another I/R group, octreotide (Sandostatin; Novartis International AG, Basel, Switzerland), 50 μg/kg intraperitoneally, was administered immediately before the reperfusion (decompression) period. At the end of the reperfusion period, rats were decapitated. Control rats with normal IAP were similarly anesthetized, but the arterial catheters that had been inserted were not inflated. Trunk blood was collected and the serum samples were stored at −80C for the determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Intestinal and liver tissue samples were removed for the determination of tissue malondialdehyde and glutathione levels, and myeloperoxidase activity.

Myeloperoxidase Activity

Myeloperoxidase activity was measured in ileal and hepatic tissues in a procedure similar to that documented by Hillegas et al.[17] Tissue samples were homogenized in 50 mmol/L potassium phosphate buffer (pH 6.0) and centrifuged at 41,400 *g* for 10 minutes; pellets were suspended in 50 mmol/L phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze-thaw cycles with sonication between cycles, the samples were centrifuged at 41,400 *g* for 10 minutes. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mmol/L phosphate buffer, o-dianisidine, and 20 mmol/L H_2O_2 solution. One unit of enzyme activity was defined as the amount of the myeloperoxidase present that caused a change in absorbance measured at 460 nm for 3 minutes. Results are expressed as U/g tissue.

Malondialdehyde and Glutathione Assays

Tissue samples were homogenized with ice-cold 150 mmol/L potassium chloride for determination of malondialdehyde and glutathione levels. The malondialdehyde levels were assayed for products of lipid peroxidation.[18] Results are expressed as nanomoles of malondialdehyde per gram of tissue. Glutathione was determined by the spectrophotometric method, which is based on the use of Ellman's reagent.[19] Results are expressed as a μmol glutathione/g tissue.

Hepatic Function Tests

Serum AST and ALT levels were determined to assess liver function by using AST and ALT (Roche Diagnostic, Mannheim, Germany) commercial kits in a Roche-Hitachi Modular Autoanalyzer (Roche Diagnostic).

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). All data were expressed as means ± standard error of the mean (SEM). Groups of data were compared using analysis of variance (ANOVA) followed by Tukey's multiple-comparison tests. Values of $P < 0.05$ were regarded as significant.

RESULTS

Hepatic Functions

Induction of high IAP (ischemia group) did not change the ALT and AST levels. However, when reperfusion was applied for 1 hour by decompressing the pressure, both values of hepatic function were found to be significantly increased with respect to control and ischemia groups ($P < 0.05$ and $P < 0.001$, respectively). On the other hand, octreotide treatment applied in the I/R group reduced the elevations in both ALT and AST levels ($P < 0.05$ and $P < 0.01$, respectively) and returned to control levels (Table 1).

Myeloperoxidase Activity

Elevated IAP in the ischemia group led to increased myeloperoxidase activity in liver tissue ($P < 0.05$), whereas intestinal myeloperoxidase activity was not changed. However, in the I/R group, myeloperoxidase activity was found to be significantly higher than in control and ischemia groups ($P < 0.001$). Treatment with octreotide reversed these elevations in both tissues and levels returned to control values ($P < 0.01$ to $P < 0.001$; Fig. 1).

Glutathione Levels

Glutathione levels in intestinal and liver tissues decreased significantly when IAP was elevated in the ischemia group ($P < 0.05$ and $P < 0.001$). Furthermore, these reductions in glutathione levels were more pronounced after the decompression in the I/R group ($P < 0.001$). Octreotide treatment significantly ($P < 0.05$ and $P < 0.01$) reversed these reductions of glutathione levels (Fig. 2).

Malondialdehyde Levels

Malondialdehyde levels, an index of lipid peroxidation, were significantly elevated in the intestinal tissue

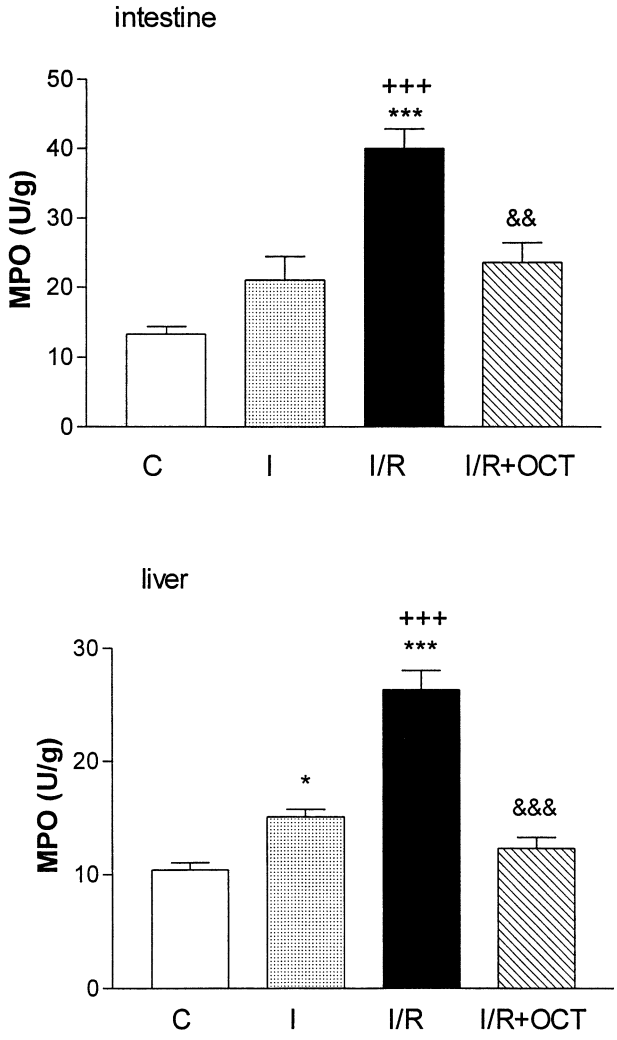
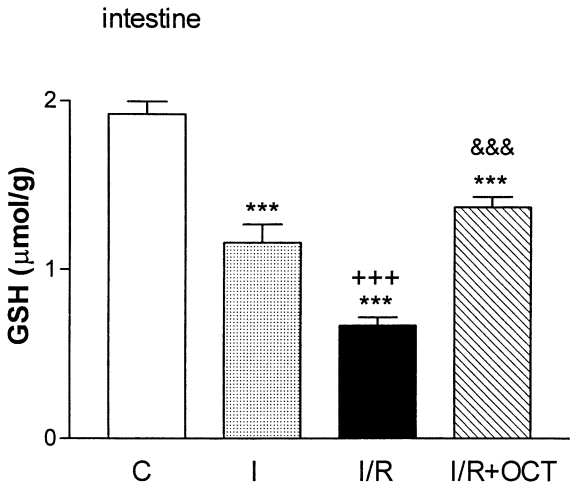


Fig. 1. Myeloperoxidase (*MPO*) activity in ischemia (*I*), ischemia/reperfusion (*I/R*), and ischemia/reperfusion plus octreotide (*I/R + OCT*)–treated groups. C = control group (rats with normal intra-aortic pressure [IAP]). I group, IAP was kept at 20 mm Hg for 1 hour; I/R group, IAP was decompressed and a 1-hour reperfusion period was allowed; I/R + OCT group, OCT (50 µg/kg intraperitoneally) was administered twice, 15 minutes before ischemia and immediately before the reperfusion period. Each group consisted of 8 rats. * = $P < 0.05$ and *** = $P < 0.001$, compared to the control group; +++ = $P < 0.001$ compared to the ischemia group; && = $P < 0.01$ and &&& = $P < 0.001$ compared to the nontreated I/R group.

Table 1. Effects of increased IAP-induced ischemia (IAP kept at 20 mm Hg for 1 hour) and I/R (IAP was decompressed and a 1 hour reperfusion period was allowed) on serum AST and ALT levels, as compared to OCT-treated (50 µg/kg intraperitoneally I/R group or control group with normal IAP

	Control (N = 8)	I Group (N = 8)	I/R (N =8)	I/R + OCT (N = 8)
AST (mg/dl)	141 ± 17	218 ± 25	295 ± 29$^{**,+}$	176 ± 6$^{\&}$
ALT (mg/dl)	69.1 ± 8.5	74.2 ± 6.1	162 ± 26$^{***,++}$	73 ± 5$^{\&\&}$

IAP = intra-abdominal pressure; I = ischemia; I/R = ischemia/reperfusion; AST = aspartate aminotransferase; ALT = alanine aminotransferase; OCT = octreodide.
$^{**}p < 0.01$, $^{***}p < 0.001$, compared to the control group;
$^{+}p < 0.05$, $^{++}p < 0.01$, compared to the ischemia group;
$^{\&}p < 0.05$, $^{\&\&}p < 0.01$, compared to the nontreated I/R group.



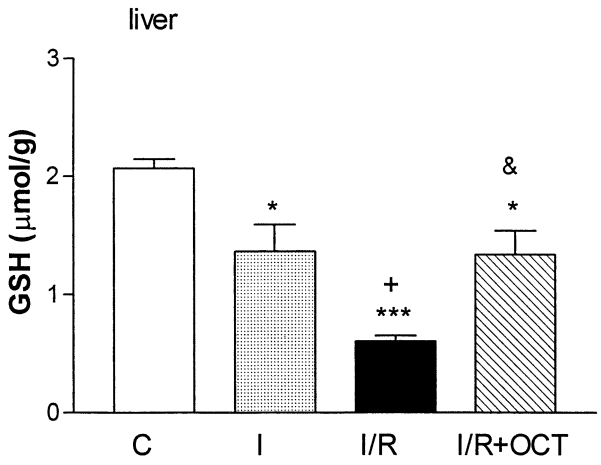
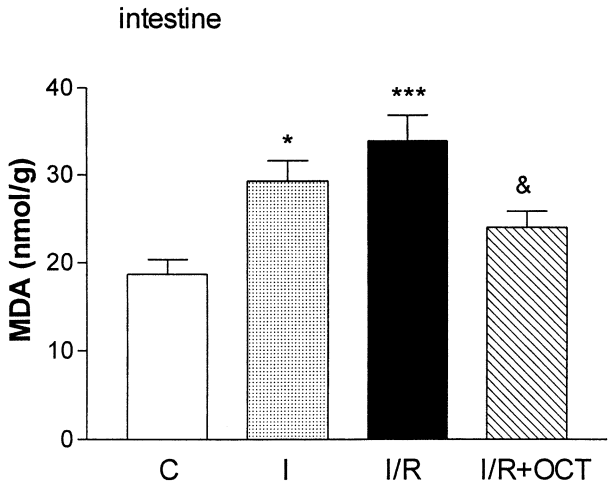


Fig. 2. Glutathione (*GSH*) levels in ischemia (*I*), ischemia/reperfusion (*I/R*), and ischemia/reperfusion plus octreotide (I/R + OCT)–treated group. C = control group (rats with normal intra-aortic pressure [IAP]). I group, IAP was kept at 20 mm Hg for 1 hour; I/R group, IAP was decompressed and a 1-hour reperfusion period was allowed; I/R + OCT group, OCT (50 μg/kg intraperitoneally) was administered twice, 15 minutes before ischemia and immediately before the reperfusion period. Each group consisted of 8 rats. * = $P < 0.05$ and *** = $P < 0.001$, compared to the control group; + = $P < 0.05$ and +++ = $P < 0.001$, compared to the ischemia group; & = $P < 0.05$ and &&& = $P < 0.001$, compared to the nontreated I/R group.



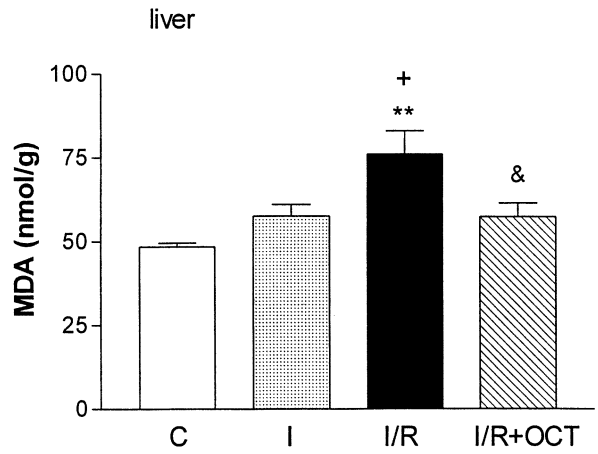


Fig. 3. Malondialdehyde (*MDA*) levels in ischemia (*I*), ischemia/reperfusion (*I/R*), and ischemia/reperfusion plus octreotide (IR + OCT)–treated groups. C = control group (rats with normal intra-aortic pressure [IAP]). I group, IAP was kept at 20 mm Hg for 1 hour; I/R group, IAP was decompressed and a 1-hour reperfusion period was allowed; I/R + OCT group, OCT (50 μg/kg intraperitoneally) was administered twice, 15 minutes before ischemia and immediately before the reperfusion period. Each group consisted of 8 rats. * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$, compared to the control group; + = $P < 0.05$, compared to the ischemia group; & = $P < 0.05$, compared to the nontreated I/R group.

($P < 0.05$) by high IAP, but remained unchanged in liver tissue. However, when the pressure was decompressed in the I/R group, malondialdehyde levels were found to be significantly elevated in both tissues ($P < 0.01$ and $P < 0.001$). Treatment with octreotide abolished the elevations in malondialdehyde levels, which did not differ from the control values ($P < 0.05$ and $P < 0.001$; Fig. 3).

DISCUSSION

The results of the present study demonstrate that oxidative tissue damage plays an important role in abdominal compartment syndrome, as assessed by increased lipid peroxidation and myeloperoxidase activity and decreased glutathione levels in the intestinal and hepatic tissues and elevated hepatic and renal

function tests. Moreover, our results also demonstrate that octreotide treatment protects against the oxidative injury due to abdominal compartment syndrome.

There are many clinical situations that can lead to increased IAP,[1] which in turn can cause fatal multiple organ failure; this is called abdominal compartment syndrome.[4] Causes of abdominal compartment syndrome include tense ascites, intestinal obstruction, large abdominal tumors. and peritoneal dialysis.[3,20] In addition, permanent gas insufflation, which is used commonly during laparoscopic surgery to provide intra-abdominal working space, elevates IAP profoundly.[21] The effects of IAP extend from pulmonary and renal dysfunction to impaired hepatic and cerebral perfusion. Moroever, intestinal ischemia, thromboembolism, and necrosis of abdominal wall muscles are reported to occur.[6] Our results support the previous findings that increased IAP leads to hepatic dysfunction, as assessed by elevated ALT levels. On the other hand, after the decompression period, increases in both ALT and AST levels indicate that reperfusion of the decompressed tissues induces a more prominent injury as compared to ischemia itself.

Reperfusion promotes generation of various reactive oxygen metabolites, which are shown to have deleterious effects on various cellular functions.[22] The organ dysfunction that accompanies this condition is associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, and parenchymal cell dysfunction and necrosis.[23] I/R elicits an acute inflammatory response characterized by activation of neutrophils. Activated neutrophils induce tissue injury through the production and release of reactive oxygen metabolites and cytotoxic proteins (e.g., proteases, myeloperoxidase, lactoferrin) into extracellular fluid.[24] Oda et al.[25] have demonstrated that abdominal compartment syndrome results in cytokine activation and polymorphonuclear neutrophil–mediated lung injury. Among several methods used to define the role of neutrophils in reperfusion tissue injury, neutrophil-specific enzyme myeloperoxidase activity was shown to increase more during the reperfusion period as compared to the ischemia period.[26,27] In the present study, myeloperoxidase activity in the intestinal and hepatic tissues of I/R groups was elevated as compared to that in control animals, and octreotide treatment significantly reversed this elevation back to control levels. These results suggest that I/R-induced oxidative damage in abdominal compartment syndrome involves the interaction of neutrophils, and the protective effects of octreotide against the organ injury are mediated, in part, by blocking tissue neutrophil infiltration.

Glutathione provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage.[28,29] Several reports indicate that tissue injury induced by various stimuli are coupled with glutathione depletion.[30] An earlier study proposed that tissue glutathione is an indicator of postischemic tissue injury.[30,31] Glutathione scavenges $O_2^{-\bullet}$ and protects protein thiol groups from oxidation. It has been reported that tissue glutathione levels and the activities of glutathione reductase and glutathione peroxidase, which are critical constituents of the glutathione-redox cycle, were significantly reduced as a result of oxidative stress.[32,33] In the present study the decrease in tissue glutathione levels after I/R is in agreement with findings in previous studies.[34–36] In octreotide-treated animals, glutathione levels showed a tendency to increase, but they were still lower than those in control animals. It appears that octreotide may help in restoring glutathione levels by inhibiting myeloperoxidase activity and subsequent lipid peroxidation. Several studies have demonstrated that I/R in the viscera is associated with lipid peroxidation: an autocatalytic mechanism leading to oxidative destruction of cellular membranes, which can lead to the production of toxic, reactive metabolites, and cell death.[36–38] Lipid peroxidation as a free radical–generating system has been suggested to be closely related to I/R-induced tissue damage, and malondialdehyde is a good indicator of the rate of lipid peroxidation. In several studies, elevated levels of lipid peroxidation products were increased from 40% to 100% above basal values after I/R.[37,38] This coincides with the results of our study showing the release of significant amounts of malondialdehyde from intestine and liver 1 hour after the abolition of IAP.[7] Our results also point out that octreotide causes a considerable inhibition of malondialdehyde production, a reduction in lipid peroxidation, and cellular injury.

Somatostatin has been recognized as a peptide that exerts a negative action on a variety of physiologic functions. However, based on the observations demonstrating that somatostatin may act as a classic endocrine hormone via the circulation, as a local regulatory factor, or as a neurotransmitter, the issue of which of its actions are physiologic and which are pharmacologic has become complicated.[12] Ferrer et al.[39] demonstrated that increased malondialdehyde levels and myeloperoxidase activities in heart and liver after intestinal I/R injury were prevented by somatostatin. The therapeutic benefits of somatostatin were also studied by Morris et al.[40] who showed that leukocyte adhesion in the areas with intermediate ischemia after intestinal I/R injury was inhibited by somatostatin.

CONCLUSION

IAP causes oxidative organ damage and octreotide, by controlling the reperfusion of abdominal organs and inhibiting neutrophil infiltration, could improve the reperfusion-induced oxidative damage. Regarding the explosive increase in the use of laparoscopic surgery that causes elevated IAP and other traumatic or surgical causes of abdominal compartment syndrome, it seems likely that octreotide has a therapeutic role as a reperfusion injury–limiting agent and must be further elucidated in IAP-induced abdominal organ injury. In this study a single dose of octreotide was administered. The effects of octreotide may be dose dependent. Further studies should be designed with different doses to reveal the effect of octreotide on I/R injury in abdominal compartment syndrome.

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Laparoscopic Splenectomy Reverses Thrombocytopenia in Patients With Hepatitis C Cirrhosis and Portal Hypertension

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Pegylated-interferon (IFN) plus ribavirin remains the most effective therapeutic regimen for patients with chronic hepatitis C infection. Thrombocytopenia is a common side effect of this treatment, often leading to discontinuation of a potentially curative therapy. We sought to determine the safety and efficacy of laparoscopic splenectomy in correcting thrombocytopenia, thus allowing completion of IFN therapy. Data were collected prospectively from September 2000 to May 2003 on all patients undergoing laparoscopic splenectomy for thrombocytopenia associated with IFN therapy and/or hepatitis C cirrhosis with portal hypertension. Demographic data, model of end-stage liver disease (MELD) score, platelet count, operative time, blood loss, spleen weight, complications, length of stay, and follow-up time were calculated. Eleven patients (7 men, 4 women) underwent laparoscopic splenectomy; their mean age was 45.4 years (range 27 to 55 years) and mean body mass index was 27 kg/m^2 (range 21 to 44 kg/m^2). All patients were Child's class A, with a mean preoperative MELD score of 9.1 (range 6 to 11). Mean operative time was 189 minutes (range 70 to 245 minutes), and blood loss averaged 141 ml (range 10 to 600 ml). A hand-assisted laparoscopic technique was used in four cases. Six patients received empiric intraoperative platelet administration. None required transfusion with packed red cells. Splenic weight averaged 1043 g (range 245 to 1650 g). Average length of stay was 2.6 days (range 1 to 6 days). Four patients had the following minor postoperative complications: self-limited atrial fibrillation (n = 1), trocar site cellulitis (n = 1), and atelectasis (n = 2). There have been no major complications over an average follow-up of 11 months (range 1 to 18 months). Mean postoperative MELD score was 8.3 (range 6 to 10). Platelet counts improved from a preoperative mean of 55,000/ul (16,000 to 88,000/μl) to 439,000/μl (200,000 to 710,000/μl) postoperatively and have remained above 100,000/μl (104,000 to 397,000/μl) during subsequent pegylated-IFN therapy. Three patients have completed a full course of IFN therapy and have obtained a sustained virologic response. Treatment is ongoing in the remaining patients. Laparoscopic splenectomy is safe in the setting of portal hypertension and thrombocytopenia associated with chronic hepatitis C infection. It can be performed with little blood loss, no need for red cell transfusion, and minimal perioperative morbidity. Laparoscopic splenectomy appears to effectively reverse thrombocytopenia and may allow these patients to safely complete IFN therapy. (J GASTROINTEST SURG 2004;8:120–126)

KEY WORDS: Laparoscopic splenectomy, hepatitis, portal hypertension, thrombocytopenia, interferon

The World Health Organization has estimated that 170 million people, or 3% of the world's population, are infected with hepatitis C virus (HCV).[1] HCV infection remains a common cause of chronic liver disease and is an increasing indication for liver transplantation. Initial treatment has typically consisted of a combination of (IFN) α-2b and ribavirin for 48 weeks.[2,3] The addition of a polyethylene glycol (pegylation) moiety to IFN improves the pharmacokinetics of this protein-based therapy allowing for a longer half-life and once-a-week dosing intervals.[4] Recent multicenter trials have demonstrated the superiority of pegylated IFN plus ribavirin compared to pegylated IFN alone or non-pegylated combination

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therapy.[5,6] Yet, patients with severe baseline thrombocytopenia may not be candidates for therapy due to the risk of exacerbation by IFN. Strict adherence to combination therapy for HCV enhances the likelihood of inducing an initial virologic response, and adherence beyond 12-24 weeks strongly benefits those with an initial response.[7] For patients with low baseline platelet counts, a full course of therapy is frequently not possible due to the development of severe IFN-induced thrombocytopenia. We sought to determine the safety and efficacy of laparoscopic splenectomy in restoring platelet counts to normal, thus allowing an uninterrupted course of IFN therapy in a select group of patients.

METHODS

Data were collected prospectively from September 2000 to May 2003 on all patients undergoing laparoscopic splenectomy for thrombocytopenia associated with IFN therapy and/or hepatitis C cirrhosis with portal hypertension. Demographic data, model of end-stage liver disease (MELD) score, platelet count, operative time, blood loss, spleen weight, complications, length of hospital stay, and follow-up time were calculated. Virologic response to IFN therapy was recorded for each patient.

Patient Selection and Preoperative Preparation

Patients considered for surgical intervention were identified by their previous inability to tolerate high-dose IFN therapy due to the development of severe thrombocytopenia (platelet count $<30{,}000/\mu l$) or deemed to be at high risk for hematologic complications due to baseline platelet counts less than 50,000/μl prior to initiating therapy. Only those with Child's class A cirrhosis without other significant medical comorbid conditions were offered splenectomy. Extensive counseling of each patient was carried out regarding the potential for perioperative morbidity, particularly bleeding complications, in the setting of cirrhosis and portal hypertension. The need for close postoperative follow-up and compliance with IFN therapy was emphasized. All were deemed to have a high likelihood of completing and responding to a full course of high-dose pegylated IFN therapy.

At least 3 weeks before surgery, each patient was vaccinated for encapsulated bacteria (pneumococcus, *H. influenza*, and meningococcus). A preoperative CT scan was performed in each patient to assess splenic size and determine the presence and extent of perisplenic varices. Prophylactic intravenous antibiotics (first-generation cephalosporin) were administered preoperatively and continued for 24 hours postoperatively.

Surgical Technique

Three left subcostal ports were used for patients managed with pure laparoscopic splenectomy. We have found the use of an additional trocar, placed posteriorly in the left flank, to be invaluable in the setting of splenomegaly and portal hypertension (Fig. 1). This port allows the assistant to elevate the enlarged spleen while the surgeon operates with two laparoscopic instruments. Patients were placed in a true lateral decubitus position. An angled (30-degree and/or 45-degree) laparoscope was used in all cases. A purely laparoscopic four-port technique was used for spleens measuring up to 20 cm in craniocaudal length or up to 19 cm in width. For spleens extending below the costal margin on preoperative CT scan or by manual palpation prior to incision, ports were positioned 4 cm below the inferior edge of the spleen, parallel to the left costal margin, but within reach of the diaphragm.

Patients with spleens measuring 20 cm or more in length in the presence of significant retroperitoneal varices were treated with a hand-assisted laparoscopic approach.[8] Patients were placed semilateral with the left flank elevated at 45 degrees. The hand-assist device was inserted through a 7 cm midline incision at the level of the lower pole of the spleen. Ports for the laparoscope and the surgeon's right hand instrument were positioned just below the tip of the spleen (Fig. 2).

Splenic attachments were divided with electrocautery and/or the ultrasonic dissector. In the majority of patients, substantial retroperitoneal varices were present, particularly along the posterior aspect of the spleen, overlying the upper pole of the kidney and the tail of the pancreas (Fig. 3). In most cases the spleen could be meticulously dissected away from its retroperitoneal attachments without injuring or dividing these varices. Once the medial and lateral attachments of the spleen were divided, the spleen was elevated away from the pancreatic tail and the hilar pedicle transected with an endoscopic linear vascular stapler. Specimens were placed into an appropriately sized impermeable retrieval bag, morcellated, and extracted. Operative time was measured from initial incision to skin closure. Splenic weight was determined by the morcellated weight of each specimen. Laparoscopic liver biopsy was performed at the conclusion of each procedure.

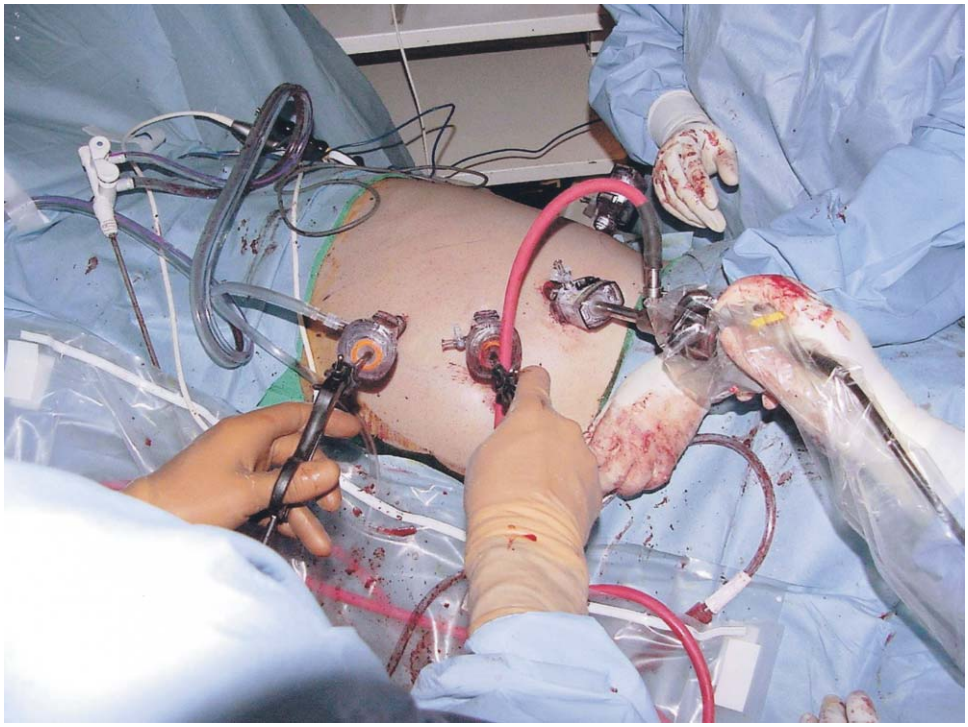

Fig. 1. Port configuration for laparoscopic splenectomy in a patient with moderate splenomegaly. Trocars are placed approximately 4 cm below the palpable tip of the spleen.

Postoperative Care

All patients were discharged when they were clinically stable, able to tolerate a regular diet, and had adequate pain control. Patients were followed in the hepatology clinic with serial platelet counts and pegylated IFN therapy resumed after full recovery from surgery (range 4 to 6 weeks). During IFN therapy, platelet counts, liver function tests and virologic responses were followed on a monthly basis. Patients were evaluated by abdominal ultrasound imaging to assess portal vein patency approximately 6 months after splenectomy.

RESULTS

Eleven patients (7 men, 4 women) underwent laparoscopic splenectomy; their mean age was 45.4 years (range 27 to 55 years) and mean BMI was 27 kg/m^2 (range 21 to 44 kg/m^2). All patients were Child's class A, with a mean model of end stage liver disease (MELD) score of 9.1 (range 6 to 11). Associated comorbid conditions included morbid obesity (n = 1) and hypertension (n = 2). Six of the 11 patients underwent splenectomy for IFN-induced thrombocytopenia, which had previously required cessation of therapy. The remaining patients presented with significantly decreased baseline platelet counts precluding initiation of IFN therapy.

A hand-assisted laparoscopic technique was used in four cases. All others were managed purely laparoscopically. Mean operative time was 189 minutes (range 70 to 245 minutes). Blood loss averaged 141 ml (range 10 to 600 ml). There were no conversions to open surgery. Six patients received empiric platelet

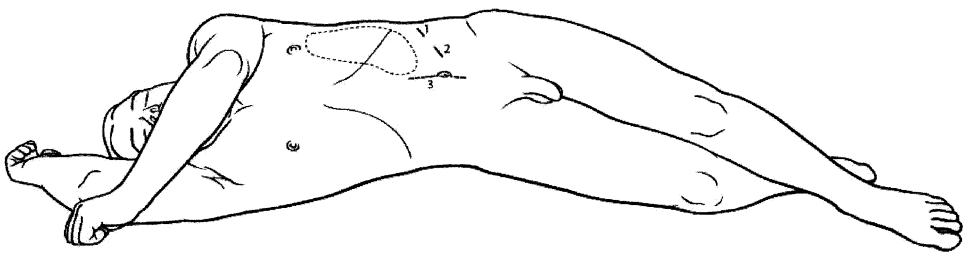

Fig. 2. Patient position and trocar placement for patients with massive splenomegaly (craniocaudal length >20 cm). The hand-assist device is oriented in the midline at the lower pole of the spleen.

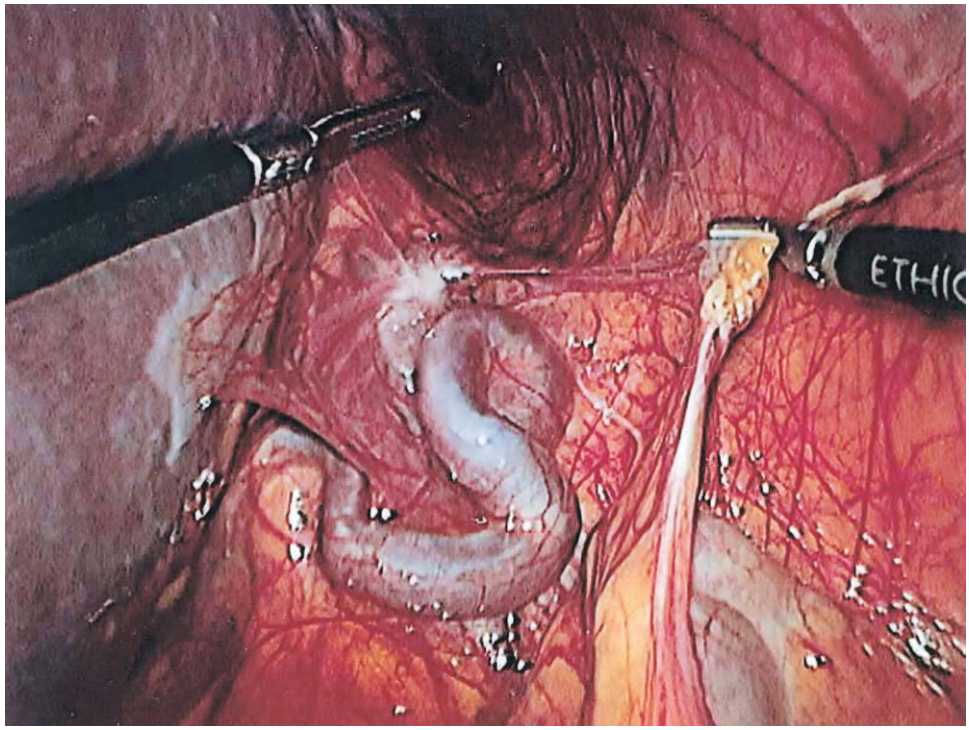

Fig. 3. Intraoperative photo of a large retroperitoneal varix involving the splenorenal ligament. The lower pole of the spleen (medial) and the splenic flexure of the colon (lateral) are adjacent to the enlarged vessel.

transfusion after splenic hilar transection. None required transfusion with packed red cells. Mean splenic weight was 1043 g (range 245 to 1650 g). Average length of stay was 2.6 days (range 1 to 6 days). Four patients had the following minor postoperative complications: self-limited atrial fibrillation (n = 1), trocar site cellulitis (n = 1), and atelectasis (n = 2). Histologic evaluation of spleen specimens universally revealed congestive splenomegaly. Ten of 11 liver biopsies demonstrated histologic evidence of cirrhosis.

Over a mean follow-up period of 11 months (range 1 to 18 months), there have been no major complications. Follow-up abdominal ultrasound imaging has been performed in 9 of 11 patients to date. One patient has radiographic evidence of portal vein thrombosis but is clinically asymptomatic. None have developed ascites postoperatively. MELD scores have been unchanged with a postoperative mean of 8.3 (range 6 to 10). Platelet counts improved from a preoperative mean of 55,000/μl (16,000 to 88,000/μl) to 439,000/μl (range 200,000 to 710,000/μl) postoperatively (Table 1).

Pegylated IFN therapy has been initiated in all patients. Platelet counts have remained greater than 100,000//μl (range 104,000 to 397,000) during treatment. Three patients have completed a full course of pegylated IFN and ribavirin therapy, and have achieved sustained virologic remission. All have undetectable (<26 c/ml) viral counts more than 6 months after treatment. The remaining patients are currently receiving IFN therapy. With the exception of one patient, all have demonstrated an initial virologic response. None have experienced recurrent thrombocytopenia.

DISCUSSION

Thrombocytopenia is a common manifestation of chronic hepatic disease, although the exact mechanisms remain incompletely understood. Portal hypertension from cirrhosis diverts the flow of blood from the portal circulation toward the enlarged spleen, resulting in hypersplenism and platelet sequestration. In healthy patients, 45% of platelet destruction occurs in the spleen.[9] Studies utilizing radiolabeled platelets in patients with chronic liver disease demonstrate an accelerated destruction of platelets by the spleen, which directly correlates with splenic volume.[10]

In addition to a low baseline platelet count in some patients with chronic HCV infection, the use of IFN to treat HCV can exacerbate the thrombocytopenia.

Table 1. Patient demographics, operative variables, and response to interferon therapy

Patient	Age (yr)	Preoperative/Postoperative platelet count μl)	Procedure	EBL (ml)	OR time (min)	IFN response
1	48	50,000/210,000	Laparoscopic	50	177	Sustained response
2	50	41,000/702,000	HALS	150	219	Sustained response
3	49	63,000/413,000	HALS	100	240	In treatment
4	46	70,000/459,000	Laparoscopic	100	228	In treatment
5	44	35,000/492,000	Laparoscopic	50	226	In treatment
6	48	88,000/462,000	Laparoscopic	50	135	Failure to respond
7	55	87,000/320,000	Laparoscopic	200	180	Sustained response
8	45	55,000/200,000	HALS	150	245	In treatment
9	27	16,000/710,000	Laparoscopic	10	70	In treatment
10	48	42,000/420,000	HALS	600	195	In treatment
11	39	62,000/464,000	Laparoscopic	100	166	In treatment
Mean	45	55,000/439,000	N/A	141	189	N/A

HALS = hand-assisted laparoscopic splenectomy; OR = operating room; EBL = estimated blood loss; IFN = interferon.

Several mechanisms for this side effect have been described. The most common reason for a drop in platelet count is IFN-induced pancytopenia resulting from bone marrow suppression and the direct inhibitory effect of IFN on megakaryocytes. Platelet counts may decrease by as much as 25% to 50% from baseline.[11] This effect is dose dependent, usually occurs within the first few weeks of IFN therapy, and leads to dose reductions or withdrawal from therapy in up to 25% of patients.[12–14]

Thrombocytopenia carries a risk of bleeding complications including hematuria, hematemesis, melena, and intracranial hemorrhage. The risk of spontaneous bleeding varies with platelet count and the individual patient's clinical risk factors for hemorrhage. Previous reports have demonstrated a dramatic increase in the frequency and severity of hemorrhage in patients with platelet counts below $20 \times 10^9/\mu l$.[15] As a result, numerous surgical procedures have been proposed to correct cirrhosis-associated thrombocytopenia. Early attempts focused on the diversion of portal flow from the spleen through portocaval anastomoses. Although these interventions were effective in decreasing splenomegaly, they did not correct thrombocytopenia in cirrhotic patients with hypersplenism.[16] Other forms of portal decompression such as transjugular intrahepatic portosystemic shunting (TIPS) have also been unsuccessful.[17] Selective shunting procedures such as the distal splenorenal shunt have demonstrated success in both resolving the thrombocytopenia associated with cirrhosis as well as reducing the incidence of variceal hemorrhage.[18,19]

Splenectomy has also been shown to correct the manifestations of hypersplenism in patients with cirrhosis.[19,20] Hashizume et al.[21] reported on 73 patients with portal hypertension who underwent splenectomy. In their series, laparoscopic splenectomies were performed to treat spontaneous bleeding complications from severe thrombocytopenia (n = 40) to correct the thrombocytopenia in those undergoing surgical treatment of hepatocellular carcinoma (n = 18), and to treat esophagogastric varices refractory to endoscopic therapy (n = 15). The investigators concluded that portal hypertension was not a contraindication to laparoscopic splenectomy and that thrombocytopenia markedly improved in all but one patient. There also appeared to be a correlation between splenic size and the degree of improvement in postoperative platelet count.

The data from this study provided the basis for the treatment plan in our patients. The primary objective of our study was to determine the safety and efficacy of laparoscopic splenectomy in alleviating thrombocytopenia in chronic HCV-infected patients, thereby allowing completion of antiviral therapy. Laparoscopic splenectomy has become the standard of care for most elective indications such as idiopathic thrombocytopenic purpura, hematologic malignancy, and abscess.[22–24] We were able to successfully employ laparoscopic splenectomy for the correction of thrombocytopenia in patients with HCV, cirrhosis, and portal hypertension. The mean postoperative platelet count after splenectomy was more than six-times the preoperative mean and has been maintained for up to 18 months after splenectomy. The magnitude and durability of platelet response has allowed our patients to undergo high-dose IFN therapy without interruption due to thrombocytopenia.

It is important to note that there were no major perioperative complications and no conversions to

open surgery in this high-risk group of patients. In addition to close monitoring of our patients for early postoperative hepatic decompensation and bleeding, all have been prospectively followed for the development of long-term postsplenectomy complications. Of primary concern, portal vein thrombosis (PVT) is a major potential complication of splenectomy and has been reported in up to 8% to 10% of patients treated with open surgery.[25–27] Although PVT can develop in any patient, it occurs most commonly in patients undergoing splenectomy for hematologic malignancy. In the only published series evaluating laparoscopic splenectomy in patients with portal hypertension, the incidence of PVT was 4%.[21] In the setting of HCV cirrhosis and portal hypertension, PVT could potentially render the patient unfit for liver transplantation should the need arise. We have been vigilant for this complication in our patients and have therefore included follow-up abdominal ultrasound examination as part of our postoperative treatment algorithm.

From a technical standpoint, laparoscopic splenectomy in patients with portal hypertension, thrombocytopenia, and splenomegaly is extremely challenging. Although there have been no intraoperative complications in our small series, it should be emphasized that our patients were highly selected and all were Child's class A, with few other medical comorbidities. Despite this, the risk for perioperative morbidity and even mortality is substantial in patients with portal hypertension and thrombocytopenia, particulary those with advanced liver disease and splenomegaly. Extreme caution must be used when manipulating the massively enlarged spleen laparoscopically. Rupture of the splenic capsule or disruption of perisplenic varices carries the risk of substantial hemorrhage. We believe that the use of a hand-assisted approach in select patients can decrease the likelihood of bleeding complications and may decrease operative time.[8] Regardless of the approach, an extensive experience with laparoscopic and hand-assisted splenectomy is essential prior to undertaking this procedure in patients with portal hypertension.

The major limitations of our study are its small size and relatively short duration of follow-up. The primary objectives of this pilot study were to assess the technical feasibility of laparoscopic splenectomy, along with the magnitude and durability of platelet response, and to evaluate for early perioperative complications. Although laparoscopic splenectomy does appear to be safe and effective in correcting thrombocytopenia in a highly selected group of patients with HCV cirrhosis and portal hypertension, it is not possible to draw firm conclusions regarding the widespread applicability of this technique. It should be noted that the patients included in this study represent only a small fraction (<2%) of patients with chronic HCV who are managed in our practice. Despite the fact that mild baseline thrombocytopenia is common in this patient population, the vast majority of patients are able to tolerate IFN therapy without interruption due to hematologic side effects. Splenectomy is considered only for those patients who have demonstrated an inability to tolerate therapy or are deemed at high risk because of severe preexisting thrombocytopenia.

CONCLUSION

Although controversial, laparoscopic splenectomy appears to offer a safe and effective method for reversing thrombocytopenia in a highly select group of patients with chronic hepatitis C infection. In our experience, surgical intervention has been associated with low complication rates and short hospital stays. Laparoscopic splenectomy has resulted in sustained normalization of platelet counts and has thereby allowed these patients to successfully undergo antiviral therapy without recurrent thrombocytopenia. At this point it is too early to draw any conclusions regarding the long-term ability of this treatment algorithm to facilitate sustained virologic response or halt the progression of liver disease due to chronic HCV infection.

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Discussion

Dr. J. Fischer (Boston, MA): About how many patients do you think this represents of your entire group of HCV-positive patients?

Dr. K. Kercher: It represents about 2% of all patients. Up to 50% of patients do present with some baseline thrombocytopenia. Only a small number have platelet counts low enough that they are not able to tolerate IFN therapy.

Dr. W. Traverso (Seattle, WA): After the great results from your group at the Carolinas Medical Center, would you consider expanding the indications for splenectomy before IFN treatment in these patients?

Dr. Kercher: As I noted, actually 5 of these 11 patients did not receive IFN therapy before proceeding to splenectomy. All of these patients had very low baseline platelet counts, on the order of 30,000, and our hepatologists thought that they were too high risk for any attempt to initiate therapy, and therefore we proceeded with splenectomy before any trial of interferon.

Our plan is to proceed with a more prospective and randomized trial in which we randomize patients to either IFN therapy and then cross over to splenectomy if they do not tolerate it or to splenectomy up front.

Bovine Pericardium Buttress Limits Recanalization of the Uncut Roux-en-Y in a Porcine Model

John M. Morton, M.D., M.P.H., Tananchai A Lucktong, M.D., Scott Trasti, D.V.M., Timothy M. Farrell, M.D.

In contrast to the traditional Roux-en-Y reconstruction, an uncut Roux-en-Y provides biliopancreatic diversion and may preserve myoelectric continuity. Previous iterations of the uncut Roux have been plagued by recanalization of the uncut staple line in the afferent small bowel. Our aim was to determine if bovine pericardium buttress prevents recanalization of the stapled small bowel partition in a porcine model. Sixteen female pigs (~30 kg) underwent a side-to-side stapled jejunojejunostomy, 20 cm distal to the ligament of Treitz, with placement of a nondivided stapled partition with a single row of 2.5 mm width staples in the intervening jejunal loop. Nine animals in the experimental group had a bovine pericardium buttressed staple line (5 permanent, 4 absorbable), whereas seven animals in the control group had a nonbuttressed staple line. At 6 or 12 weeks, necropsy was performed and the primary outcome, staple line recanalization, was assessed grossly and histologically. Statistical analysis was performed by means of the chi-square test. There were no major complications and all animals gained weight. Overall, eight of nine bovine pericardium buttressed staple lines were grossly and histologically intact at necropsy, whereas all nonbuttressed uncut staple lines had recanalized completely ($P < 0.05$). At 6 weeks, both permanent (N = 4) and absorbable (N = 3) buttress preparations prevented recanalization. At 12 weeks the permanent buttress remained closed (N = 1), but the absorbable buttress had allowed partial recanalization (N = 1). The use of bovine pericardium buttress will prevent small bowel recanalization of uncut small bowel staple lines at early follow-up. Pilot data at intermediate follow-up suggest permanent buttress is more durable than absorbable buttress. These results warrant investigation of bovine pericardium for intestinal applications in humans. (J GASTROINTEST SURG 2004;8:127–131)

KEY WORDS: Uncut Roux-en-Y, bovine pericardium

Roux-en-Y reconstruction (Fig. 1) is used as a remedial operation for postgastrectomy complications such as bile gastritis, early satiety, postvagotomy diarrhea, dumping, gastric atony, and afferent/efferent limb obstruction. However, the traditional Roux-en-Y may impair gastric emptying and create aberrant peristalsis in the Roux limb.[1,2] The so-called "Roux stasis syndrome," which is marked by postprandial nausea, emesis, and abdominal pain, has been attributed to myoelectric discontinuity between the duodenal pacemaker and ectopic pacemakers, with secondary jejunal pacemakers creating orad-propagating contractions in the Roux limb.[3]

The uncut Roux-en-Y reconstruction (Fig. 2) has been proposed as a means to maintain the benefits of diversion of biliopancreatic secretions while preserving myoelectric continuity between the duodenal pacemaker and the segment of jejunum serving as the Roux limb.[4,5] The uncut Roux-en-Y compares favorably with traditional Roux-en-Y with regard to

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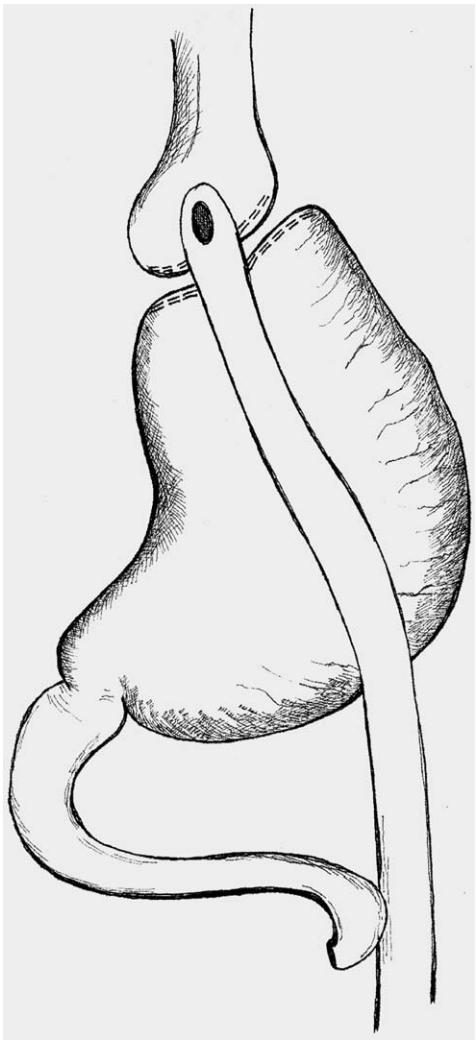

Fig. 1. Standard Roux-en-Y.

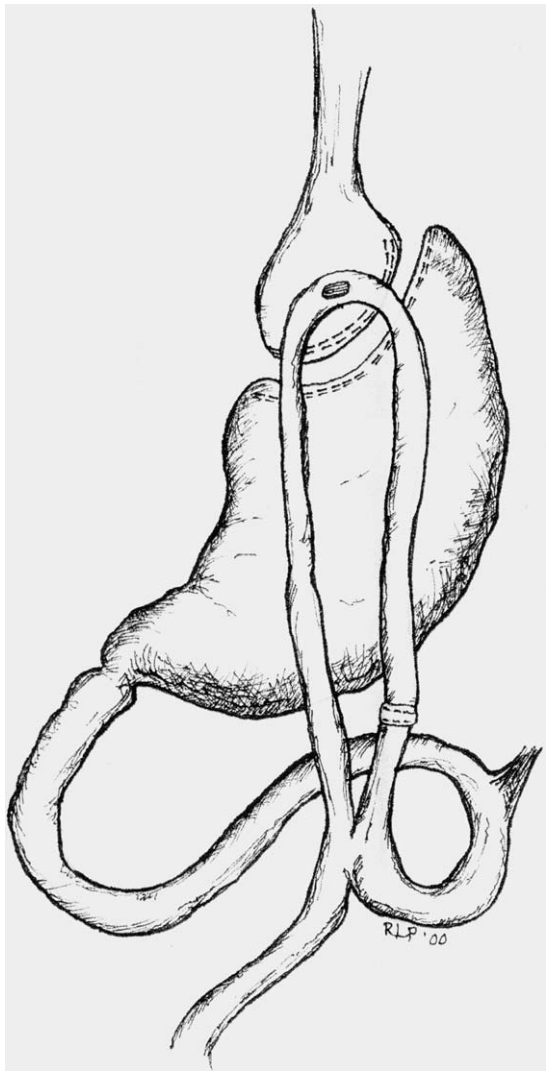

Fig. 2. Uncut Roux-en-Y.

early postoperative morbidity and symptomatic improvement.[6,7] However, efforts to employ the uncut Roux-en-Y have been plagued by recanalization of the small bowel partition despite multiple modifications in the technique.[8–10]

Polytetrafluoroethylene (PTFE) buttress has been used as a means to prevent recanalization in a pig model[11]; however, application in humans is limited by concerns for infection or erosion of the PTFE. The aim of this study was to determine whether the placement of bovine pericardium buttress would prevent recanalization of the afferent small bowel.

METHODS

The University of North Carolina Institutional Animal Care and Use Committee approved the protocol for surgical procedures and postoperative care. Sixteen domestic pigs were used (25 to 35 kg), with nine serving as experimental animals and seven as controls.

Animals were sedated with ketamine at a dosage of 22 to 30 mg/kg intramuscularly, and general anesthesia was maintained with 1.5% to 2.0% isoflurane and oxygen. A short upper midline laparotomy was made, and the ligament of Treitz was identified. Twenty centimeters distal to the ligament of Treitz, a side-to-side jejunojejunostomy was fashioned using a linear stapler (ETS Flex 45, single row, 2.5 mm width; Ethicon Endosurgery, Cincinnati, OH) to create a common channel, and 3-0 vicryl and 3-0 silk sutures were used to close the remaining enterotomy in two layers. To create the experimental staple line, a noncutting linear stapler (EZ 45 No-Knife, single row, 2.5 mm width; Ethicon Endosurgery) was fired in the proximal aspect of the intervening jejunal loop. In

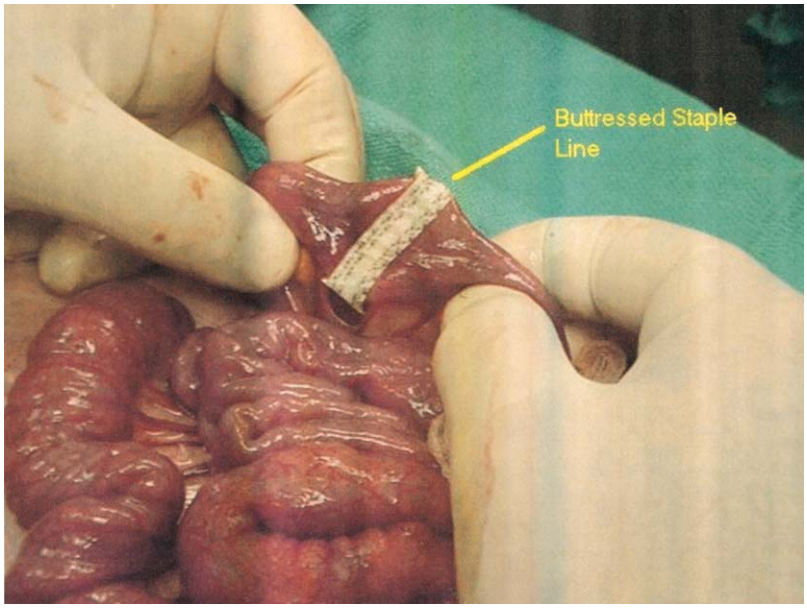

Fig. 3. Bovine pericardium buttress was positioned within both sides of the staple line resulting in an extraserosal "sandwich" on firing.

the seven control animals, no buttress material was applied. In the nine experimental animals, bovine pericardium buttress was positioned within both sides of the staple line resulting in an extraserosal "sandwich" on firing (Fig. 3). Five experimental animals received permanent bovine pericardium buttress consisting of Peri-Strips Dry (Bio-Vascular, Inc., Saint Paul, MN), and four animals received absorbable bovine pericardium buttress consisting of Veritas (Bio-Vascular, Inc.). In all cases, buttress material extending beyond the bowel wall was trimmed flush. The abdomen was closed with a running No. 1 polydioxanone suture.

At 6 weeks, necropsies were performed in seven experimental and five control animals. Two experimental and two control animals were allowed to survive 12 weeks to provide pilot intermediate data to guide future experiments. In each case, adhesion formation was assessed grossly, and the operative site was harvested. The stapled partition was assessed for recanalization grossly by opening the jejunal loop above and below the partition site and inspecting the area for patency. Additionally, histologic sections were prepared and evaluated by a blinded veterinary pathologist for evaluation of tissue ingrowth and inflammatory cell infiltration.

Regarding our primary outcome of staple line recanalization, statistical analysis was performed using the chi-square test, with $P < 0.05$ set as the level of significance.

RESULTS

All animals tolerated the procedure well and resumed a normal diet within 24 hours. No complications required intervention before necropsy. All animals gained weight as expected.

Serosal adhesions in both experimental and control groups were minimal. No differences were noted in luminal appearance. Staple line results are presented in Table 1. At 6 weeks, all control animals (N = 5) demonstrated complete recanalization of the staple line, whereas all buttressed staple lines (4 Peri-Strips, 3 Veritas) remained intact. Comparison of experimental and control groups at 6 weeks revealed a significant difference in our primary outcome of staple line recanalization, ($P < 0.05$, χ^2).

Histologic examination of the Peri-Strips–buttressed staple lines confirmed absence of recanalization and demonstrated minimal to moderate tissue reaction. In the Veritas-reinforced staple lines, tissue reaction was difficult to identify, and there appeared to be evidence of tissue ingrowth into the Veritas collagen matrix (Fig. 4).

Table 1. Staple line integrity

Group	6 wk	12 wk
Control (n = 7)	5 open	2 open
Experimental (n = 9)	7 closed	1 closed, 1 open
P value	<0.05	N/A*

*Twelve-week data are observational, given the small numbers; N/A = not applicable.

In the animals followed for 12 weeks, both control staple lines were completely open, and the Veritas-buttressed staple line had partially recanalized, whereas the Peri-Strips staple line remained closed (Fig. 5). No statistical analyses were attempted at the intermediate time point because of the small number of animals.

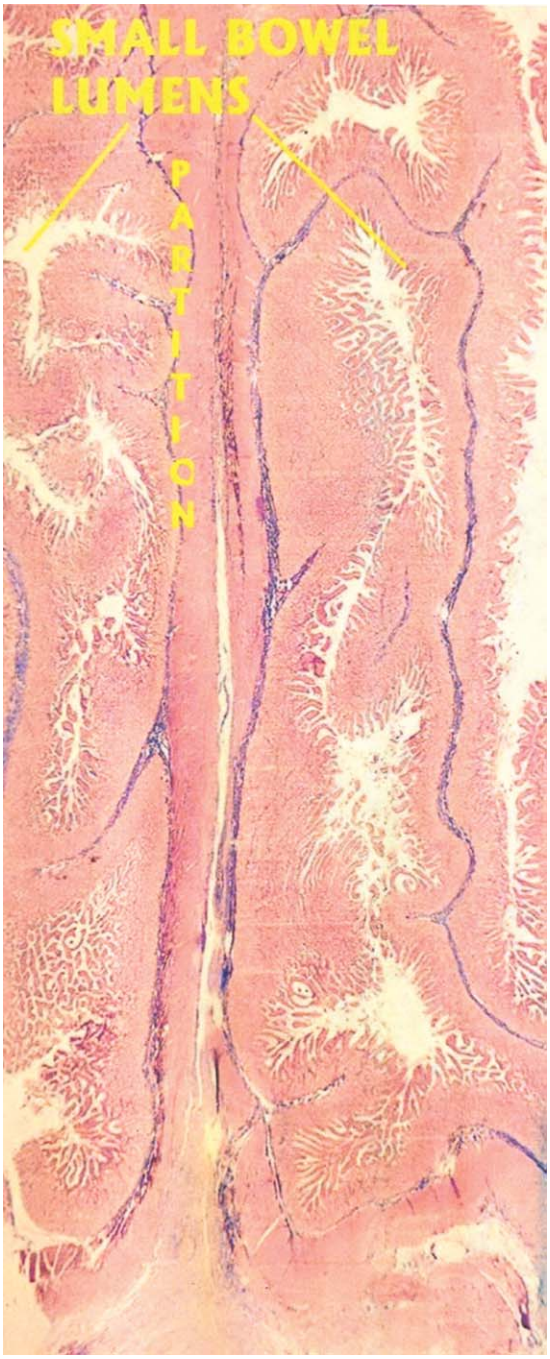

Fig. 4. Microscopic appearance of a representative staple line buttressed with absorbable bovine pericardium (Veritas, Bio-Vascular, Inc., Saint Paul, MN) shows evidence of tissue ingrowth with minimal tissue reaction.

DISCUSSION

The uncut Roux-en-Y may have advantages over traditional Roux-en-Y reconstruction because myoelectric continuity is maintained and Roux stasis is less likely, but experience has shown that the uncut Roux limb partition may dehisce. In this study, early (6 weeks) recanalization was prevented by the use of either permanent or absorbable bovine pericardium as a staple line buttress. Although intermediate (12 weeks) follow-up does not allow statistical comparison, the occurrence of partition failure in the staple line buttressed with absorbable material implies that the durability of the partition may vary with the persistence of the prosthetic material.

If results of longer-duration trials with permanent buttress material in animals and humans are comparable to these early studies in animals, the uncut Roux reconstruction may be applied in postgastrectomy syndromes and other clinical situations where the Roux-en-Y has proven efficacy, with the potential benefit of reducing the likelihood of Roux stasis. In addition, the uncut Roux procedure may be ideally suited for certain laparoscopic operations, such as

Fig. 5. Staple line buttressed with permanent bovine pericardium (Peri-Strips) at 12 weeks.

gastric bypass for morbid obesity, where an antecolic, ante-gastric Roux limb will be more easily fashioned with reduced risk for certain internal hernias.

Other potential enteral applications for permanent bovine pericardium buttress include use on the stomach in gastric partitioning procedures such as vertical banded gastroplasty and nonisolated gastric bypass, where high rates of staple line dehiscence in the past have inspired evolution toward divided staple lines despite the higher risk of gastric leakage.

The absorbable bovine pericardium product tested (Veritas), which has a very low tissue profile, is a non–cross-linked bovine pericardium product that is minimally immunogenic. As it is absorbed, Veritas is apt to have tissue ingrowth. Veritas bovine pericardium has been employed successfully for urologic slings but not in gastrointestinal applications. If further studies confirm its tendency to maintain staple line integrity at 6 weeks and its ability to be reabsorbed and replaced by native tissue by 12 weeks, Veritas may be ideally suited for reinforcing high-risk enteral stapled anastomoses such as those fashioned in patients on steroids for inflammatory bowel disease. Our results, and these potential applications for further use, warrant additional investigation of both permanent and absorbable preparations of bovine pericardium for intestinal application in humans.

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Laparoscopic Nissen Fundoplication for Treating Reflux in Lung Transplant Recipients

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Gastroesophageal reflux disease may contribute to pulmonary injury and the development of bronchiolitis obliterans syndrome in lung transplant patients. As a result, such individuals are increasingly likely to undergo corrective gastrointestinal surgery. The present study collected outcome data for 28 lung transplant patients with documented reflux who underwent an uncomplicated laparoscopic Nissen fundoplication at our institution. The results were compared to data from 63 nontransplant reflux patients who had undergone the procedure over the same time period. All Nissen fundoplications were conducted by the same surgeon. There were no intraoperative or perioperative deaths in either patient group. Operative parameters did not differ but the postoperative hospital stay was significantly greater for the lung transplant patients ($P < 0.05$). Seven transplant patients (25%) were readmitted within 30 days compared to two readmissions (3.2%) in the reflux group. Five transplant patients (17.9%) have died, all from pulmonary complications; on average, death occurred 15.5 months after the Nissen surgery. There have been no deaths in the reflux group. These data indicate that laparoscopic Nissen fundoplication can be performed on lung transplant recipients to treat reflux. The average hospital stay is longer and there are more frequent readmissions in this population, but this does not appear to be due to any Nissen-related morbidity. (J GASTROINTEST SURG 2004;8:132–137)

KEY WORDS: Fundoplication, gastroesophageal reflux, laparoscopy, lung transplantation

Compared to patients receiving heart or cadaveric kidney transplants, lung transplant recipients have a decreased 5-year survival rate (U.S. Scientific Registry of Transplant Recipients 2000 Annual Report). A significant contributor to this poor prognosis is bronchiolitis obliterans syndrome (BOS). BOS is defined as a 20% or greater decline in pulmonary function without evidence of infection, rejection, or anastomotic narrowing.[1,2] The most common sign of BOS is decreased forced expiratory volume in 1 second (FEV_1), the overt result of chronic, obstructive, fibrotic scarring of the lungs. After infection, BOS is the second most common cause of death in lung transplant patients, and it is the leading cause of long-term mortality (i.e., >6 months post-lung transplant).[1,2] The prevalence of BOS among lung transplant patients is approximately 50%, and the traditional treatment of augmented immunosuppression is rarely successful and often complicated by infection. Although risk factors for BOS have been identified (e.g., multiple episodes of acute rejection, cytomegalovirus infection, mismatched HLA, and/or frequent episodes of pneumonia), its etiology remains unknown.[1,2]

Recent studies have identified a possible contributor to BOS and with it a putative treatment. End-stage pulmonary disease is associated with a significant incidence of gastroesophageal reflux disease (GERD). For example, 50% of patients with idiopathic pulmonary fibrosis have been reported to suffer from GERD,[3] the prevalence of which increases among patients who have received lung transplants.[4] Chronic aspiration is

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also a concern in heart-lung recipients.[5] By extension, preliminary reports indicate that antireflux surgery in lung transplant patients with GERD is an effective means of improving pulmonary function and BOS status, and prolonging survival.[6,7] Given that laparoscopic Nissen fundoplication is one of the most common surgical treatments for reflux disease, the question arises as to how safe and effective this therapy is for such a high-risk population. In the present study, we addressed this question by conducting a retrospective review of the records from lung transplant patients who underwent laparoscopic Nissen fundoplication at our institution.

MATERIAL AND METHODS

Duke University Medical Center is a tertiary care hospital that conducts approximately 40 lung transplant operations per year. Nationwide, just over 10,000 lung transplants were performed between 1988 and 2002 (data supplied by the Organ Procurement and Transplantation Network). From our cohort we identified 28 lung transplant recipients (only one received their transplant outside of Duke Medical Center) who subsequently underwent an uncomplicated laparoscopic Nissen fundoplication (i.e., patients without a concurrent pyloroplasty or open procedure for separate intra-abdominal pathology) between June 1997 and February 2002; six were single and 22 were bilateral lung transplants; the gastrointestinal surgeries were performed by one of us (W.S.E.). No transplant patient was excluded from this study on the basis of pulmonary symptoms or respiratory complications. Results for these transplant patients were then compared to data from a control group made up of 63 reflux patients. These individuals were also patients of W.S.E. and had no history of lung transplantation. They comprised the total number of uncomplicated laparoscopic Nissen fundoplications he conducted during the survey period.

Patients in both groups had presented with symptoms consistent with reflux, and surgical treatment was initiated after a positive 24-hour esophageal pH study and esophageal manometry. For the chart reviews, specific parameters compared between groups included demographic information, hiatal hernia status, operative time, estimated blood loss, length of postoperative hospital stay (LOS), readmission rates, and postoperative symptoms. In addition to contrasting transplant recipients to control patients, data from single lung and bilateral lung transplant recipients were compared to each other.

Control and transplant data are presented as group means $\pm$ standard deviation (SD) or other standard comparative measures as noted. Group differences in the parametric data (age, operating time, and blood loss) were tested for using Student's t test. To assess for differences in LOS, values were compared using the Mann-Whitney test for ordinal data. For the other ordinal data (sex, presence/absence of hiatal hernia, 30-day readmission rates), 2×2 contingency tables with two analyses were used to assess frequency differences between the two treatment groups.[8] For all comparisons, a statistically significant difference was presumed to exist at $P < 0.05$.

RESULTS

The transplant reflux group consisted of individuals who had either single (n = 6) or bilateral (n = 22) lung transplantation before undergoing laparoscopic Nissen fundoplication. On average, the time between the two surgeries was 18 months (median 8.75 months) with a range of 1 to 84 months. After receiving the lung transplant, patients were placed on immunosuppression therapy consisting of daily administration of prednisone (20 mg), azathioprine (1 to 2 mg/kg), and cyclosporine (5 to 10 mg/kg); toward the end of the study period tacrolimus and mycophenolate were incorporated into the regimen.

As a group, the transplant recipients tolerated the Nissen fundoplication well with no intraoperative or perioperative deaths and no significant operative complications. Prior to the gastrointestinal surgery, each lung transplant patient underwent a thorough evaluation to confirm his or her ability to withstand the procedure. Anesthetic management took into account the high-risk status of these patients.[9] Each underwent a rapid sequence induction with the cuff placed below the vocal cords to avoid traumatizing the tracheal anastomosis. Volume loading and crystalloid infusions were minimized to prevent accumulation of interstitial fluid; diuretics were administered at the discretion of the attending anesthesiologist. Particular attention was paid to providing and maintaining adequate ventilation (e.g., SaO_2 >97%) and favored reduced pressure over volume control to lessen the risk of barotrauma. In most patients, invasive monitoring (e.g., insertion of a central line) was not conducted. For the actual surgery, the suture material, trocar positioning, length of wrap, and caliber of crural closure were the same as in the control patients. A harmonic scalpel was used to maximize hemostasis. In general, the fundoplication performed in the transplant patients was designed to be slightly tighter than its standard counterpart to fully ensure the absence of micro- and macroaspiration; decisions in this regard were made by the surgeon on a case-by-case basis.

The demographic and operative characteristics of the lung transplant recipients and control patients are presented in columns 2 and 3 of Table 1. There were no significant group differences in age, sex distribution, or the presence or absence of hiatal hernia at the time of fundoplication (in all cases, $P > 0.05$). The intraoperative measurements of estimated blood loss and duration of surgery were also similar. The one area that differed in the groups was the LOS: lung transplant patients spent a significantly longer period of time recovering from the procedure ($P < 0.05$). The clinical events that necessitated this increase included pneumonia (n = 3), urinary tract infection (n = 2), nausea (n = 2), postoperative ileus (n = 1), adjustment of cyclosporine levels (n = 1), and respiratory therapy (n = 1). Columns 5 and 6 break down the parameters of the transplant group into single and bilateral recipients. Comparisons between these two subgroups (columns 5 and 6) revealed no significant differences for any of the measures investigated.

After discharge, transplant patients had a significantly higher rate of readmission compared to the control patients ($\chi^2 = 10.4$; $P = 0.001$) with 7 (25%) of the 28 transplant patients returning to the hospital compared with only 2 (3.2%) of the 63 control patients; these data are depicted graphically in Fig. 1. For the transplant group, the reasons for readmission included *Pseudomonas* pneumonia (n = 2), acute rejection (n = 1), pneumonia with acute rejection (n = 1), deep venous thrombosis (n = 1), recurrent pancreatitis (n = 1), and nausea/vomiting (n = 1). All of these readmissions were bilateral lung transplant patients. In the nontransplant group, one patient was readmitted for renal insufficiency whereas the other came in for observation and pain management.

Up to this point and excluding routine surveillance for Barrett's esophagus, few patients in either group have received post-Nissen follow-up assessment for GERD. In the transplant group, one patient complained of symptoms consistent with heartburn, whereas another patient continued to have decreases in FEV_1 and persistent abdominal pain. Additional 24-hour esophageal pH monitoring and esophageal manometry assessments of these two individuals yielded normal results. The patient with decreased FEV_1 and abdominal pain was found to have antibody-mediated chronic rejection of the lungs and subsequently underwent a second lung transplantation. In addition, five of the lung transplant patients have died, with the cause of death identified as chronic rejection (n = 3), aspergillosis (n = 1), or lymphoma complicated by pneumonia (n = 1). The mean duration from laparoscopic Nissen fundoplication to death in these patients was 15.5 months. Among the nontransplant patients, three have continued to complain of heartburn. These patients were found to have normal esophageal pH and normal manometric findings (n = 2) or declined further study (n = 1). To date, no deaths have occurred in the nontransplant group.

Pulmonary function studies of lung transplant patients after antireflux therapy (i.e., both open and closed surgical procedures) have been conducted and improvements have been noted; these group data are reported elsewhere.[6,7] FEV_1 assessment of the present population that underwent an uncomplicated laparoscopic Nissen fundoplication is currently underway. Preliminary results indicate that, for most individuals, FEV_1 was improved or at least maintained 12 months after the reflux surgery.[10]

DISCUSSION

There is an increasing amount of research that supports an interrelationship between gastrointestinal dysfunction and pulmonary abnormalities.[11] A high incidence of general (e.g., heartburn, chest pain) and specific (elevated 24-hour pH) reflux symptoms

Table 1. Demographic and operative data for lung transplant and control patients

Parameter	Control (n = 63)	Transplant (n = 28)	*P* value	Single lung (n = 6)	Bilateral lung (n = 22)	*P* value
Age (yr)	48 ± 14	44 ± 14	$P = 0.15$	54 ± 11	41 ± 14	0.06
Male/female	33/30	16/12	$P > 0.05$	4/2	12/10	>0.05
Hiatal hernia (%)	46 (73%)	22 (78%)	$P > 0.05$	6 (100%)	18 (73%)	>0.05
Operative time (min)	122 ± 22	118 ± 24	$P = 0.51$	121 ± 31	117 ± 22	0.74
	n = 59	n = 26			n = 20	
Estimated blood loss (ml)	69 ± 55	76 ± 40	$P = 0.58$	76 ± 51	76 ± 37	1.00
	n = 58	n = 26			n = 20	
LOS (days)	0.71 (range 0–4)	2.89 (range 1–17)	$P < 0.05$	2.67 (range 1–9)	2.95 (range 1–17)	>0.05

The various data are presented as means ± SD, ratios, percentages, or means and ranges as noted. When a specific point of information was missing from a patient's chart, the group size (n) was adjusted accordingly. LOS = Length of hospital stay.

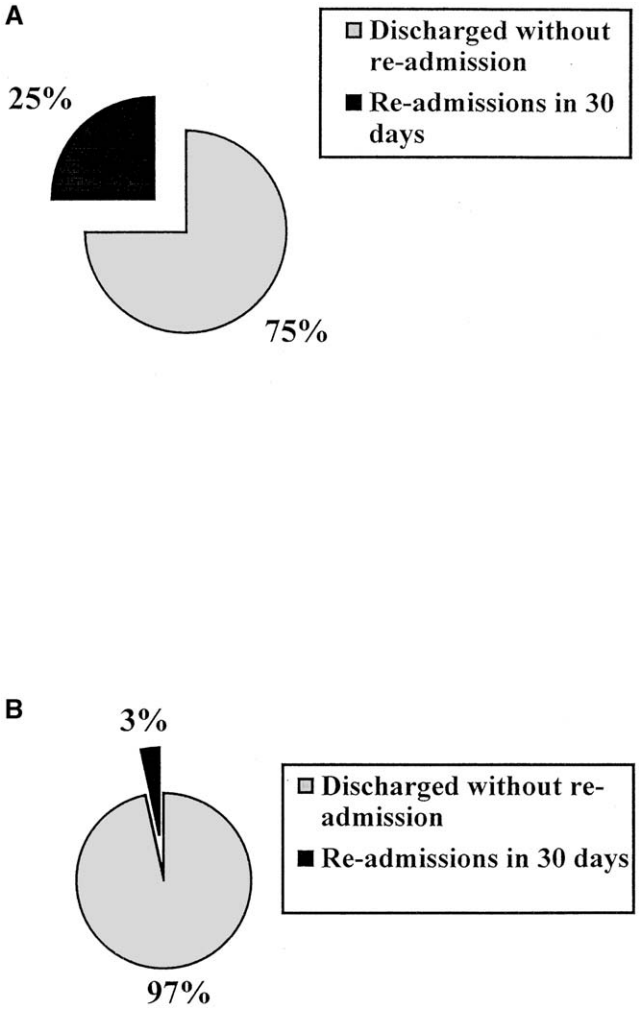

Fig. 1. Readmission rates in lung transplant recipients (**A**) and control patients (**B**) up to 30 days after laparoscopic Nissen fundoplication. For various causes detailed in the text, transplant patients had a significantly higher rate of readmission than control patients ($\chi^2 = 10.4$, $P = 0.001$).

have been reported among patients with idiopathic pulmonary fibrosis,[3] chronic bronchitis,[12] recurrent pneumonia,[11] asthma,[13,14] and, as noted earlier, lung transplant recipients.[4] Despite this apparent association, GERD may go undiagnosed in respiratory patients who do not have the classic symptoms.[13] Indeed, cough may be the only presenting complaint.[15] One study reported that of the 77 patients evaluated with a primary complaint of cough, wheezing, and/or recurrent pneumonia, 70% were subsequently determined to have occult GERD.[16]

Reflux and lung disease appear to be linked, although the actual causality and timeline are unclear. Patients with end-stage lung disease have a high incidence of GERD, but this incidence appears to increase after lung transplantation.[4] These observations are not limited to transplant recipients; severe gastrointestinal complications have also been noted in persons with emphysema who underwent lung volume reduction surgery.[17] Such findings make it difficult to determine whether or not reflux contributed to the original lung disease, or vice versa. Persons presenting with GERD do not routinely undergo pulmonary testing, but such testing may be warranted to elucidate this relationship.

The lack of a clear etiology has not precluded researchers from treating GERD with the goal of improving both gastrointestinal and pulmonary function. The majority of these therapies have been applied to asthmatic patients with varying results. Perrin-Fayolle et al.[18] reported a 25% cure rate for patients with intrinsic asthma associated with nocturnal crises and signs of reflux prior to the onset of asthma, who underwent Nissen transabdominal gastropexy; an additional 16% of the 44 patients studied showed marked improvements in their FEV_1 scores. However, systematic reviews of the past 30

years of published research suggest that GERD therapy has only modest benefits in that surgery or medical treatments may reduce reflux symptoms in patients with asthma but have no effect on pulmonary function.[19,20] In contrast to the asthma literature, reflux treatment of lung transplant recipients is a relatively new concept; the first report of GERD as cause of allograft dysfunction and its apparent reversal after Nissen fundoplication was published in 2000.[21] Since then, numerous transplant patients have undergone surgical treatment of GERD with the end result being a significant increase in actuarial survival compared to the general lung transplant population.[6]

It is now well established that laparoscopic Nissen fundoplication is a safe and effective treatment for GERD in the general population.[22,23] The present study expands this experience to include lung transplant recipients undergoing an uncomplicated laparoscopic Nissen fundoplication. For our patients, the procedure was associated with minimal complications and no intraoperative or perioperative deaths. Preexisting fibrotic scar tissue at the hiatus and an increase in fatty/friable tissue (from prolonged steroid use) augmented the difficulty of the dissection, yet in our cohort this did not increase the operative time or estimated blood loss (see Table 1). Compared to the group of control patients, the transplant patients did have a longer postoperative hospital stay, but this was a reflection of their general high-risk status as apposed to specific events associated with reflux-surgery. Likewise the higher rate of readmission was also related to their pulmonary rather than gastrointestinal status. Support for this latter observation comes from the fact that all readmissions were bilateral lung recipients. On the whole, both groups enjoyed a successful resolution of their reflux symptoms. In addition, preliminary follow-up data indicate that the majority of transplant patients have improved or at least maintained their level of pulmonary function[10]; further assessments are ongoing.

At present, one question that cannot be answered is when should the lung transplant patient undergo Nissen fundoplication? For our patients, the period between the two operations ranged from 1 to 84 months with the decision to proceed with the gastrointestinal procedure based on the appearance (and confirmation) of GERD. Whether such treatment should be applied to all lung transplant recipients as a prophylactic measure remains to be determined. To help answer this question, we have conducted (and continue to conduct) pH studies on a significant proportion of our lung transplant patients.[6] It is our hope that continued research in this area will result in improved allograft function and increased survival of lung transplant recipients.

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