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SSAT CONTROVERSY IN GI SURGERY DEBATE

Open Versus Minimally Invasive Esophagectomy: What Is the Best Approach? Frame the Issue

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Abstract Surgical resection continues to be the gold standard treatment approach for early invasive and locoregional esophageal cancer. Esophagectomy has historically had a reputation as a complex operation with high mortality and morbidity. Increasingly, results from high-volume specialized centers have demonstrated that mortality rates of below 4% should be expected and that patients can potentially demonstrate excellent levels of quality of life following surgical resection. Up until recently, virtually all surgical resections were done utilizing an open approach utilizing either a transthoracic or a transhiatal operation. Over the past several years, however, a variety of fully minimally invasive or hybrid procedures have been advocated with a view of improving mortality and morbidity outcomes. In the absence of either randomized or controlled prospective comparisons, this series of papers will review current perceptions of the advantages of both minimally invasive and open surgery for the treatment of esophageal cancer.

Keywords Esophageal cancer · Esophagectomy · Minimally invasive surgery

The outcomes of surgical resection for esophageal cancer have historically been an outlier compared to other cancer-related operations with respect to documented levels of morbidity and mortality. This has resulted in esophageal resection being one of two cancer-related operations routinely monitored by the independent consumer group Leapfrog (http://www.leapfroggroup.org/). The assessment of the "best approach" is nothing new, as multiple comparisons between open transhiatal and transthoracic esophageal resection have been carried out with no significant difference noted in morbidity or mortality, even in randomized controlled trials.

Minimally invasive esophageal resection has progressively evolved over the last two decades with a wide variety of hybrid and totally minimally invasive approaches now being reported. A recent international survey involving 269 surgeons indicated that the majority of surgeons continued to favor open approaches (78%). However, 14% indicated a preference for minimally invasive resection and 8% had no preference.[1] Pertinently, unlike comparisons of open approaches in the past, there is currently no good randomized comparison of minimally invasive and open techniques.

Historical issues associated with open surgery have highlighted the high attendant morbidity and mortality levels. There are also concerns regarding extended post-operative pain and recovery and a perception (possibly unjustified) of poor long-term quality of life. Advocates of minimally invasive resections indicate the potential for improved outcomes, although concerns over the effect of the attendant learning curve and whether minimally invasive approaches are an equivalent cancer operation are consistently raised. The specific learning curve associated with minimally invasive esophageal resection has the potential to be more complex as many thoracic surgeons have limited laparoscopic experience and many upper GI/general surgeons have minimal exposure to complex thoracoscopic procedures. This has led some units to "share" the procedure, with one team doing the laparoscopic abdominal portion, and another responsible for the thoracoscopic component.

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Perusals of the currently published outcomes for a variety of minimally invasive esophageal resections suggests that these operations are longer but do provide the potential for decreased pain, shorter length of stay, and fewer pulmonary complications. There are also suggestions that the minimally invasive approach can be associated with less blood loss and possibly a lower mortality rate. Most importantly, recent reports of survival data and assessment of lymph nodes resected at the time of surgery are equivalent to open operations. Equally interesting is the fact that recently reported levels of overall morbidity and length of stay, an issue in which the minimally invasive approach was supposed to have a measurable impact, remain comparable.

A recent extensive review of evidence-based surgical treatment of esophageal cancer has highlighted these potential advantages of minimally invasive surgery but also appropriately cautioned that some of these series may have a "patient selection bias" in that more straightforward patients, i.e., with less comorbidities and earlier tumors, may be more prevalent in reports of the early experience in minimally invasive resections. In addition, there could be the potential for a "publication bias" in that the most experienced surgical centers are more likely to have initiated the transition to minimally invasive approaches, while other centers who have attempted the transition to minimally invasive techniques with poorer outcomes are less likely to have published.[2]

In addition, although the approaches to open surgery are well-established with long track records, many of the minimally invasive approaches, even in the most experienced centers, have demonstrated an evolution over time from hybrid approaches to fully- and minimally-invasive techniques. There have also been progressive changes in anastomotic location and even patient positioning which, although understandable, make meaningful comparisons challenging.

Another potentially pertinent, but under-recognized, issue in the transition from open to minimally invasive esophageal resection is the potential effect on the surgeons themselves. There are very few operations which are longer and more physically taxing than esophagectomy. A recent assessment has demonstrated that 87% of surgeons doing regular, high-volume minimally invasive practices will experience some type of occupational injury or disability.[3] This issue could be particularly important in a practice performing a high volume of minimally invasive esophageal resections.

Taking a Moment to Look at the Big Picture

It is a favorite pastime among surgeons to critically analyze the technical advantages of one surgical approach over another. This issue is almost always worthy of periodic review; however, we cannot continue to ignore the fact that the place of esophageal resection in the treatment of high-grade dysplasia, early, as well as locoregional, esophageal cancer is being increasingly challenged. Typically, this is due to a perception of unacceptable morbidity and mortality rates associated with esophagectomy as well as advancements in endoscopic treatment techniques and chemo-radiation protocols. Publications from high-volume centers have repeatedly demonstrated that the mortality rates for esophageal resection can, and should, be below 4%. However, a nationwide study on the trends in esophageal surgery demonstrated that as recently as 2006, the operative mortality for esophageal resection in the USA was 7%.[4] Endoscopic techniques have made major inroads with respect to the treatment of high-grade dysplasia, and in some centers, intramucosal and T1a invasive cancers.[5] In addition, there are a number of studies indicating that definitive radiation chemotherapy utilized in physiologically fit patients with T1a and T1b malignancies, who would otherwise be considered good surgical candidates, can demonstrate similar long-term survival data to surgery.[6] Locoregional esophageal cancer T2-3 N0-1 is typically treated with neoadjuvant therapy and esophageal resection. However, neoadjuvant chemoradiotherapy can demonstrate complete responses in 18–40% of cases. More to the point, randomized-controlled trials of definitive chemoradiation versus trimodality therapy in the treatment of esophageal cancer show no significant differences in median and 2–3 years survivorship.[7,8]

These issues highlight that payors and patients will increasingly have non-surgical options for the treatment of their esophageal malignancy. Therefore, comparisons in technical approaches are appropriate; but ultimately, irrespective of approach, surgeons must demonstrate and document continued improvements in mortality, morbidity, survivorship, quality of life, and patient satisfaction associated with surgical management.

Summary

There is no doubt that the evolution of minimally invasive approaches has been a profound advancement in many areas of surgery. A recent review by the Dutch Health Inspectorate suggests that national health authorities and the public in general have less tolerance for the ramifications of learning curves, especially in the absence of level 1 evidence documenting specific benefit.[9] The COST and CCLOR randomized trials provided evidence-based data to shape the application of minimally invasive techniques in the management of colon cancer. Two recent meta-analyses of the current literature comparing minimally invasive and open techniques associated with esophageal resection

indicated that the "jury remains out" as to the best approach, and that randomized-controlled trials will be required to provide a meaningful basis for making a definitive decision.[10,11]

An indication of the current status of esophageal resection in the management of esophageal cancer occurred in a recent publication which documents that in the era between 1997 and 2002, when the incidence of esophageal cancer was increasing 2% per year, the overall number of esophageal resections carried out in the USA actually decreased.[12] As this debate continues, surgeons should be cognizant that whatever technical evolution we adopt, we need to continue to demonstrate a consistent improvement in clinical outcomes or we risk being marginalized in the overall treatment of esophageal cancer.

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SSAT CONTROVERSY IN GI SURGERY DEBATE

"Open" Esophagectomy

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Abstract "Open" esophagectomy has been the standard of care for treatment of esophageal carcinoma against which evolving minimally invasive surgical, endoscopic, and non-operative therapies must be compared. In experienced hands and with appropriate patient selection, "open" esophagectomy can achieve good rates of cure with low mortality, acceptable morbidity, and good long-term quality of life.

Keywords Esophagectomy · Esophageal cancer · MIE

Introduction

"Open" esophagectomy, performed by any of a number of methods, has been the standard for esophageal resection and reconstruction for decades. Given the multitude of operative approaches, technical details and esophageal replacement conduits, "open" esophagectomy does not refer to a single type of procedure, and the operation can be tailored to the needs of the individual patient and the experience of the surgeon. Any assessment of outcomes after "open" esophagectomy, therefore, must be specific to the type of operation performed. Taken in aggregate, such data are the baseline against which new and evolving minimally invasive surgical, endoscopic, and non-operative therapies must be compared.

A thorough discussion of outcomes following esophageal resection and reconstruction should assess a number of different factors (Table 1). Also important to consider is the distinction between the outcomes obtained in centers of expertise achieving the best results reported in the literature versus more typical results obtained in lower volume and less expert units. Of all of the outcome measures regarding esophagectomy for cancer, data are widely available for perioperative mortality/morbidity, cure and recurrence rates, and quality of life (QOL) whereas data for other measures, specifically comparing operative approaches, are sparse.

Perioperative Mortality

Esophagectomy historically was associated with some of the highest perioperative mortality and morbidity rates reported in the literature.[1] The past two decades, however, have brought progressively decreasing rates of perioperative death particularly from specialty and high-volume centers. Today, mortality rates following open esophagectomy of 1–2% are reported from the best centers in the USA and Europe.[2,3] Population-based data, however, would suggest mortality rates averaging 7–10% following esophagectomy performed in the community at large.[4–6]

Cure

As with all malignancies, the ability to cure esophageal cancer is highly dependent upon the stage of disease at the time of initial presentation to the physician. Given the shift in epidemiology of esophageal cancer over the past 3–4 decades, the institution of screening and surveillance programs for Barrett's esophagus, and liberal utilization of flexible upper endoscopy for assessment of foregut symptoms, esophageal cancer is increasingly being detected at an early stage. While the ability to cure esophageal cancer with resection

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Table 1 Outcomes measures after esophagectomy

1 Perioperative mortality and morbidity
2 Cure rates
3 Local–regional recurrences
4 Pain
5 Recovery time
6 Quality of life
7 Costs
8 Reproducibility
9 Learning curve

historically was poor, more recent data would suggest 5-year survival rates in the range of 50% when performed in specialty centers for esophageal adenocarcinoma, particularly when an en bloc lymphadenectomy is a part of the procedure.[7] A nihilistic attitude toward the role of esophageal resection in providing cure, therefore, is not appropriate.

Local–Regional Recurrence Rates

The rate of local–regional recurrence following esophagectomy for cancer is highly dependent upon the tumor stage, the achievement of a complete (R0) resection with negative surgical margins, and the aggressiveness of regional lymphadenectomy. When an en bloc resection of the esophagus is performed with extensive abdominal and mediastinal lymphadenectomy, with or without cervical lymphadenectomy, local–regional recurrence rates of 1–10% have been reported. With less aggressive surgical approaches such as transhiatal esophagectomy, higher recurrence rates in the range of 25–45% have been found (Table 2).

Table 2 Reported ability of transthoracic en bloc esophagectomy and transhiatal esophagectomy to control loca1–regional disease

Lead author	Number	Local recurrence (%)
En bloc esophagectomy		
Matsubara (1994)	171	10
Altorki (2001)	111	8
Hagen (2001)	100	1
Collard (2001)	324	4
Swanson (2001)	250	5.6
Range		1–10%
Transhiatal esophagectomy		
Hulscher(2001)	137	23
Becker (1987)	35	31
Gignoux (1987)	56	47
Nygaard (1992)	186	35
Range		23–47%

Quality of Life

Esophagectomy is a potentially morbid operation that can negatively impact long-term QOL. Of note, however, are data that suggest that esophagectomy can be performed with no significant detriment to QOL when averaged over large populations.[8] In addition, less invasive or aggressive surgical approaches, such as transhiatal esophagectomy with or without sparing of the vagus nerves, may be utilized when appropriate in an effort to lessen the long-term impact of operative intervention.[9]

Personal Observations on Minimally Invasive Esophagectomy

As minimally invasive esophagectomy (MIE) techniques are refined and become more widely utilized, surgeons should acknowledge which aspects of the perioperative and long-term outcomes might be improved by such approaches, which aspects likely will not be improved or could be worsened, and which aspects we know little about. Based on the available experience and data, MIE appears to decrease pain and recovery time relative to a thoracotomy or laparotomy. On the other hand, MIE likely does not reduce mortality or many of the morbidities inherent to esophagectomy such as anastomotic complications or gastrointestinal side effects. Long-term quality of life is likely unchanged relative to open procedures. Cure rates do not appear to be improved by minimally invasive approaches, and little data are available regarding local-regional recurrence rates. In addition, data are sparse regarding procedural costs (including personnel) and operative time relative to open techniques, particularly as the learning curve is being ascended. Concern clearly exists regarding complications during the learning curve phase for a particular surgeon, and such details are likely under-reported. Until more extensive experience is gained at lower volume centers, whether the results reported for MIE at a select few high-volume centers can be extrapolated to the population at large also remains unknown.

Conclusions

"Open" esophagectomy remains the standard of care for treatment of esophageal carcinoma against which alternative surgical approaches and non-operative therapies need to be compared. In experienced hands and with appropriate patient selection, "open" esophagectomy can be done with low mortality, low local-regional recurrence rates, good cure rates, and good QOL. The choice of procedure, including operative approach, esophageal replacement conduit, preservation or removal of vagus nerves, and extent of lymphadenectomy, should be tailored to the needs of the individual patient and

the patient's disease process. Surgeons need to track their outcomes so that meaningful data can be provided to the patients and their referring physicians as treatment decisions are being made.

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SSAT CONTROVERSY IN GI SURGERY DEBATE

Open Versus Minimally Invasive Esophagectomy: What is the Best Approach? Minimally Invasive Esophagectomy

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Abstract
Introduction Esophageal cancer is a complex disease that is typically discovered at a late stage and is associated with a poor overall survival rate.
Discussion Regardless of surgical approach, esophagectomy carries a significant morbidity and mortality. The surgeon should choose the surgical approach based on her comfort level, training and experience. Further investigation is required to evaluate the translatability of minimally invasive esophagectomy on a large scale.

Keywords Minimally invasive esophagectomy · Esophagectomy · Esophageal cancer · Laparoscopy · Thoracoscopy

The incidence of adenocarcinoma of the esophagus has increased faster than any other solid organ tumor over the last three decades, and the role of surgery has been challenged over the last several years, as evidenced by endoscopic interventions and the use of definitive chemotherapy and radiation without surgery. In an era of increasing scrutiny on outcomes and emphasis on evidence-based interventions, the optimal surgical technique for esophageal resection has to be critically evaluated not only for assessing best surgical outcomes but also to identify stage-specific patient groups who will benefit most from surgical resection.

Traditionally, esophagectomy has been performed with open transthoracic and transhiatal approaches. Randomized controlled trails by Hulscher et al. ([1]), as well as Omloo and colleagues ([2]), have failed to show survival advantage with either technique. The management strategy and the operative approach undertaken by surgeons across the USA and 14 other countries have shown considerable variability in a recent publication by Enestvedt1 et al. ([3]). These studies highlight the lack of consensus among surgeons for the management of resectable esophageal cancer. It is likely that the lethality of esophageal cancer, coupled with its relative rarity, limits our ability to carry out meaningful comparisons of approaches to treatment. The fact that no difference was found between open transhiatal and transthoracic approaches in a randomized controlled trial is really a testament to the advanced stage at which this cancer is typically discovered and the fact that surgery is relatively ineffective in rendering a definitive cure. However, recently, minimally invasive techniques have been reported by some high-volume centers with acceptable outcomes and may represent a means by which to reduce procedure-related morbidity, without compromising the disease-free survival rates currently achieved with the multitude of open approaches.

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Minimally invasive esophagectomy (MIE) was first described by Depaula and colleagues ([4]). Luketich et al. ([5]) reported their initial experience with MIE in 2003. In this report of 222 patients, thoracoscopic mobilization was performed, followed by laparoscopy and a stapled EEA neck anastomosis. Laparoscopy includes celiac/left gastric node dissection, gastric mobilization, conduit creation, pyloroplasty, and jejunostomy tube placement. Seventy-eight (35.1%) patients had neoadjuvant chemotherapy and 36 (16.2%) had radiation. Conversion to open technique was required in 16 (7.2%) patients. Operative mortality was 1.3% and median hospital stay was 7 days. Overall anastomotic leak rate was 11.7%. Midseries, a narrower gastric tube, was used to improve gastric drainage without a pyloroplasty in 58 patients. Sixteen (25.9%) of these patients had an anastomotic leak compared to 10 (6.1%) in whom a standard gastric conduit was created. Minor complications like atrial fibrillation, atelectasis, and wound infection were observed in 53 (23.9%) patients. Major complications including anastomotic leak, pneumonia, chylothorax, and vocal cord palsy were seen in 71 (32%) of patients. Stage-specific survival was similar to published reports. These outcomes compare favorably to reported series of open esophagectomy.

There is no randomized trial comparing MIE with open techniques. In a retrospective review of MIE vs. open esophagectomy, Nguyen et al. ([6]) showed that MIE patients had a shorter operative time, less blood transfusion requirement, and shorter hospitalization. This study had fewer than 20 patients in each arm. Other reports on MIE have been case series mainly focusing on technique and single-center outcomes. In a systemic review of all reported MIE series, Verhage et al. ([7]) evaluated the outcomes comparing MIE with open approaches—transthoracic Ivor-Lewis approach and transhiatal esophagectomy. Ten case-controlled studies and one systemic review were included in the analysis after assessing Pubmed, Embase, and the Cochrane library. MIE was associated with decreased blood loss (577 ml for open vs. 312 ml for MIE). Intensive care unit (ICU) stay was shorter with MIE (7.6 vs. 4.5 days) and so was the length of hospitalization (19.6 vs. 14.9 days). Overall complication rate was lower with MIE compared to open approach (60.4% vs. 43.8%). Mean lymph node retrieval was higher with MIE compared to open (20.2 vs. 23.8).

MIE has also been shown to have a lower morbidity compared to open approach ([8]). In this meta-analysis, 1,008 patients were compared in two groups: (1) MIE vs. transthoracic and (2) thoracoscopic esophagectomy vs. transthoracic open approach. Thirty-day mortality, number of harvested lymph nodes, and 3-year survival were similar in the two comparisons. Open approach was associated with a less stricture rate ($p<0.001$). Another meta-analysis compared esophagectomy outcomes separating operative approach in three groups: (1) MIE vs. transthoracic, (2) thoracoscopy and laparotomy vs. transthoracic, and (3) laparosocopic vs. open transhiatal esophagectomy ([9]). One controlled clinical trial and nine case–control studies were evaluated, totaling 1,061 patients. In groups 1 and 2, there was a trend towards a reduced mortality with the MIE group ($p=0.64$ and 0.34, respectively). In group 1, there was no significant difference among MIE and transthoracic arms for major morbidity or pulmonary complications ($p=0.78$ and 0.91, respectively). In group 2, MIE patients had significantly reduced anastomotic leak rate ($p=0.03$). It should be noted that there are several overlapping publications in this meta-analysis and one by Verhage et al. ([7]) described above.

Overall, MIE techniques have evolved over the years and are being performed at select high-volume centers. In case series, it has been demonstrated to have less operative blood loss, shorter length of stay, and in some reports, lesser morbidity. Mortality rates are comparable or lower at high-volume centers with extensive experience. The oncologic completeness of the MIE approach shows it to be equal to open approach with comparable survival and similar number of lymph nodes harvested. However, there are no randomized trials comparing MIE to open approach. Most of the reported case series come from select high-volume centers. There could be a patient selection bias in that patients with less comorbidities and favorable anatomy received MIE, whereas sicker patients who received neoadjuvant therapy or with bulky disease had open resection. MIE is a long and technically challenging operation that has a substantial learning curve.

Considering these issues and the knowledge that esophagectomy, irrespective of the approach, still carries a significant morbidity and mortality for an aggressive malignancy with poor long-term survival rates, the surgeon should choose the surgical approach based on his comfort, training, and experience. Further studies are required in evaluating the efficacy and safety of MIE in patients with esophageal cancer. As disease-based specialists, the responsibility is ours not to be dizzied by trying to identify the superior approach to esophagectomy, but instead, to focus on methods of screening and early detection, such that our therapies result in definitive cure.

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2010 SSAT POSTER PRESENTATION

Postoperative Impedance–pH Testing is Unreliable After Nissen Fundoplication With or Without Giant Hiatal Hernia Repair

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Abstract
Introduction Combined 24-h multichannel intralumenal impedance–pH monitoring (MII-pH) is gaining popularity as a diagnostic tool for gastroesophageal reflux. Since the surgical reduction of hiatal hernias and creation of a fundoplication anatomically restores the gastroesophageal reflux barrier, one would assume that it effectively stops all reflux regardless of composition. Our aim is to evaluate the results of routine MII-pH testing in successful Nissen fundoplication patients.
Material and Methods Sixty-two patients with normal acid exposure, confirmed by 24-h pH testing, after Nissen fundoplication were evaluated with symptomatic questionnaire, esophageal manometry and MII-pH testing more than 6 months after surgery. Patients were grouped into normal and abnormal based on postoperative impedance results. Patients with Nissen alone were separately compared to patients with Nissen+giant hiatal hernia (GHH).
Results Twenty-nine (47%) patients exhibited abnormal impedance after successful Nissen fundoplication. Abnormal impedance was associated with GHH repair, lower bolus pressures, and lower distal esophageal contraction amplitudes.
Conclusion Postoperative testing with the standard MII-pH catheters using published normative values seems to be clinically irrelevant. Clinicians should analyze the results of routine MII-pH testing in the setting of a fundoplication critically as the current technology is associated with a high false positive rate.

Keywords Fundoplication · Impedance · MII-pH · GERD

Introduction

Gastroesophageal reflux disease (GERD), defined as chronic retrograde movement of gastric contents into the esophagus, causes symptoms which contribute to a decreased quality of life in a relatively large portion of the population. The vast body of literature concerning the pathophysiology of GERD emphasizes the central role of acid reflux in association with complications such as reflux esophagitis and strictures. While acid suppression may be an effective treatment of GERD for many patients, a large proportion still has persistent symptoms that fail to respond adequately to even high-dose proton pump inhibition.[1,2] Many of these patients will have volume regurgitation despite acid suppression while others will have non-acid reflux. This has led to a growing interest in the use of combined 24-h multichannel intralumenal impedance–pH monitoring (MII-pH) to evaluate patients who are dissatisfied with acid suppression alone for a variety of reasons. Recent studies have demonstrated that combining preoperative impedance testing with traditional pH monitoring can help identify patients who are likely to achieve a favorable result with anti-reflux surgery.[3–6] Consequently, MII-pH testing has become an important diagnostic tool to detect gastroesophageal reflux, particularly non-acid reflux in medically refractory patients.

Laparoscopic fundoplication, commonly with concomitant hiatal hernia repair, is widely accepted as the surgical treatment

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of choice for patients with severe GERD.[7–9] Objective confirmation of successful anti-reflux surgery has traditionally relied on postoperative 24-h pH monitoring as the gold standard to demonstrate normalization of pH in the distal esophagus. Since the surgical reduction of hiatal hernias and creation of a fundoplication anatomically restores the gastro-esophageal reflux barrier, one would assume that it effectively stops all reflux regardless of composition. Hypothetically, patients with normalization of acid reflux after anti-reflux surgery should also have normal impedance results using MII-pH technology. The aim of this study is to evaluate the results of routine MII-pH testing in otherwise successful Nissen fundoplication patients, defined by symptom relief and normalization of esophageal acid exposure.

Material and Methods

Patients were selected from an IRB approved electronic database (Microsoft Access; Microsoft Corp., Redmond, WA) of over 3,000 patients who underwent laparoscopic esophageal procedures at our institution. Comprehensive preoperative, intraoperative, and postoperative data are recorded prospectively on standard forms. MII-pH technology was introduced to our esophageal testing laboratory and incorporated into our routine preoperative and postoperative protocols in 2008. All patients who underwent laparoscopic Nissen fundoplication and had postoperative MII-pH testing more than 6 months after surgery with a normal pH make up the study cohort. Patients who had concurrent giant hiatal hernia (type III) repairs were included and are referred to as "giant hiatal hernias" (GHH). Patients with esophageal lengthening procedures or other esophagogastric surgery were excluded.

Interventions and Data Acquisition

All patients had standard laparoscopic Nissen fundoplication with posterior crural closure under the direction of the senior investigators (CMD or LLS) as previously described.[10,11] Briefly, the gastroesophageal junction and mediastinal esophagus was widely mobilized to achieve intra-abdominal esophageal length greater than 2 cm without tension. The vagal nerves were preserved. The posterior crura was approximated with permanent suture in all operations. Excision of the hiatal hernia sac and routine incorporation of biologic mesh was used to reinforce the crural close in cases of giant hiatal hernias. The gastrosplenic ligament was divided, and a floppy 360-degree fundoplication was secured over a large bougie (54-60f).

Solid state high-resolution esophageal manometry was performed and analyzed using the ManoScan/ManoView system (Sierra Scientific, Los Angeles, CA, USA) to locate and characterize the lower esophageal sphincter (LES) and body motility. A ManoScan Z catheter was passed transnasally into the esophagus and stomach, and patients completed ten 5-ml water swallows in a supine position following a 6-h fast. Any incomplete or disrupted swallows were repeated.

Ambulatory MII-pH monitoring was performed using the AccuTrac pH-Z system (Sierra Scientific, Los Angeles, CA, USA). A 50-Hz AccuFET pH 6-impedance, 1 pH catheter was used, with impedance electrodes located at heights of 3, 5, 7, 9, 15, and 17 cm above the LES. The combined MII-pH catheter was passed transnasally and positioned such that the pH electrode was 5 cm above the manometrically determined upper border of the LES. The patients were asked to continue normal activity and keep a diary of precise eating times, time spent in upright and supine positions, and any reflux symptoms for consecutive 24 h. Tracings were collected and analyzed using the AccuView analysis software. Each tracing was further reviewed by hand to confirm the computer-generated results. A reflux event was recorded if the impedance decreased in ohms by 60% below the baseline for less than 2 s and returned to at least 40% for 5 s afterwards per software recommended settings. Antegrade events and events during a meal were excluded. All patients were off antisecretory medications for at least 7 days prior and during the procedure. Meal times were excluded from the 24-h MII-pH monitoring data.

Patients were interviewed at the time of routine postoperative testing by a manometry nurse (AG) specialist and severity of GERD symptoms were recorded using a 0–5-point scale: 0, never; 1, rarely (one to two times a year); 2,

Table 1 Patient demographics

	Normal impedance (n=33)	Abnormal impedance (n=29)	P value
Age (years)	53.7±14.7	60±12.6	0.075
BMI	28.5±6.0	30.6±4.7	0.131
Follow-up (days after surgery)	357.3±292.6	449.54±768.6	0.718
Gender	M, 11 (33%)	M, 5 (17%)	0.273
	F, 22 (67%)	F, 24 (83%)	

Demographic data in patients who had normal pH testing after Nissen fundoplication

Table 2 Postoperative high-resolution manometry results

	Normal impedance (n=33)	Abnormal impedance (n=29)	P value
Peristalsis (% complete)	88.4±13.7	85.6±17.7	0.489
Distal esophageal contraction amplitude (mmHg)	106.6±54.0	72.7±28.0	0.005
Bolus pressure (mmHg)	38.0±14.0	30.4±7.7	0.042
Lower esophageal sphincter resting pressure (mmHg)	18.9±13.9	17.0±10.6	0.562
Lower esophageal sphincter residual pressure (mmHg)	13.51±7.1	13.8±6.4	0.895

High-resolution manometry data in patients who had normal pH testing after Nissen fundoplication

sometimes (one to two times a month); 3, often (one to two times a week); 4, daily (not continuous); 5, always (daily, continuous).

Data Analysis

By design, all patients in the cohort had a normal postoperative pH score defined as percent time, pH<4, less than 4.4%, and/or a DeMeester Score of less than 14.7. Patients were then divided into two groups for data analysis based on published impedance normal values:[12] "normal impedance group," those experiencing less than 73 total impedance events of any time duration over the 24-h monitoring period, and "abnormal impedance group," those experiencing greater than or equal to 74 total events. The data were also analyzed by dividing the cohort into those with and without concomitant GHH repair. The Nissen-only group was then analyzed separately. A univariate analysis and an unpaired t test in PASW Statistics 18 (SPSS Inc., Chicago, IL, USA), with a two-sided error probability of $p<0.05$ was used to compare groups. Liquid MII-pH impedance events were defined as a rapid decline in impedance to less than 50% of baseline esophageal impedance, progressing over time from distally to proximally in at least two distal channels. Impedance events were categorized into acid (pH<4.0), weak acid (pH 4–7), and non-acid (pH>7). The number of impedance events of any time duration and the number of impedance events lasting >5 min were recorded for all three pH impedance categories.

Results

Normal vs Abnormal Impedance

Sixty-two patients met the study criteria (normal pH on MII-pH testing after Nissen fundoplication) and were analyzed. Twenty-nine (47%) of these patients exhibited abnormal postoperative impedance. There were no significant differences in demographic factors (Table 1). Esophageal peristalsis and LES parameters were equivalent, but the normal impedance group demonstrated significantly higher bolus pressures and distal esophageal contraction amplitudes when compared to the abnormal group (Table 2). By design, the pHemetry data are normal in both groups (Table 3). The ratio of the type of event (non-acid, weak acid, acid) was almost identical in both groups, with less than 5% of total episodes in either group being acidic. Excellent symptomatic results were reported by both groups. There was no difference in GERD symptomatology, whether abnormal impedance was present (Table 4).

Nissen Only vs Nissen±GHH Repair

Of the 62 patients analyzed, 21 had a concomitant giant hiatal hernia repair. A significantly higher number of GHH patients were in the abnormal group (21% vs 48%, p= 0.025). When the entire cohort was regrouped into Nissen-only and Nissen-GHH groups, similar differences identified in the normal vs abnormal analysis (Table 5). Similar trends

Table 3 Postoperative MII-pH results

	Normal impedance (n=33)	Abnormal impedance (n=29)	P value
No. of all events >5 min	1.5±2.5	4.0±9.3	0.141
No. of all events of all durations	33.4±19.4	156.4±74.6	0.000
No. of acid events	1.45±4.0 (4%)	2.33±4.2 (1%)	0.414
No. of weak acid events	26.2±18.3 (79%)	132.3±75.9 (85%)	0.000
No. of non-acid events	5.7±8.7 (17%)	21.7±24.3 (14%)	0.001
% time pH<4.0	0.07±0.1	0.5±0.8	0.008
DeMeester score	0.4±0.4	1.9±3.1	0.008
Gastric pH	2.3±1.1	2.4±1.0	0.663

MII-pH data. The composition of reflux events as a percentage of total reflux events is shown in parenthesis

Table 4 Postoperative GERD symptomatology

	Normal impedance (n=33)	Abnormal impedance (n=29)	P value
Dysphagia solid	0.6±0.7	0.7±0.9	0.583
Dysphagia liquid	0.2±0.6	0.6±1.1	0.195
Regurgitation	0.2±0.6	0.2±0.6	0.827
Heartburn	0.3±0.7	0.2±0.6	0.259

Symptom outcomes using the 0–5 scale with 5 being the most frequent/severe

emerged when comparing the GHH vs Nissen-only groups as when comparing the abnormal vs normal impedance groups. Specifically, manometric characteristics of the LES, peristalsis, and clinical symptomatology were the same regardless of the additional GHH repair, but manometric bolus pressure and distal esophageal contraction amplitude were higher in the Nissen-only group. A significantly higher percentage of GHH patients experienced abnormal impedance events compared to Nissen-only patients (Table 6). No significant difference was found in the type of impedance event (non-acid, weak acid, acid), although the distribution was again similar, with both groups experiencing very few total acid events. The odds of having abnormal impedance events was significantly higher in patients with concomitant GHH (odds ratio (OR), 3.4; confidence interval (CI), 1.14–10.49; p=0.028), age≥56, (OR, 3.02; CI, 1.05–8.85; p=0.039), and lower distal esophageal contraction amplitude≤81.6 (OR, 2.79; CI, 0.09–0.9; p=0.039) on univariate analysis. However, none of these factors were significant on multivariate regression analysis.

Nissen Only

Of the 62 patients analyzed, 41 patients had Nissen only without GHH (all had sufficient mobilization of gastro-esophageal junction into the abdomen and posterior crural closure). There were no significant differences in baseline demographics (Table 6), high-resolution manometric features (Table 7), or postoperative symptomatology (Table 8). By design, the MII-pH data show meaningful differences only for impedance (Table 9).

Discussion

For over three decades, it has been possible to detect and detail the degree of acidic gastroesophageal reflux by measuring the presence of hydrogen ions using intra-esophageal catheter-mounted pH sensors.[13] However, this limits the detection of reflux to acid events only, and neglects to record acid events neutralized by food, bile, or acid suppression therapy. As the distal esophageal pH probe measures acid concentration, only at 1 pH sensor, it is also limited in its ability to determine bolus volume, direction of movement, or composition.

Combined 24-h multichannel intraluminal impedance–pH monitoring (MII-pH) technology detects changes in resistance to alternating current flow between two electrodes when they are bridged by a liquid and/or gas bolus. Commonly, catheters use one antimony pH electrode and six impedance electrodes to provide the highest sensitivity for the detection of physical properties (gas, liquid, mixed), chemical properties (acidity), height of refluxate, bolus presence, and clearance with a high reproducibility. Multichannel intraluminal impedance coupled with pH monitoring (MII-pH), therefore, provides more information than pH testing alone. MII-pH catheters have impedance sensors at various heights above the gastro-esophageal junction, measuring changes in current when two sensors are bridged by liquid or gas. These fluctuations distinguish the physical properties of refluxate, the height of refluxate, esophageal motility, and bolus clearance with a high reproducibility. Because of this, MII-pH is being increasingly used in the evaluation of reflux patients, but its use in the postoperative setting is still being explored.

Table 5 Postoperative comparison of impedance and HRM data in Nissen-only vs Nissen+giant hiatal hernia groups

	Nissen only (n=41)	Nissen+GHH (n=21)	P value
No. of abnormal postop impedance patients	14 (34%)	15 (71%)	0.025
Bolus pressure (mmHg)	36.8±12.1	28.1±9.1	0.038
Distal esophageal contraction amplitude (mmHg)	102.0±52.6	71.3±26.2	0.013
No. of all events of all durations	75.9±81.6	112.6±74.1	0.092
No. of acid events	2.1±4.5 (3%)	1.4±3.2 (1%)	0.564
No. of weak acid events	64.3±74.5 (83%)	93.7±73.6 (84%)	0.150
No. of non-acid events	11.2±19.8 (14%)	16.0±17.8 (15%)	0.366

Comparison of objective outcomes following Nissen and Nissen+GHH in patients with normal 24-h pH. The composition of reflux events as a percentage of total reflux events is shown in parenthesis

Table 6 Patient demographics: Nissen only

	Normal impedance (n=26)	Abnormal impedance (n=15)	P value
Age (years)	50.7±13.7	57.5±13.2	0.131
BMI	28.6±5.9	31.2±5.5	0.180
Follow-up (days after surgery)	365.2±326.5	677.2±1,050.6	0.168
Gender	M, 8 (31%)	M, 4 (27%)	0.788
	F, 18 (69%)	F, 11 (73%)	

Our surgical group has performed laparoscopic Nissen fundoplication surgery since 1991. In November of 2008, we began implementing MII-pH as part of our routine postoperative testing protocol to evaluate surgical outcomes. Since fundoplication and hiatal hernia repair physically recreate the gastroesophageal reflux barrier, one would expect that successful, asymptomatic Nissen fundoplication patients with normal postoperative pH studies would also have normal impedance results. However, we demonstrate that in this group, (those without symptoms and with a normal pH), impedance results are abnormal in nearly 50% of patients. These events call into question the clinical value of MII-pH in evaluating outcomes in Nissen fundoplication surgery.

One potential flaw in our study is the lack of preoperative MII-pH data for comparison. Strictly speaking, acid exposure should not be used as a marker for reflux if the patient is a non-acid refluxer. Unfortunately, only ten patients in this data set had preoperative impedance testing simply due to the timing of acquisition of the technology into our lab. In addition, while pH testing is a routine part of the preoperative evaluation for straightforward nissen patients, we do not routinely subject patients to 24 h testing if the primary indication is a symptomatic giant hiatal hernia. For many patients, the presence of a GHH likely represents a different underlying disease than pure GERD patients, namely a primary diaphragmatic failure. Consequently, only four of the 21 giant hiatal hernia patients had preoperative 24-h pH testing alone (all abnormal) and one had preoperative MII-pH (abnormal acid and abnormal non-acid reflux). Justification for obtaining routine postoperative acid reflux testing in these patients is that the dissection of the hiatal region to reduce the hernia alone may weaken the reflux barrier and could actually generate gastroesophageal reflux postoperatively. Still, it could be argued that we cannot be certain that the absence of acid reflux represents an intact reflux barrier for the 17 untested patients overall. However, when looking only at those patients who had documented abnormal acid reflux preoperatively, a group for which postoperative acid normalization should represent wrap integrity, 12/29 (41%) still had an abnormal postoperative impedance result in the setting of excellent symptomatic outcomes and normalization of esophageal acid exposure. It is likely that there is a flaw in the testing modality, perhaps due to anatomic variation in GHH repairs that make it more likely to have a falsely abnormal impedance result.

Del Genio et al. evaluated 15 consecutive patients who underwent a laparoscopic Nissen–Rosetti procedure with pre- and postoperative MII-pH and water perfusion manometry.[14] As expected, acid exposure and non-acid reflux significantly decreased after surgery, and most patients were normalized. To further investigate the effect of fundoplication on impedance, they carefully analyzed each reflux episode and characterized them either as true reflux events or false positives (swallow induced reflux, intraesophageal reflux, or no retrograde movement). After this laborious analysis, the mean postoperative, non-acid reflux events decreased from 39 to only 8.3 demonstrating that the fundoplications were competent to all types of reflux. This implies that the initial computerized analysis of MII-pH was too sensitive. The authors hypothesize that there is a "phenomenon of the tail inversion" in which a bolus propelled through the esophagus is forced cephalad as it hits the fundoplication. This would suggest that the tighter the wrap the more abnormal the impedance would be. However, our data show that subjective measures of dysphagia and objective LES pressures, both indicators of esophageal outflow restrictions, are not worse in patients

Table 7 Postoperative high-resolution manometry results: Nissen only

	Normal impedance (n=26)	Abnormal impedance (n=15)	P value
Peristalsis (% complete)	88.4±14.1	90.0±15.4	0.751
Distal esophageal contraction amplitude (mmHg)	110.6±58.6	86.3±32.4	0.189
Bolus pressure (mmHg)	39.3±14.1	32.7±6.7	0.159
Lower esophageal sphincter resting pressure (mmHg)	19.0±14.8	18.8±12.2	0.960
Lower esophageal sphincter residual pressure (mmHg)	13.0±6.7	14.7±7.2	0.488

Table 8 Postoperative GERD symptomatology: Nissen only

	Normal impedance (n=26)	Abnormal impedance (n=15)	P value
Dysphagia solid	0.7±0.8	0.7±0.9	0.830
Dysphagia liquid	0.3±0.7	0.6±1.2	0.251
Regurgitation	0.2±0.6	0.2±0.8	0.873
Heartburn	0.3±0.6	0.2±0.8	0.746

with high numbers of postoperative impedance events. Furthermore, manometric bolus pressure, a direct measure of outflow resistance, was actually lower in patients with high postoperative impedance events.

Still, the concept of "tail inversion" definitely seems reasonable. There does seem to be a change in the composition of the refluxate following antireflux surgery favoring non-acid and weak acid events despite normal gastric pH further supporting the integrity of the wrap as an antireflux barrier. Compared to published normal preoperative values, (33% weak acid, 0% non-acid, and 67% acid),[12] all postoperative patients in this study exhibited a similar ratio of 79–85% weak acid, 14–17% non-acid, and 1–4% acid in there reflux events. This again suggests that preoperative normal values may not be valid in the postoperative evaluation of antireflux surgery.

The idea that anatomic changes created by surgery explain the abnormality of postoperative impedance is supported by our findings demonstrating a higher percentage of abnormal impedance reflux events in patients following giant hiatal hernia repair with fundoplication compared to fundoplication alone. In fact, otherwise asymptomatic Nissen-GHH patients were 3.4 times more likely to exhibit abnormal impedance than those undergoing successful Nissen-alone. It may be that the anatomy of the esophagus after GHH repair is affected by the crural closure, particularly with a large posterior cruroplasty when compared to patients who have a fundoplication alone. The esophagus is sometimes angulated as it passes over the closure which may create a subtle obstacle in the esophageal outflow path. This distortion may cause a reservoir to form in the distal esophagus which could affect clearance further by enhancing retrograde escape. This trapping of fluid and bolus remnants before finally passing through the wrap may be misinterpreted as non-acid reflux with current impedance technology. These subtle changes are not clinically significant attesting to the high sensitivity of impedance testing.

The popularity of esophageal impedance testing has risen dramatically over the past 5 years with "high tech" units emerging even in nonspecialized centers. Its increased sensitivity for gastroesophageal reflux has expanded the role of antireflux surgery with excellent outcomes for patients with symptomatic non-acid reflux,[5,6] but users should be aware of the complexities in data analysis which may inadvertently lead to a high rate of false positive results, particularly in the postoperative setting. There are a multitude of technical subtleties that need to be considered when applying the MII-pH technology, and there is a discrepancy between seemingly small details which may greatly impact results and ultimate clinical recommendations. One issue we have identified is a lack of standardization in thresholds for impedance events. Since reviewing this data, we have changed our threshold for impedance events to decrease the sensitivity for postoperative patients. We agree with the recommendations of del Genio et al. that each event must be carefully scrutinized by a trained analyst to delete false positives yet, even with careful manual review of each episode, we still saw a high number of impedance events after successful surgery. Particular caution must be taken with computer-generated reports or reports from unknown esophageal testing facilities.

Another way to improve the accuracy of the MII-pH system might be to place the impedance sensors across the distal esophageal high-pressure zone to allow correlation between true esophageal gastric events and intra-esophageal "ghost" events. While there are several versions of available impedance catheters with varying number and spacing of impedance detectors, there is not currently one with impedance sensors that span the gastroesophageal junction or fundoplication while maintaining the pH sensor in the standard location 5 cm above the LES.

Table 9 Postoperative MII-pH results: Nissen only

	Normal impedance (n=26)	Abnormal impedance (n=15)	P value
No. of all events >5 min	1.6±2.7	4.3±11.8	0.287
No. of all events of all durations	31.5±19.3	164.8±86.5	0.000
No of acid events	1.8±4.5 (6%)	2.6±4.8 (1%)	0.607
No. of weak acid events	25.0±16.5 (77%)	139.8±84.7 (85%)	0.000
No. of non-acid events	5.6±8.8 (17%)	22.5±29.7 (14%)	0.010
% time pH<4.0	0.08±0.2	0.6±0.9	0.005
DeMeester score	0.4±0.5	0.7±0.5	0.140
Gastric pH	2.1±1.0	2.7±1.3	0.130

Our data indicate that the application of preoperative normative impedance values to patients after fundoplication is not useful. Since we have found the clinical utility of using current impedance catheters for routine postoperative testing not worth their increase in cost, we currently recommend only selective use and if used, careful analysis of the results. For now, the best method for demonstrating an intact, functioning fundoplication remains the gold standard 24-h pH test.

Conclusion

Postoperative testing with the standard MII-pH catheters using published normative values seems to be clinically irrelevant. Clinicians should analyze critically the results of routine MII-pH testing in the setting of a fundoplication, as the current technology is associated with a high false positive rate. Since a fundoplication should stop all reflux regardless of composition, positioning the impedance sensors below and above the wrap may be more useful.

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SSAT POSTER PRESENTATION

Laparoscopic and Endoscopic Pyloroplasty for Gastroparesis Results in Sustained Symptom Improvement

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Abstract
Background Gastroparesis is a chronic digestive disorder with symptoms of nausea, vomiting, bloating, and abdominal pain resulting in a poor quality of life. Surgeons are increasingly asked to treat patients with gastroparesis as medical options have become limited due to safety concerns of many prokinetics. Surgical options include gastric stimulator implantation, sub-total gastrectomy, and pyloroplasty. We report our experience with minimally invasive pyloroplasty as sole surgical treatment for adult gastroparesis.
Materials and Methods A retrospective review of prospectively collected data of 28 patients who underwent minimally invasive pyloroplasty alone as treatment for gastroparesis from Jan 2007 to Sept 2010. Pre- and postoperative symptom severity score (SSS), gastric emptying scintigraphy (GES), and medication use were reviewed.
Results A laparoscopic Heineke–Mikulicz pyloroplasty was performed in 26 patients. A laparoscopic assisted, flexible trans-oral endoscopic circular stapled pyloroplasty was used in two patients. Prokinetic use was significantly reduced from 89% to 14% (p=<0.0001). The mean GES $T1/2$ decreased from 320 to 112 min (p=0.001) and normalized in 71%. Significant improvements in the SSS were seen at 1 month for nausea (p=<0.0001), vomiting (p=<0.0001), bloating (p=0.0023), abdominal pain (p=<0.0001), and gastroesophageal reflux disease (GERD) symptoms (p=0.0143). Significant improvement persisted at 3 months for nausea (p=<0.0001), vomiting (p=<0.0001), bloating (p=0.0004), abdominal pain (p=0.0001) and GERD symptoms (p=0.013). The average length of stay was 3.71 days. Overall, 83% of patients' indicated that they saw improvement at 1 month follow-up.
Conclusion Minimally invasive pyloroplasty provides excellent outcomes for patients with gastroparesis and should be considered as a primary treatment along with diet and medications as it is effective and does not eliminate the option for additional surgical options in the future for refractory disease. With technological advancements, a totally endoscopic pyloroplasty may be a less invasive option.

Keywords Gastroparesis · Pyloroplasty · Laparoscopy · Endoscopy · Surgery

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Introduction

Gastroparesis is a chronic digestive disorder best defined as severe nausea, vomiting, bloating, and abdominal pain in the setting of objectively delayed gastric emptying without mechanical gastric outlet obstruction. For unknown reasons, there has been an explosive increase in the number of patients presenting with this diagnosis over the last two decades. Etiology of this problem includes diabetic gastropathy, post surgical (vagal compromise) and increasingly, idiopathic. By and large, there is no cure for gastroparesis and the goal of treatment should focus on symptom control. Current first-line treatment strategies include prokinetic agents, antiemetic

medications, diabetes control, weaning of narcotics and dietary modifications. Unfortunately, prokinetic options have been dramatically reduced over recent years due to safety concerns[1–5] making alternative therapies even more important.

Antral and pyloric dysfunctions have been demonstrated in the pathogenesis of gastroparesis. Endoscopic therapies directed at the pylorus such as dilation and intra-pyloric botulinum toxin A injection (Botox) have been shown to improve gastric emptying temporarily.[6–8] Pyloroplasty has long been recognized as an effective and permanent gastric drainage procedure for mechanical obstructions and electively vagotomized stomachs.[9] However, reports of pyloroplasty for gastroparesis are limited. We present our experience with minimally invasive pyloroplasty as a primary treatment for gastroparesis.

Materials and Methods

Patient population A retrospective review of prospectively collected data of patients with gastroparesis who were referred for evaluation to our foregut center was queried to identify patients who underwent pyloroplasty between January 2007 and September 2010. Patients who had prior gastric surgery or who underwent concurrent anti-reflux surgery were excluded. Gastroparesis was defined by the presence of symptoms AND an abnormal radionuclide gastric emptying scintigraphy (GES) in the absence of gastric outlet obstruction on upper endoscopy. Baseline demographics, pre and postoperative symptom scores and medication use were prospectively collected on data-collection forms which are maintained in an electronic database system (Microsoft Access 2003, Microsoft Corporation, Redmond, USA). Symptoms were recorded with a standardized gastroesophageal symptom assessment tool using a scale of 0–4 with higher ordinal values representing greater frequency of symptoms.[10] An additional gastroparesis specific symptom assessment tool was also filled out by the patient at each visit (Medtronic, Minneapolis, MN). Postoperative clinical data was collected at 1 and 3 months after surgery. Symptom improvement was defined as reduction individual symptom scores as well as in the combined symptom severity score. A radionuclide-labeled meal GES was routinely offered 3 months after surgery. The majority of GES labs reported a $T1/2$, or a $T1/2$ estimate using a best fit linear regression model, although a 4 h emptying time was preferred and collected whenever possible. An abnormal 4-h GES was defined by a >10% retention at 4 h. Individual institution normal values provided on the reports were used to characterize results if only a $T1/2$ was available. Statistical significance was assigned using the online statistics tool, graphpad. The Fischer's exact test was used to detect differences in pre- and postoperative prokinetic use and paired t test to assess differences in GES $T1/2$ and symptom severity score and 1 and 3 months follow-up. If a patient was dismissed from follow-up after only one visit, the last reported score was assumed for the 3 month data.

Surgical technique The surgical procedure consisted of either a laparoscopic modified Heineke–Mikulicz pyloroplasty (HMP), robot-assisted laparoscopic HMP or a laparoscopic assisted trans-oral flexible endoscopic stapled pyloroplasty by one of two foregut surgeons (CMD and LLS). Laparoscopic and robotic HMP was performed using four to five trocars placed in standard foregut surgery arrangement (Fig. 1). The pylorus is mobilized from its superior and inferior peritoneal attachments to decrease tension on the suture line. A gentle kocher maneuver is performed when necessary to obtain adequate visualization and facilitate a tension free closure, particularly in patients with a prior cholecystectomy. A 5-cm full-thickness pyloromyotomy is made using an ultrasonic shear. The pyloromytomy is then closed transversely using a running 2–0 monofilament absorbable sutures for a single layer closure. The closure is started in the middle of the closure line to avoid "frame-shifting" with subsequent narrowing of the pyloroplasty (Fig. 2). Intra-operative endoscopic suture

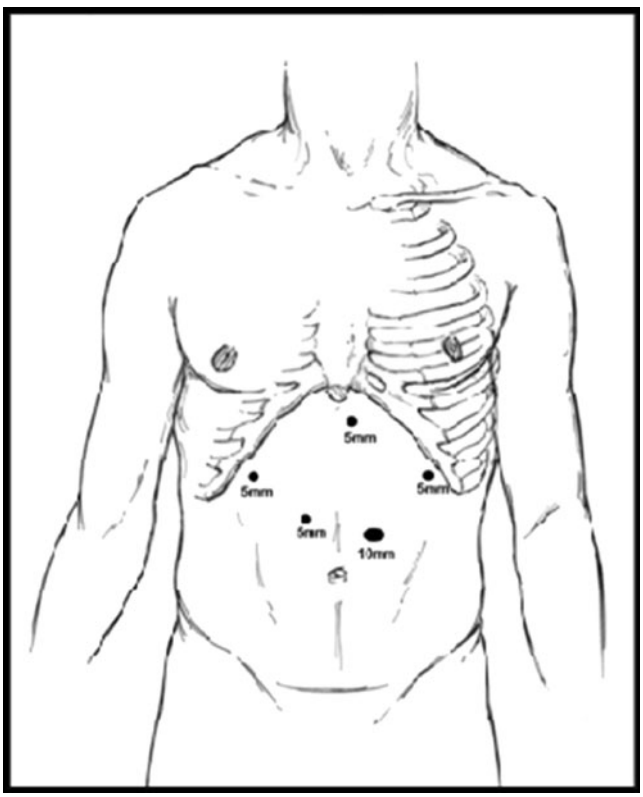

Fig. 1 Typical laparoscopic post site placement for laparoscopic pyloroplasty

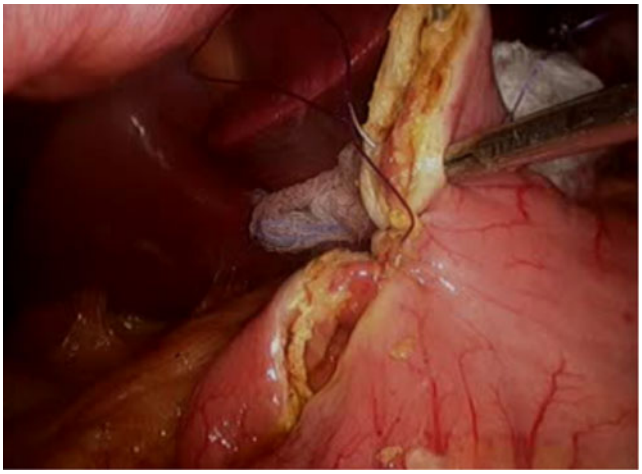

Fig. 2 Intra-operative image demonstrating laparoscopic pyloromyotomy closure technique

line leak test is performed with endoscopic air insufflation under saline and/or methylene blue. A 15-french closed suction drain is placed near the suture line.

The laparoscopic assisted trans-oral stapled pyloroplasty is performed using a flexible 21 mm circular powered stapler (Power Medical Interventions, Langhorn, PA; Fig. 3). The stapler is passed transorally into the stomach and positioned across the pylorus with laparoscopic assistance. The anvil is then opened and a suture held between two laparoscopic graspers, is used to compress the pylorus into the stapler while the stapler is closed and fired. This results in a partial pylorectomy of the anterior wall of the pylorus and is similar to previously described for open surgery.[11]

Postoperative care includes nasogastric tube gastric decompression for 24 h, daily drain amylase levels and a gastrograffin upper GI study on postoperative day 1 or 2. A suture line leak is defined as an amylase level >1,000 or contrast extravasation on upper GI. Outcomes such as length of stay, leak rate, obstruction rate, and need for re-operation were also recorded.

Fig. 3 Flexible trans-oral circular stapler (Power Medical Interventions, Langhorn, PA)

Upon discharge, patients are placed on a pureed diet for 2 weeks then advance to gastroparesis diet (low fat, low fiber, small frequent meals). They are advised to maintain prokinetic and antiemetic medications as needed. Routine postoperative clinic visit occurs 3–4 weeks after surgery and again at 3 months with a postoperative GES.

Results

One hundred forty-two gastroparesis patients had a pyloroplasty during the study interval. One hundred fourteen were excluded due to concurrent or prior gastric or esophageal surgery, most commonly anti-reflux procedures. The remaining 28 patients who had pyloroplasty alone make up the study cohort. Five had previous or concurrent feeding jejunostomy tubes. One diabetic patient had a previous gastric stimulator implantation without improvement.

The mean patient age was 41 years and 86 were female. The mean body mass index was 26. Twenty-one patients were identified with idiopathic gastroparesis and seven with diabetic gastroparesis. A laparoscopic Heineke–Mikulicz pyloroplasty with running absorbable suture was performed in 25 patients. A laparoscopic assisted flexible trans-oral endoscopic circular stapler pyloroplasty was used in two patients. One patient had robot-assisted laparoscopic pyloroplasty.

The average length of stay was 3.71 days. There were no major surgical complications. Specifically, no suture line leaks occurred. One patient underwent a negative laparoscopic/endoscopic re-exploration due to biochemical suspicion of a leak; however, no leak was identified. One patient developed transient obstruction due to gastro-duodenal edema requiring gastric decompression. This resolved by 3 months with eventual resolution of all gastroparetic symptoms. Three patients later underwent gastric stimulator (Enterra, Medtronic) placement for refractory gastroparesis symptoms (one of them had normal postoperative GES. but a GES was not repeated in the remaining two). One patient (3.6%) required a fundoplication for persistent gastroesophageal reflux disease (GERD) symptoms 6 months after pyloroplasty.

Twenty-five patients filled out symptom score sheets at 1 month (89%). Of these, 83% of patients had symptom improvement at their 1 month follow-up. Seventeen patients were discharged from clinic after the 1 month follow-up due to symptom improvement or resolution. Of the 11 who returned, 9 now reported significant symptom improvement for an overall improvement rate of and 92% at 3 month follow-up. Significant improvements in the

symptom severity score were seen at 1 month for nausea (p =<0.0001), vomiting (p=<0.0001), bloating (p=0.0023), abdominal pain (p=<0.0001), and GERD symptoms (p= 0.0143). Significant improvement persisted at 3 months for nausea (p=<0.0001), vomiting (p=<0.0001), bloating (p= 0.0004), abdominal pain (p=0.0001), and GERD symptoms (p=0.013). In a sub-analysis of the diabetic patients alone (n=7), significant improvements were seen at 1 month for nausea (p=0.05) and abdominal pain (p=0.007) and at 3 months for nausea (p=0.05), bloating (p=0.05), and abdominal pain (p=0.04). Seven patients reported problematic diarrhea before surgery. This improved or resolved in five and remained stable in two. No patients reported a worsening of diarrhea symptoms after pyloroplasty.

Prokinetic use was significantly reduced from 89% pre operatively to 14% postoperatively (p=<0.0001). When the diabetic patients were analyzed alone, there was a consistent reduction in prokinetic usage from 100% pre operatively to 14% postoperatively (p=0.0047). Postoperative GES was routinely offered at 3 months for objective assessment. Results were available for 14 patients and were normal in 71%. Overall, the mean GES ($T1/2$) decreased from 320 min preoperatively to 112 min postoperatively (p=0.001). The mean GES ($T1/2$) specifically for diabetic patients (available for four of seven patients) decreased from 220 min pre operatively to 74 min postoperatively (p=0.18). No clinical differences were apparent between patients who had a postoperative GES and patients who declined (Fig. 4).

Discussion

Gastroparesis can be a debilitating gastrointestinal disorder presenting with chronic nausea, vomiting, bloating and abdominal pain. Patients with this condition are frequently disabled by it and typically are enormous consumers of medical resources. Treatments are primarily aimed at symptom control via medical and dietary management. Unfortunately, medical options have recently diminished due to safety concerns regarding cisapride and metaclopramide. This has resulted in increased referrals to GI surgeons for recommendations regarding treatment options. Options for surgical treatments range from gastrostomy/feeding jejunostomy placement to sub-total gastrectomy. Placing PEG tubes for venting and a jejunostomy for feeding is not a good choice due to inevitable tube complications, the cost of enteral feeding and an overall failure to improve the patient's quality of life. Interestingly, gastric resection is also associated with less than encouraging outcomes and is used only as a last resort.[12–15]

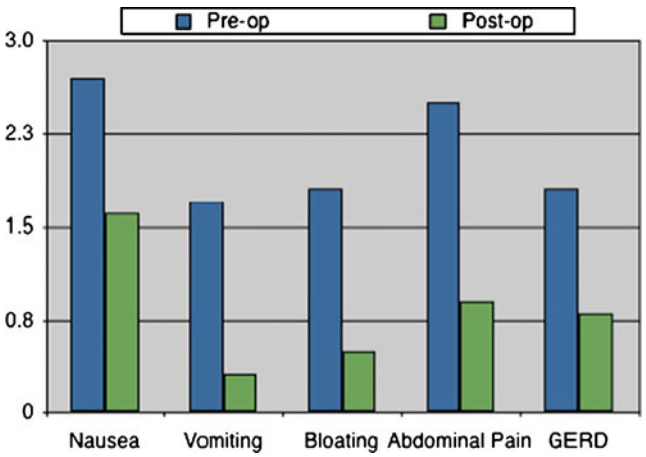

Fig. 4 Gastroparetic symptom scores before and 3 months after laparoscopic pyloroplasty

Another surgical intervention for gastroparesis is an implantable gastric stimulator (Enterra, Medtronic, Shoreview, MN). The gastric stimulator consists of a subcutaneous pulse generator connected to two electrical leads that are surgically secured to the anterior stomach wall near the greater curvature. The leads are typically placed laparoscopically qualifying this as a minimally invasive option. The Enterra device has been shown to provide a significant short-term decrease in symptoms, particularly for nausea, in a majority of patients but long-term results may be somewhat less favorable.[16–19] Twenty percent of patients will receive little to no benefit from the device and to date there are no reliable predictors of failure, however the use of endoscopically placed temporary leads may prove useful in predicting response to the stimulator prior to permanent implantation, but data on this is limited.[20] Device related complications are reported in 15% of patients and can include bowel obstruction, perforation, lead migration, or wound complications. In addition, removal of the device is necessary prior to magnetic resonance imaging. Finally, the device is available only as a "humanitarian exemption" device which requires IRB approval, branding it as "investigational", and this and its cost has made it unappealing for most insurance companies. This has made access to the device less than universal. Although there seems to be a role for gastric stimulation in the treatment of refractory gastroparesis symptoms—particularly nausea—there are a substantial number of patients who cannot access the technology or for whom it does not work.

For these reasons, we would argue that a laparoscopic pyloroplasty is an effective first-line surgical option for select patients with gastroparesis who fail medical treatment. Objective improvements in gastric emptying have previously been correlated with symptom improvement in patients with gastroparesis.[16,21] It has been shown that increased pyloric tone results in slower gastric emptying[22] and that pyloric disruption improves forward flow.[23]

Surgical pyloroplasty has been used as a gastric drainage procedure for decades. Its use in the vagotomized stomach, particularly in the treatment of ulcer disease, has been well documented.[24] From its initial description in the 1,800 s, the Heineke–Mikulicz pyloroplasty has been the most common pyloroplasty technique and it can be performed laparoscopically. However, since the advent of potent antacid medications and H pylori treatment, pyloroplasty, especially laparoscopic pyloroplasty, is not commonly performed by surgeons today.

While there are many publications describing pyloroplasty for pediatric patients with delayed gastric emptying,[25,26] reports are limited regarding its use in adults with primary gastric emptying problems.[27] Published reports of laparoscopic pyloroplasty mostly describe its use as an adjunct to anti-reflux surgery in the GERD population with poor gastric emptying. We and others have shown that pyloroplasty is an excellent remedy for uncomfortable bloating and other symptoms in patients with gastroparesis undergoing anti-reflux surgery although there is a higher incidence of mild diarrhea after surgery.[28,29] Pyloroplasty combined with fundoplication in lung transplant patients has been reported to decrease aspiration in patients with concurrent gastroparesis and reflux.[30] Interestingly, post-pyloroplasty diarrhea was not a problem in the current series (pyloroplasty without concurrent fundoplication) and actually seemed to improve in many patients who reported preoperative diarrhea.

Our data explores the role of pyloroplasty in patients whose chief complaint was gastroparetic symptoms. It should be noted that in spite of being a high volume tertiary referral center for esophageal and gastric diseases, indications for an isolated pyloroplasty is unusual. Out of 142 patients having a laparoscopic pyloroplasty during the study's time frame, only 28 (20%) had isolated problems with delayed gastric emptying. The majority of patients seen had delayed emptying incident to complex gastro-esophageal reflux disease. For patients with significant subjective and objective features of GERD (defective lower esophageal sphincter, patulous gastroesophageal flap valve or significant hiatal hernias on endoscopy, and most importantly abnormal acid exposure on 24 h pH) but also with documented abnormal gastric emptying, we perform a fundoplication and a pyloroplasty to minimize postoperative symptoms and recurrence secondary to gastric distension or vomiting. To maintain a homogenous study population, patients with fundoplication and pyloroplasty were excluded from this analysis. In this series, 68% of patients had mild preoperative symptoms of GERD and all but one had complete relief of reflux symptoms after pyloroplasty. The one patient who continued to complain of GERD after pyloroplasty subsequently had a successful fundoplication.

Our data represents the largest reported series exploring the role of minimally invasive pyloroplasty as primary surgical therapy for gastroparesis. We demonstrate that pyloroplasty can be done safely with a low complication rate. The technique presented requires advanced laparoscopic suturing skills and is certainly associated with an, as of yet undefined, learning curve. However, liberal use of intra-operative endoscopic leak testing should identify a majority of suture line problems allowing for the safe application of technique. Robotic assistance, if a robot is available at the surgeon's institution, may be helpful for those who have been unable to master laparoscopic suturing and are therefore reluctant to perform this option.

The ever increasing challenge to achieve the "least" invasive surgical strategy continues to inspire. Single incision pyloromyotomy in pediatrics has already been reported.[31] Here, we also present a new technique of trans-oral pyloroplasty using a flexible endoscopic approach. Although these two cases were definitely laparoscopic assisted, the potential to perform a safe, simple trans-oral pyloroplasty is imaginable. The feasibility of such a concept has been reported by Park et al. in an animate survival model using a needle knife and T tag sutures.[32] Our minimally invasive adaptation of a circular stapled pyloroplasty, originally described in 1995 as an open technique by Potter,[11] was made possible by using an endoscopic, flexible, powered stapler with laparoscopic assistance. The main difficulty we encountered using the flexible stapler was lack of steerability and poor endoscopic visualization. Further refinements of this technique have been halted due to the current unavailability of the flexible powered stapler but we hope to resume work on this promising technique in the future.

Although the present data demonstrates decreased symptoms for a majority of patients with gastroparesis treated with laparoscopic pyloroplasty, there are limitations to the study. First, while we attempted to objectify the clinical data, digestive symptoms are notoriously difficult to assess. The Gastroparesis Cardinal Symptom Index has been developed and validated to address this problem.[33] Future studies should utilize this or similar standardized assessment tools to improve the quality of the data and provide a vehicle for comparison of results across studies. Secondly, the quality of radionuclide GES is known to differ amongst radiology centers due to a wide variability in testing protocols and normal values. Despite our continual plea to adhere to published recommendations for standardized meals and a 4-h emptying measurement,[34] many patients are referred from distant locations and repeating the test for every patient is not feasible despite known inaccuracies. Third, our study does not take into account anatomic variations which may interfere with successful emptying after pyloroplasty. In some cases, the stomach is

enlarged and atonic to such a degree that emptying will not improve even with a pyloroplasty due to the inability of the stomach to propel the food to the relatively high lying outlet. This phenomenon has been recognized for decades when Mayo described mobilization of the pylorus and securing it to the umbilicus to promote gravity drainage in 1905.[35] Such a "J shaped" stomach may be better served by a sub-total gastrectomy and gastrojejunostomy.

Gastroparetic patients can be a difficult and heterogeneous group to care for and we believe multiple treatment approaches are needed to optimize results. This series shows that pyloroplasty, combined with medical and dietary management, can help decrease all symptoms associated with gastroparesis and does not preclude additional surgical procedures when needed. In some cases, temporary gastric decompression or J tubes were required but all were removed within 6 months. One patient went on to have a fundoplication for continued reflux and three patients with severe persistent nausea had gastric stimulator implantation. Although there were no suture line leaks in this series, we prefer not to implant the stimulator at the same time as the pyloroplasty due to the potential infection risk but also because a majority of our patients do not seem to need the stimulator afterwards. It is likely that with time and more experience, the treatment algorithms for this patient population will mature and therapies will be tailored to individual characteristics. Although our results are encouraging, the long-term outcomes for gastroparetic patients treated with pyloroplasty are unknown and following this initial cohort will be helpful in determining the ultimate role for this procedure.

Conclusion

Laparoscopic pyloroplasty has definite objective and subjective benefits in the treatment of patients with gastroparesis. Pyloroplasty should be considered in the armamentarium of therapies as it is effective and does not interfere with more aggressive surgical approaches or stimulator implantation for refractory cases. Minimally invasive approaches will continue to evolve as technology advances to further improve outcomes including totally endoscopic pyloroplasty.

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ORIGINAL ARTICLE

Three-Step Esophagojejunal Anastomosis with Atraumatic Anvil Insertion Technique After Laparoscopic Total Gastrectomy

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Abstract
Background Esophagojejunostomy during laparoscopic total gastrectomy (LATG) using a circular stapler is a difficult procedure for which there remains no widely accepted standard technique. Based upon our experience with esophagogastrostomy during laparoscopic proximal gastrectomy, we have applied a modified lift-up method to LATG.
Material and methods Esophagojejunostomy using a modified lift-up method was performed during LATG in 41 patients with early gastric cancer, from July 2005 to June 2010. The lift-up technique comprises three steps, which together reduce the difficulty of anvil insertion by lifting up the nasogastric tube connected to the anvil head.
Results During the early stages of the present study, some patients who underwent LATG with the modified lift-up method developed anastomotic leakage, with stenosis occurring in two cases (4.9%) and three cases (7.3%), respectively. All patients who developed complications showed improvement following conservative treatment with no surgical procedure. The anastomotic leaks occurred during the later periods of the study. There was no mortality in the present study.
Conclusions Our modified lift-up technique facilitates circular-stapled esophagojejunostomy in LATG and could provide a more feasible and safe option for an established procedure, especially for preventing anastomotic leak.

Keywords Gastric cancer · Laparoscopic total gastrectomy · Esophagojejunal anastomosis

Introduction

Laparoscopic distal gastrectomy (LADG) for early gastric cancer has been widely used since first performed by Kitano et al. in 1991 because it is associated with less postoperative pain, an earlier return of bowel function, shorter periods of hospitalization, and better cosmetic results.[1] In contrast, laparoscopic total gastrectomy (LATG) for upper gastric cancer is only performed at a limited number of hospitals, and only a few studies have analyzed surgical outcomes of this approach,[2–5] primarily due to the increased technical difficulty associated with LATG compared to LADG, especially in reconstructions accompanied by esophagojejunostomy, for which there is currently no widely accepted standard technique during LATG.[6,7]

The EEA stapler is a widely used instrument with a well-described low incidence of anastomotic leak in both open and laparoscopic rectal surgery. In contrast to open gastrectomy, an often troubling technical challenge with LATG is difficulty inserting the anvil head into the transected esophageal lumen after placing a purse-string suture because of the narrow working space and limited forceps handling. Excessive force employed to insert the anvil head into a noncompliant esophagus may result in mucosal tears causing disastrous anastomotic leakage.[8]

Drawing from our experience of esophagogastrostomy during laparoscopic proximal gastrectomy,[9] we have applied the anastomotic technique to LATG. This modifica-

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tion reduces the difficulty of anvil insertion by lifting up the nasogastric tube connected to the anvil head, thus avoiding the subsequent esophageal damage and eliminating the need to introduce a purse-string suture. Herein we report our initial experience of 41 cases of LATG with esophagojejunostomy using our modified lift-up anvil insertion without purse-string suture.

Material and Methods

Patients

Esophagojejunostomy involving the lifting up of the anvil insertion (modified from a previously described lift-up method[9]) was performed during LATG on 41 patients with early gastric cancer in the Department of Gastroenterological Surgery at the Cancer Institute Ariake Hospital, Tokyo, Japan, from July 2005 to June 2010. This modified lift-up method during LATG was introduced in 2005 for the treatment of gastric cancer and was only carried out by experienced laparoscopic surgeons. All tumors in this study were classified histologically as adenocarcinomas that had invaded only the mucosa or submucosa of the stomach without lymph node metastasis (cT1, cN0). LATG was indicated if the cancer was located in the upper or middle third of the stomach. We evaluated tumor location and the depth of tumor invasion on the basis of endoscopy results, an upper gastrointestinal series, and endoscopic ultrasonography. Distant metastases were evaluated by abdominal ultrasonography and computed tomography. Patients with early gastric cancer with extra indication of endoscopic resection, such as patients with submucosal cancer or mucosal cancer that was histologically confirmed as poorly differentiated adenocarcinoma, were treated with this procedure.

Surgical Procedure

A modified D2 dissection, according to the en bloc technique, was performed in the LATG patients. Dissected lymph nodes were classified according to the Japanese Classification of Gastric Carcinoma, 13th edition.[10] The extension of systemic lymph node dissection was as follows: Group 1 lymph nodes included the right and left paracardial lymph nodes, lymph nodes along the lesser curvature, lymph nodes along the short gastric vessels, lymph nodes along the right and left gastroepiploic vessels, and the supra- and infra-pyloric lymph nodes; selective group 2 lymph nodes included the lymph nodes along the left gastric artery, lymph nodes along the common hepatic artery, lymph nodes around the celiac artery, lymph nodes along the proximal splenic artery, and the part of lymph nodes along the distal splenic artery.

Following dissection of lymph nodes along the greater and lesser curvatures of the stomach, the duodenum was divided by the EndoGIA Universal 60-mm stapler (Covidien Japan, Tokyo, Japan), and the stomach was ready for total gastrectomy, achieved in three steps. In the first step, the vagus nerve was carefully exposed and divided to avoid injuring the muscle layer of the esophagus, and then the right-hand wall of the esophagus just above the esophagogastric junction was incised using an ultrasonically activated coagulation device (Harmonic ACE; Johnson & Johnson Japan, Tokyo, Japan). The transected line was previously marked by blue dye along the minor axis of the esophagus. The nasogastric tube was then identified and easily pulled out through the incision on the esophageal wall into the abdominal cavity. The umbilical port was extended to a 3-cm incision, and a wound retractor was placed into the wound as an operation port. The nasogastric tube was exteriorized through this port site.

Then the head of the anvil was prepared with 2–0 Ti-Cron sutures (Covidien, Norwalk, CT, USA), and the tail of the anvil was capped with a 10-cm length of nasogastric tube (Fig. 1). The anvil suture was then tied to the nasogastric tube. To prevent slippage of the esophageal mucosa off the esophageal muscle layer during insertion of the anvil head, a stitch was sutured through the esophageal wall. The nasogastric tube was then gently pulled by the other surgeon at the anesthetic position while the tail of the anvil was pushed into the esophageal lumen by the grasper of the first surgeon (Fig. 2). This procedure made insertion of the anvil smooth and atraumatic.

In the final step, when the tail of the anvil reached the esophagus over the diaphragmatic crus, the length of nasogastric tube connected to the tail of the anvil was lifted up and the distal esophagus was transected using an Endo GIA Universal 60-mm stapler (Covidien Japan, Tokyo, Japan). To keep the entry hole of the anvil shaft as tight as possible, the esophageal incision was tightly sutured by two stitches before transection using a linear stapler (Fig. 3). The transaction line was decided along the rectangular marking to the axis of the esophagus to prevent a beak-shaped esophageal stump. This approach makes it

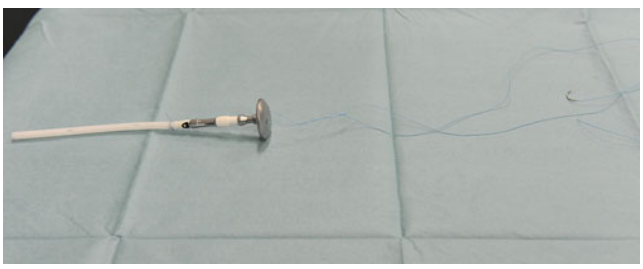

Fig. 1 The head of the anvil is prepared with 2–0 Ti-Cron sutures, and the tail of the anvil is capped with a 10-cm length of nasogastric tube

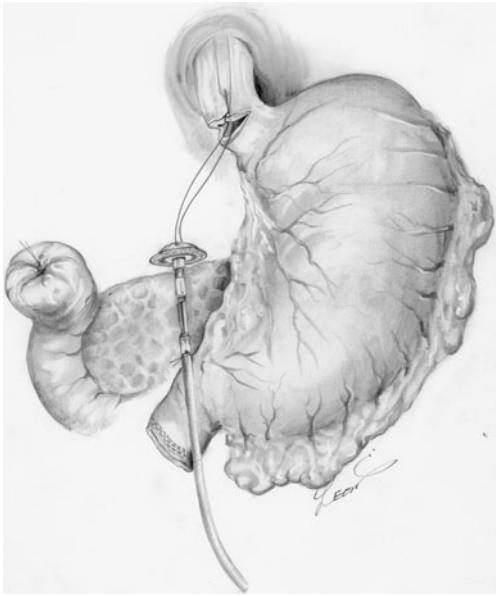

Fig. 2 The anvil suture is tied to the nasogastric tube. To prevent slipping of the esophageal mucosa off the esophageal muscle layer at the insertion of the anvil head, the stitch is sutured through the esophageal wall. The nasogastric tube is then gently pulled by the other surgeon at the anesthetic position while the tail of the anvil is pushed into the esophageal lumen by the grasper of the primary surgeon

possible for the anvil head to be replaced correctly into the esophagus, thus avoiding the need for an additional purse-string suture. The anvil was then positioned correctly by pulling on the length of tubing. Total gastrectomy was completed via these three steps.

Following gastrectomy, the jejunum was transected about 25 cm distal to the ligament of the Treiz, and the third jejunal artery was divided to avoid tension at the esophagojejunum anastomosis while maintaining continuation of the marginal jejunal artery under laparoscopy. The stomach and the distal jejunum were exteriorized through the umbilical port. The jejunum was returned and raised through the antecolic route after insertion of the main body of the mechanical circular stapler (Premium Plus CEEA stapler, 25 mm in diameter; Covidien Japan; Fig. 4), while the shaft of the PCEEA remained external where it could be controlled from the outside of the abdomen. A pneumoperitoneum was created for anastomosis, the esophagojejunum anastomosis was performed laparoscopically (Fig. 5), and then the stick of the raised jejunum was closed using an Endo GIA Universal 60-mm stapler (Fig. 6). The status of the anastomosis and the absence of twisting of the raised small intestine were confirmed also under laparoscopy. A Roux-en-Y anastomosis was performed using an Endo GIA Universal 60-mm stapler under direct vision through the minilaparotomy port at the umbilical site. After hemostasis and washing, a Pertersen hole was sutured by several stitches laparoscopically to complete the operation.

Clinical Analysis

The following clinical data were obtained from medical records: gender, age, body mass index, preoperative complications, and clinical staging of patients. Operative findings such as operation time, estimated blood loss, and number of dissected lymph nodes were also recorded. To assess the postoperative clinical course, postoperative complications, the numbers of days to resumption of oral intake, and length of postoperative hospital stay were recorded. Data were presented as means ± standard error (SE).

Fig. 3 Transection of the esophagus. To keep the entry hole of the anvil shaft as tight as possible, the esophageal incision is tightly sutured by two stitches before transection using a linear stapler. The transaction line is marked along the rectangular marking to the axis of the esophagus to prevent a beak-shaped esophageal stump

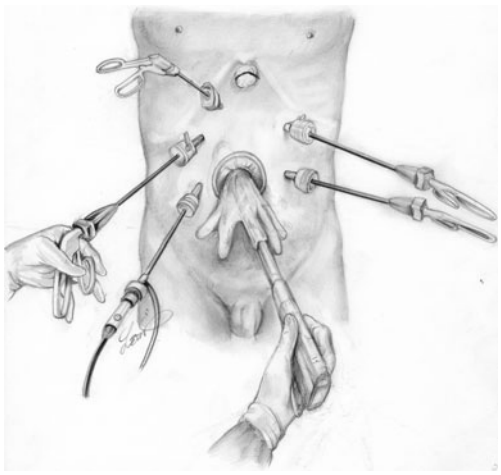

Fig. 4 The jejunum is returned and raised through the antecolic route after insertion of the main body of the mechanical circular stapler (Premium Plus CEEA stapler, 25 mm in diameter), while the shaft of the PCEEA remains externally where it can be controlled from the outside of the abdomen

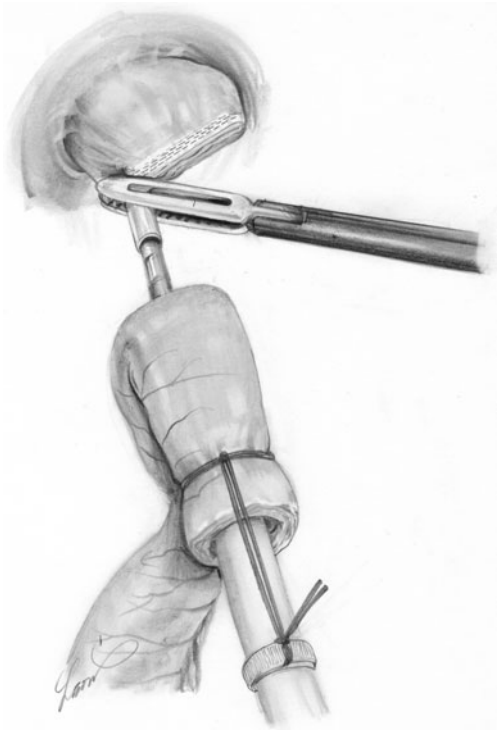

Fig. 5 A pneumoperitoneum is created for anastomosis, and the esophagojejunum anastomosis is performed laparoscopically. Appropriate approximation is confirmed by introducing a laparoscopy through the right and left quadrant

Results

Surgical Results

Patient demographics and clinical histories including age, gender, body mass index, preoperative complications, and clinical staging are presented in Table 1. Only one patient of 41 was diagnosed as clinical stage IB, with all others diagnosed as stage IA. Table 2 summarizes the operative and the postoperative data. The average operation time and estimated blood loss during LATG using the modified lift-up method was 298.6±10.1 min and 85.9±15.2 ml, respectively. None of the patients required conversion to open surgery.

The incidence of postoperative complications is shown in Table 2. Pancreatic fistula, anastomotic leakage, and stenosis occurred in three cases (7.3%), two cases (4.9%), and two cases (7.3%), respectively. Two patients who developed anastomotic leakage were improved by conservative treatment with no further surgical procedure. The

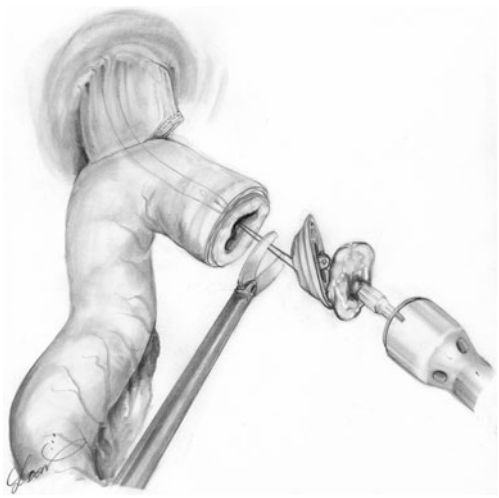

Fig. 6 The stick of the raised jejunum is closed using an Endo GIA Universal 60-mm stapler

Table 1 Characteristics of patients undergoing laparoscopic total gastrectomy reconstructed with a modified lift-up method

Number of cases	41
Sex	
Male/female	31/10
Age (years)	65.8±1.6
Body mass index (kg/m^2)	23.8±0.6
Preoperative complication	
Diabetes	3 (7.3%)
Ischemic heart disease	0 (0.0%)
Hypertension	14 (34.1%)
Cirrhosis	1 (2.4%)
Asthma	2 (4.9%)
History of abdominal operation	9 (22.0%)
Clinical staging	
IA	40 (97.6%)
IB	1 (2.4%)

Data are presented as means±SE. Body mass index = Body weight/Height^2 (kg/m^2)

Table 2 Operative data from patients undergoing laparoscopic total gastrectomy reconstructed with a modified lift-up method

	n=41
Operation time (min)	298.6±10.1
Intra-operative blood loss (ml)	85.9±15.2
Total number of resected lymph nodes	42.8±2.3
Postoperative complications	
Pancreatic fistula	3 (7.3%)
Pneumonia	0 (0%)
Intra-abdominal abscess	2 (4.9%)
Anastomotic leakage	2 (4.9%)
Anastomotic stenosis	3 (7.3%)
Anastomotic bleeding	0 (0%)
Bowel obstruction	0 (0%)
Time until start of oral intake (days)	2.7±0.5
Postoperative hospital stay (days)	16.9±1.5

Data are presented as means±SE

three patients who developed stenosis of the esophagojejunal anastomosis needed endoscopic balloon dilatation of an anastomotic stricture, 30 to 67 days postoperatively. Two patients (4.9%) developed intra-abdominal abscesses without any obvious causative factor including anastomotic failure and pancreatic fistula. Other postoperative complications including pneumonia, anastomotic bleeding, and bowel obstruction were not found in any of the present patients. There was no mortality reported in the present study. The mean time until oral intake resumed was 2.7± 0.5 days, and the mean length of postoperative hospital stay was 16.9±1.5 days.

Discussion

Esophagojejunostomy performed laparoscopically is a complicated procedure requiring a circular stapling device and other instruments, and therefore, laparoscopic approaches have not to date been considered appropriate. However, even under direct vision, esophagojejunostomy can still be a difficult procedure, especially in obese patients with a thick abdominal wall.[11,12] A laparoscopic approach with total gastrectomy and a magnified view would be an excellent option to overcome this problem.

Although stapling devices for EEA have facilitated more rapid and reliable re-establishment of esophagojejunal continuity following esophageal resections, various intra-operative problems can arise in laparoscopic procedure, especially with insertion of the anvil into the esophageal lumen and the proper placement of esophageal purse-string sutures. Our newly developed modified lift-up technique simplifies insertion of the anvil without any manipulation of the esophagus, and simultaneously pushing the tail and pulling the head of the anvil via the nasogastric tube could reduce the need for excessive force. Since the esophagus is not yet completely transected during the insertion of the anvil, this procedure may be technically simple and safe without laparotomy. Consequently, the esophagus can be transected using a linear stapling device, thus totally avoiding the need for purse-string sutures and ensuring that placement of the anvil on the esophageal stump is completed.

A new commercially available device, the Orvil package™ (Covidien), consisting of the 25-mm anvil with the head pretilted and the tip attached to an 18-Fr orogastric tube, permits the esophagojejunal stapled anastomosis with the anvil introduced through the mouth into a nasogastric tube. Although this method theoretically permits an esophagojejunal stapled anastomosis with a 25-mm EEA, difficulties are sometimes encountered in introducing the anvil to the lower esophagus because of narrowing at locations such as the larynx and esophagus at the tracheal bifurcation level. It is also necessary for the tilted anvil head to tilt back automatically into the flat position in the narrow esophageal lumen when it is attached to the main body of the stapler. Thus, esophageal mucosal injury could be caused by a relatively large EEA being introduced into the esophageal lumen. Our technique facilitates the introduction of an adequately sized anvil without the risk of esophageal mucosal injury associated with open gastric surgery.

Anastomotic leakage and stricture are the most important anastomosis-related complications after total gastrectomy, and both were encountered during the present series. Several important points are relevant for preventing anastomotic leakage, including avoiding esophageal injury at the insertion of anvil head, keeping the entry hole of the anvil shaft as tight as possible, and avoiding tension at the esophagojejunum anastomosis. We have modified the previously reported lift-up method with attention to these respects, following our improved results with respect to anastomosis. The stricture rate with a stapler anastomosis was also reported to be high.[13] Although there could be several factors contributing to the stricture of esophagojejunal anastomosis including blood flow, tension of the anastomosis, and the stapler size, special attention should be paid to the anastomotic diameter. The narrow space of an esophageal anastomotic site with the beak-shaped stump could cause stricture after esophagojejunostomy. We therefore also modified our transection of the esophagus and marked the transected line by blue dye along the minor axis of the esophagus.

Conclusion

In conclusion, our modified lift-up technique facilitates circular-stapled esophagojejunostomy in LATG and could be a more feasible and safe method which in particular may avoid anastomotic insufficiency.

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ORIGINAL ARTICLE

Impact of Lymphatic Vessel Invasion on Survival in Curative Resected Gastric Cancer

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Abstract
Background Lymphatic vessel invasion (LV) has been regarded as a prognostic factor in some solid tumors. The aim was to clarify the impact of lymphatic vessel invasion on survival in curative resected gastric cancer.
Methods In this retrospective study, we reviewed the records of 1,024 patients who underwent curative resection for gastric cancer. Among all of the studied patients, 285 of them (27.8%) had lymphatic vessel invasion.
Results There were significant differences in tumor size, tumor location, depth of invasion, and lymph node metastasis (LN) between the patients with lymphatic vessel invasion and those without. The 5-year survival rates in patients were 80.1%, 59.2%, 40.9%, and 30.5% for LN−LV−, LN−LV+, LN+LV−, and LN+LV+ group, respectively. Multivariate analysis revealed that age, tumor location, the depth of invasion, and lymph node metastasis were independent prognostic factors for curative resected gastric cancer. Lymphatic vessel invasion was not an independent prognostic factor in node-positive gastric cancer; however, it was true in node-negative gastric cancer.
Conclusion Lymphatic vessel invasion is one of the independent prognostic factors for node-negative gastric cancer after curative resection.

Keywords Gastric cancer · Lymph node metastasis · Lymphatic vessel invasion · Prognosis

Introduction

The survival of patients with gastric cancer has been improved by early detection, rational lymphadenectomy, chemotherapy, and molecular-targeted therapy.[1,2] However, gastric cancer still remains a major cause of cancer mortality worldwide.[3] Some potential prognostic factors, such as tumor size, depth of tumor invasion, status of lymph node, and histological type, have been evaluated in patients with gastric cancer.[4,5] It is clear that the status of lymph node metastasis (LN) is one of the most important prognostic factors for gastric cancer. The lymphatic system plays an important role in the tumor spread and the tumor recurrence. Recently, lymphatic vessel invasion (LV) has been regarded as a prognostic factor in some solid tumors.[6,7] Although there have been several studies on the lymphatic vessel invasion in gastric cancer,[8,9] the prognostic value of lymphatic vessel invasion has not been fully verified and the studies to this topic are still rare. Many questions related to lymphatic vessel invasion in gastric cancer are waiting to be answered.

The aim of our study was to clarify the influence of lymphatic vessel invasion on the prognosis of the gastric cancer through a large number of patients after curative resection. We compared the clinicopathological features of patients with and without lymphatic vessel invasion and studied the prognostic values of lymphatic vessel invasion in node-negative and also node-positive gastric cancer.

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Patients and Methods

Patients

A total of 1,024 patients with gastric cancer who underwent gastrectomy from January 1996 to December 2005 at Xiangya Hospital of Central South University were retrospectively evaluated in this study. The selection criteria for inclusion included (a) patients who received the curative resection, (b) the resected specimens of the patients were pathologically examined, and (c) the medical records and follow-up data were complete and available. We excluded the patients who have evidences of distant metastasis at operation or died in the postoperative period (30 days). The latest follow-up was carried out in December 2010. The median follow-up was 62 months (range, 2–178 months). The study protocol was approved by the Ethics Committee of Xiangya Hospital, Central South University (Changsha, China).

Lymphatic vessel invasion was determined by the presence of tumor cells in the lumen of lymphatic vessels, which were lined by endothelial cells, with or without lymphocytes. The original histopathology reports of the specimens and the recorded histopathological data of all cases were reviewed. One pathologist re-examined the histopathological slides to confirm the status of the lymphatic vessels for all the cases. If there was a disagreement, another pathologist would be invited to review the slides, and a consensus was reached between the two pathologists.

Clinicopathological characteristics such as age, gender, tumor size, tumor location, type of gastrectomy, histological grade, depth of invasion, and lymph node metastasis were compared in the presence and absence of lymphatic vessel invasion. The depth of tumor invasion and lymph node metastasis were classified according to the seventh edition TNM staging of the American Joint Committee on Cancer and the International Union Against Cancer.[10] The tumor histology was categorized as two groups: differentiated type, which included papillary adenocarcinoma and tubular adenocarcinoma, and undifferentiated type, which included poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and mucinous adenocarcinoma.

Statistical Analysis

All statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Clinicopathological characteristics were analyzed by chi-square tests and independent *t* tests. According to the status of LN and presence or absence of LV, the patients were divided into four groups, namely LN−LV− group, LN−LV+ group, LN+LV− group, and LN+LV+ group. The Kaplan–Meier method and log rank test were adopted for the analysis of the survival rate comparison. Multivariate analysis was performed with the Cox proportional hazards regression model. A *P* value of less than 0.05 was considered statistically significant.

Results

Clinicopathological Characteristics of the Patients

Among the 1,024 patients with gastric cancer in our study, the male-to-female ratio was 2.51, and the mean age was 56.7 years (range, 26–80 years). Lymphatic vessel invasion was observed in 285 of 1,024 cases (27.8%). As show in Table 1, the mean tumor size (5.7 cm) of the patients with lymphatic vessel invasion was significantly larger than that (5.0 cm) of the patients without lymphatic vessel invasion ($P<0.001$), and 8.8% cases in patients with lymphatic vessel invasion occupied the total stomach. For the patients with lymphatic vessel invasion, poorer differentiated tumors ($P<0.001$), deeper tumor invasion ($P<0.001$), and more lymph node metastasis ($P<0.001$) were noted compared to the patients without lymphatic vessel invasion. There were no significant differences in terms of age ($P=0.242$), gender ($P=0.910$), and gastrectomy type ($P=0.069$) between the patients with and without lymphatic vessel invasion.

Survival Analysis

As shown in Fig. 1, in node-negative gastric cancer, the 5-year survival rate of the patients with lymphatic vessel invasion (LN−LV+ group) was significantly lower than that of the patients without lymphatic vessel invasion (LN−LV− group; 59.2% vs 80.1%; $P<0.001$). For the patients with node-positive gastric cancer, the 5-year survival rate was significantly lower in the cases with lymphatic vessel invasion (LN+LV+ group) than in those without lymphatic vessel invasion (LN+LV− group; 30.5% vs 40.9%; $P=0.008$).

Prognostic Factors in Gastric Cancer

Using multivariate analysis, age ($P=0.007$), tumor location ($P=0.003$), the depth of tumor invasion ($P<0.001$), and lymph node metastasis ($P<0.001$) were identified as the independent prognostic factors for patients with gastric cancer after curative resection (Table 2). In addition, we identified age ($P=0.031$), depth of invasion ($P<0.001$), and lymphatic vessel invasion ($P=0.016$) as independent prognostic factors in node-negative gastric cancer (Table 3). While in gastric cancer with positive lymph nodes, it was found that tumor size ($P=0.025$), tumor location ($P=0.014$), and depth of invasion ($P<0.001$) were independent prognostic factors (Table 4).

Table 1 Clinicopathological characteristics of patients with gastric cancer according to lymphatic vessel invasion

Characteristics	Lymphatic vessel invasion		*P* value
	Absent (*n*=739; %)	Present (*n*=285; %)	
Age (years)	56.4±10.4	57.3±9.6	0.242
Gender			0.910
Male	529 (71.6)	203 (71.2)	
Female	210 (28.4)	82 (28.8)	
Tumor size (cm)	5.0±2.7	5.7±2.9	<0.001
Tumor location			0.045
Lower	499 (67.5)	187 (65.6)	
Middle	124 (16.8)	48 (16.8)	
Upper	83 (11.2)	25 (8.8)	
Entire	33 (4.5)	25 (8.8)	
Type of gastrectomy			0.069
Partial	633 (85.7)	231 (81.0)	
Total	106 (14.3)	54 (18.9)	
Histological grade			<0.001
Differentiated	304 (41.1)	79 (27.7)	
Undifferentiated	435 (58.9)	206 (72.3)	
Depth of invasion			<0.001
T1	137 (18.5)	7 (2.5)	
T2	166 (22.5)	34 (11.9)	
T3	236 (31.9)	97 (34.0)	
T4	200 (27.1)	147 (51.6)	
Lymph node metastasis			<0.001
N0	331 (44.8)	49 (17.2)	
N1	144 (19.5)	48 (16.8)	
N2	135 (18.3)	96 (33.7)	
N3	129 (17.4)	92 (32.3)	

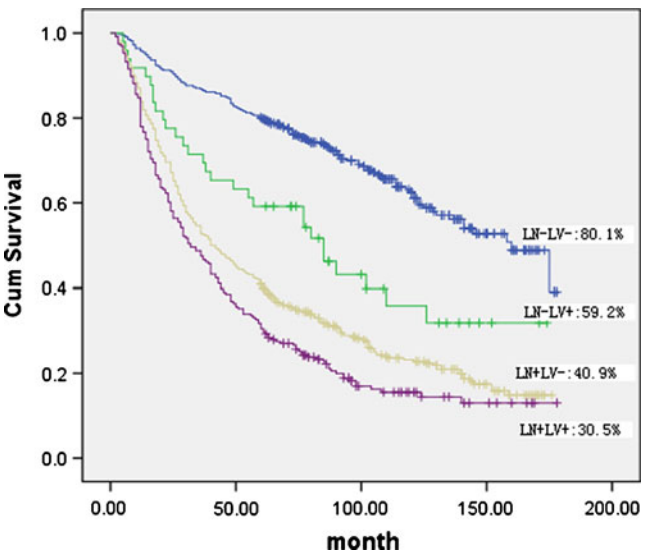

Fig. 1 Cumulative survival curves for curative resected gastric cancer

Discussion

Gastric cancer is one of the most common malignancies and is also a major cause of the cancer-related death worldwide.[3] Lymphatic vessel invasion is one of the key steps in the tumor spread and metastasis and correlates with the recurrence and survival in some solid tumors.[6,7] Investigations have discussed the influence of lymphatic vessel invasion on the prognosis in gastric cancer; however, the studies are rare and the results are controversial.

In our study, lymphatic vessel invasion was defined as the presence of tumor cells in the lumen of lymphatic vessels, which were lined by endothelial cells, with or without lymphocytes, and the sections of the tumor at the maximal diameter were examined. Theoretically, the method to identify lymphatic vessels invasion in a tumor is to examine all the tumor tissues from surgical specimen. However, it is a huge workload to examine all serial sections from the tumor tissues of all the patients, which is actually impractical. Lymphatic vessel invasion is associated with tumor aggressiveness, so the sections of the tumor

Table 2 Multivariate analysis of prognostic factors in patients with gastric cancer after curative resection

Factors	Hazard ratio (95% CI)	*P* value
Age (years)		
≤60	1	
>60	1.242 (1.061–1.453)	0.007
Tumor location		0.003
Lower	1	
Middle	1.033 (0.836–1.277)	0.760
Upper	1.394 (1.099–1.769)	0.006
Entire	1.554 (1.145–2.110)	0.005
Depth of invasion		<0.001
T1	1	
T2	1.459 (1.002–2.126)	0.049
T3	2.274 (1.597–3.237)	<0.001
T4	2.863 (2.002–4.095)	<0.001
Lymph node metastasis		<0.001
N0	1	
N1	1.642 (1.284–2.101)	<0.001
N2	2.357 (1.870–2.970)	<0.001
N3	3.919 (3.105–4.947)	<0.001

at the maximal diameter are representative. We consider the use of multiple sections of the tumor at the maximal diameter to be the most suitable method of sampling to evaluate lymphatic invasion.

Several authors reported that tumor size tended to be similar in patients with lymphatic vessel invasion and those without.[9] In our study, the mean tumor size of the patients with lymphatic vessel invasion tended to be larger than that of those without lymphatic vessel invasion (5.7 vs 5.0 cm; $P<0.001$; Table 1). Our results showed that there were a large proportion of undifferentiated types in patients with lymphatic vessel invasion. In contrast, other studies stated

Table 3 Multivariate analysis of prognostic factors in patients with node-negative gastric cancer

Factors	Hazard ratio (95% CI)	*P* value
Age (years)		
≤60	1	
>60	1.448 (1.035–2.027)	0.031
Depth of invasion		<0.001
T1	1	
T2	1.561 (0.950–2.566)	0.079
T3	2.195 (1.381–3.490)	0.001
T4	3.016 (1.858–4.896)	<0.001
Lymphatic vessel invasion		
Absent	1	
Present	1.684 (1.103–2.572)	0.016

Table 4 Multivariate analysis of prognostic factors in patients with node-positive gastric cancer

Factors	Hazard ratio (95% CI)	*P* value
Tumor size (cm)		
≤5	1	
>5	1.235 (1.027–1.484)	0.025
Tumor location		0.014
Lower	1	
Middle	0.997 (0.780–1.274)	0.982
Upper	1.393 (1.060–1.832)	0.018
Entire	1.522 (1.085–2.137)	0.015
Depth of invasion		<0.001
T1	1	
T2	1.436 (0.738–2.793)	0.287
T3	2.213 (1.167–4.194)	0.015
T4	3.113 (1.648–5.888)	<0.001

that there was no correlation between lymphatic vessel invasion and tumor histological grade in gastric cancer.[8,9,11] Meanwhile, we found that lymphatic vessel invasion had a strong link with the depth of tumor invasion ($P<0.001$; Table 1), and similar observation has also been noted in the report of Borie et al.[8] Furthermore, in our study, the patients with lymphatic vessel invasion had a larger proportion of subserosa (T3) and serosa (T4) invasion than those without lymphatic vessel invasion. One explanation to this is that the subserosa is a thin layer of loose connective tissue which lies below the muscularis and contains lymphatic vessels, blood vessels, and nerve fibers,[12] and when the tumor breaks through muscularis and invades subserosa, the probability of invading vessels will significantly increase. In many solid tumors, a positive association between lymphatic vessel invasion and lymph node metastasis has been observed.[13,14] One of the early steps in metastatic process is the penetration of tumor cells into the lymphatic vessels which are in and around the primary tumor site.[15] Then tumor cells are carried through the lymphatic vessels to the regional lymph nodes as tumor emboli. Lymphatic vessel invasion also correlated with lymph node metastasis ($P<0.001$; Table 1) in our study.

All these results indicated that lymphatic vessel invasion is strongly associated with unfavorable cancer characteristics in patients with gastric cancer. Consequently, the patients with lymphatic vessel invasion had worse prognosis than those without lymphatic vessel invasion (35.4% vs 58.5%; $P<0.001$), which is consistent with previous reports.[11] Because survival of gastric cancer is significantly influenced by lymph node metastasis, we evaluated prognosis according to lymphatic vessel invasion under the same conditions of lymph node metastasis. For the patients with node-negative gastric cancer, the 5-year

survival rates were significantly different between those with lymphatic vessel invasion and those without (59.2% vs 80.1%; $P<0.001$). Even for the node-positive gastric cancer, the 5-year survival of patients with lymphatic vessel invasion was also significantly lower than those without lymphatic vessel invasion (30.5% vs 40.9%; $P=0.008$). In addition, the difference of the survival curves between the node-negative cases with lymphatic vessel invasion and the node-positive cases without lymphatic vessel invasion was significant ($P=0.016$; Fig. 1).

In our study, multivariate analysis indicated that lymph vessel invasion is not an independent poor prognostic factor for the survival of the patients with gastric cancer after curative resection. The independent prognostic factors were age, tumor location, the depth of tumor invasion, and lymph node metastasis (Table 2). Through further analysis, some interesting phenomenon was observed. We identified lymphatic vessel invasion as an independent prognostic factor for patients with lymph node-negative gastric cancer after curative resection (Table 3). Although the patients with node-negative gastric cancer underwent the curative surgery, lots of them still suffered from the tumor recurrence through several recurrent routes including lymphatic spreading, hematogenous spreading, and locoregional recurrence. Some authors reported that lymphatic vessel invasion was an independent risk factor for recurrence and poor prognosis for patients with the node-negative gastric cancer.[11] Our results were consistent with previous studies. Invasion and metastasis are the major causes of death for the patients with gastric cancer and the important factors affecting treatment and prognosis. The gastric cancer patients with lymphatic vessel invasion have worse prognosis than those without, which might be explained by more aggressive biological behavior and increased probability of cancer recurrence in these tumors. However, lymphatic vessel invasion, affecting the survival of patients with lymph node positive, was not observed as an independent prognostic factor for survival in node-positive gastric cancer (Table 4). These findings might be explained by that lymph node metastasis is more important than lymph vessel invasion in affecting the prognosis of gastric cancer. Under the condition of no lymph node metastasis, lymphatic vessel invasion will affect the survival of patients with gastric cancer as an independent prognostic factor.

Tumor cells can penetrate vessels, invade surrounding tissue, and escape to the distance. The presence of lymphatic vessel invasion indicates that tumor cells have already invaded the lymphatic system; therefore, radical gastrectomy should remove the tumor lesion, the regional lymph nodes, and the involved surrounding tissue completely as whole resection in order to avoid the tumor cells falling into the abdominal cavity from the vessels. No lymph node metastasis does not mean no lymphatic spread and can neither negate the value of lymphadenectomy. In addition, preoperative chemotherapy and postoperative chemotherapy may have a positive effect that can eliminate the micrometastasis and free tumor cells in lymphatic system.[16]

Conclusion

This research demonstrated that lymphatic vessel invasion is one of the independent prognostic factors for node-negative gastric cancer after curative resection. The detection of lymphatic vessel invasion in gastric cancer, especially in lymph node-negative gastric cancer, will be helpful to predict patient prognosis more accurately and identify the patients at high risk for recurrence and metastasis and establish the individualized treatment plan and monitor plan.

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ORIGINAL ARTICLE

Prophylactic Cholecystectomy, a Mandatory Step in Morbidly Obese Patients Undergoing Laparoscopic Roux-en-Y Gastric Bypass?

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Abstract
Background The aim of this study was to determine the incidence of symptomatic gallstone disease requiring cholecystectomy (CCE) after laparoscopic Roux-en-Y gastric bypass (LRYGBP) and to identify the peri-operative risk factors associated with postoperative symptomatic gallstone disease.
Methods Between August 2003 and November 2009, 724 patients underwent LRYGBP at the Groeninge Hospital. Preoperative ultrasound was performed in 600 of 641 patients without history of CCE and 120 (20.0%) were diagnosed with cholecystolithiasis.
Result Six hundred twenty-five patients were included, 43(6.9%) developed delayed symptoms related to biliary disease. Of these 43 patients, 39 underwent post-LRYGBP CCE. Of these 39 patients, 9 (7.5%) had a positive ultrasound prior to LRYGBP. Multivariate analysis identified weight loss at 3 months post-LRYGB of more than 50% of excess weight [HR (95% CI), 2.04 (1.04–4.28); $p=0.037$) as the sole significant independent predictor of delayed symptomatic cholecystolithiasis.
Conclusions Symptomatic gallstone disease occurred only in 6.9% of patients post-LRYGBP. Multivariate analysis identified weight loss at 3 months post-LRYGBP of more than 50% of excess weight as the sole significant independent predictor of delayed symptomatic cholecystolithiasis. Prophylactic CCE should not be recommended at the time of LRYGBP.

Keywords Laparoscopic gastric bypass · Prophylactic cholecystectomy

Introduction

Morbid obesity predisposes patients to comorbid diseases affecting almost every organ system including type 2 diabetes, cardiovascular disease, hypertension, hyperlipidemia, hypoventilation syndrome, asthma, sleep apnea, stroke, pseudotumor cerebri, arthritis, several types of cancers, gallbladder disease, urinary incontinence, and depression.[1,2] Surgery has been the only effective treatment for morbid obesity with long-term sustained weight loss and postoperative complete resolution or significant improvement in obesity-related comorbidities. Because of the increasing prevalence of morbid obesity, the number of bariatric operations performed each year is growing rapidly.[3,4] Roux-en-Y gastric bypass (RYGBP) is considered by many surgeons to be the "gold standard" bariatric procedure.

Cholecystectomy (CCE) is one of the most performed abdominal operations today.[5] Prophylactic CCE at the time of gastric bypass has been proposed, but there are conflicting views regarding this strategy.[6] In the morbidly obese patient, prophylactic laparoscopic CCE can be difficult. Often the gallbladder is engulfed by the large liver and is difficult to dissect laparoscopically.[7] Historically, it has been proposed

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that up to one third of patients develop gallstones after RYGBP.[6,8–10] However, contradictory reports have been published regarding the incidence of symptomatic gallstone disease requiring CCE with reported rates ranging between 3% and 28%.[9,11–14] From a clinical point of view, it is important to recognize risk factors in the morbid obese patient, predictive for the development of symptomatic gallbladder disease, particularly in asymptomatic patients planned to undergo laparoscopic Roux-en-Y gastric bypass (LRYGBP).

The aim of the present study was to determine the incidence of symptomatic gallstone disease requiring CCE after LRYGBP and to identify the peri-operative risk factors associated with postoperative symptomatic gallstone disease. This would enable surgeons to perform prophylactic CCE at the time of LRYGBP only in selected high-risk patient groups.

Methods

Demographics

Between August 2003 and November 2009, 724 patients (201 males, 523 females) underwent LRYGBP at the Groeninge Hospital, Kortrijk, Belgium. Mean age of patients at the time of surgery was 39.7 (range, 15.9–68.3) years. All operations were performed by three surgeons (FV, DD, and FVR). Mean BMI (in kilograms per square meter) of the patients was 42.3 (range, 21.8–64.0).

Preoperative ultrasound was performed in 600 of 641 patients without history of CCE. Of these 600 patients, 120 (20.0%) were diagnosed with cholecystolithiasis. Of these 120 patients, 16 (13.3%) underwent CCE simultaneously with LRYGBP.

Follow-Up

All patients followed a strict follow-up protocol at our outpatient clinic. Return visits were scheduled at 1, 3, 6, and 12 months after surgery. After the first year, an annual checkup was organized. At present, 577 of 625 patients (92.3%) eligible for evaluation were still in follow-up. The median follow-up time was 51 months (range, 6–78 months).

Statistics

Statistical calculations were carried out using JMP version 8.0.1 for Mac (SAS, 2009). An exact 95% confidence interval was constructed for the percentage of post-RYGBP CCE. Patients who underwent CCE prior to or simultaneously with LRYGBP were excluded from multivariate Cox regression analysis (N=99). A priori dichotomizations were considered for continuous and ordinal variables. First, for a set of variables, the relation with the occurrence of post-LRYGBP CCE was verified in a univariate Cox regression analysis. Variables significant at the 0.10 level in the univariate analysis were combined into a multivariable model.

Demographic and clinical variables considered were: age (years), gender (male/female), preoperative weight (in kilograms), preoperative length (in centimeters), BMI (in kilograms per square centimeter), preoperative excess weight (in kilograms), cardiovascular disease (yes/no), dyslipidemia (yes/no), diabetes mellitus (yes/no), thyroid disease (yes/no), lithiasis on preoperative ultrasound (yes/no), percent of excess weight loss (EWL) at 1 month, percent of EWL at 3 months, >50% EWL at 1 month (yes/no), and >50% EWL at 3 months (yes/no). No variable selection techniques were used to reduce the final multivariable model. Two-sided p values less than 0.05 were considered statistically significant.

Results

After exclusion, 625 patients were included. Of these 625 patients, 43 (6.9%, 95% CI, 4.9%–8.9%) developed delayed symptoms related to biliary disease after a median follow-up of 51 months (range, 6–78 months). Of these 43 patients, 39 underwent uncomplicated post-LRYGBP CCE. Of these 39 patients, only 9 (7.5%) had a positive ultrasound prior to LRYGBP. On the other hand, 28 patients who underwent post-LRYGBP CCE had no signs of gallstone disease on ultrasound prior to LRYGBP. Two of the 39 patients who underwent post-LRYGBP CCE did not undergo an abdominal ultrasound prior to LRYGBP. The mean (±SD) time between LRYGBP and post-LRYGBP CCE was 17.4±13.1 months.

Univariate Analysis (Tables 1 and 2)

Univariate Cox regression (Table 2) identified a history of cardiovascular disease (p=0.10), dyslipidemia (p=0.086), diabetes mellitus (p=0.020), and weight loss at 3 months post-LRYGBP of more than 50% of excess weight (p=0.020) to have a p value<0.1 in the univariate analysis and were withheld for multivariate analysis.

Multivariate Analysis (Table 2)

Multivariate analysis (Table 2) identified weight loss at 3 months post-LRYGBP of more than 50% of excess weight [HR (95% CI), 2.04 (1.04–4.28); p=0.037) as the sole significant independent predictor of delayed cholecystectomy post-LRYGBP in our study cohort. No further attempts were made to reduce the number of variables in the final model.

Table 1 Demographics, clinical, and postoperative parameters

	CCE (n=39)	No CCE (n=586)	All (n=625)	p value
Age (range) (years)	38.8 (20.5–64.8)	38.1 (15.9–68.3)	38.1 (15.9–68.3)	0.41
Gender (M:F)	9:30	185:401	194:431	0.26
Preop BMI (range) (kg/m^2)	41.9 (35.5–53.3)	41.4 (21.8–64.0)	41.5 (21.8–64.0)	0.99
Cardiovascular disease	0	26 (4.4%)	26 (4.2%)	0.40
Dyslipidemia	5 (12.8%)	158 (27.0%)	163 (26.1%)	0.06
Diabetes mellitus	1 (2.5%)	82 (14.0%)	83 (13.3%)	0.05
Preop US (+)	9 (24.3%)	95 (17.4%)	104 (17.8%)	0.27
% EWL at 1 month (IQR)	31.5 (21.4–39.1)	28.4 (21.3–35.4)	28.6 (21.4–35.7)	0.94
% EWL at 3 months (IQR)	56.7 (44.7–62.8)	50.8 (42.1–60.9)	51.3 (42.2–61.4)	0.85
>50% EWL at 1 month	2 (5.1%)	27 (4.6%)	29 (4.6%)	0.70
>50% EWL at 3 months	28 (71.8%)	309 (52.7%)	337 (53.9%)	0.03

CCE cholecystectomy, *M* male, *F* female, *Preop* preoperative, *Preop US* (+) bile stones one preoperative ultrasound, *EWL* excess weight loss, *IQR* interquartile range, *BMI* body mass index

Discussion

Gallbladder disease is one of the most frequent obesity-related comorbid conditions.[8,15,16]Rapid weight loss in morbid obese patients is a well-established risk factor for the development of gallstones.[9]Many factors have been proposed which may cause the development of cholesterol gallstones in the morbid obese patient undergoing gastric bypass surgery. Cholesterol supersaturation of hepatic bile may be the inciting factor for stone development. This was studied by Shiffman et al. by comparing bile samples taken from patients at the time of gastric bypass surgery with samples taken from patients that developed stones after they had a RYGBP.[9,17]Furthermore, morbidly obese patients have increased secretion of calcium and mucin within the gallbladder during the phase of rapid weight loss. Together with an increased concentration of prostaglandins and arachidonic acid in the bile, these elevated levels of mucin and calcium can promote gallstone formation.[15,18]Dieting and fasting in obese patients also lead to gallbladder stasis by resistance to cholecystokinin as obesity is a cholecystokinin-resistant state. Furthermore, hypocaloric meals which are used during periods of dieting or postoperatively in bariatric patients can also cause gallbladder stasis.[19]

In Europe, ultrasound studies demonstrated gallstones in 10–15% of adults. In our obese patient population, preoperative ultrasound was performed in 600 of 641 patients without history of CCE. Twenty percent of patients were diagnosed with cholecystolithiasis. But since postoperative gallstone formation itself is not a clinically relevant outcome, in our study, we focused on symptomatic gallbladder disease requiring cholecystectomy following LRYGBP. Larger studies demonstrated that in asymptomatic bariatric patients followed for symptomatic gallbladder disease, 3.8–11.5% will become symptomatic requiring subsequent CCE.[6,15,20,21]In our series, 6.9% of patients developed symptomatic gallbladder disease. Furthermore, a recent review by Sakorafas et al. indicated that the progression of asymptomatic patients to symptomatic disease is relatively low.[22]

The traditional risk factors for gallstone formation in the general population such as age, female gender, and obesity were not associated with symptomatic gallstone disease in our study group. In our study cohort, multivariate analysis

Table 2 Results of univariate and multivariate Cox regression analysis

Parameters	Univariate		Multivariate	
	HR (95% CI)	p value	HR (95% CI)	p value
Cardiovascular disease	<0. 001 (0–1.42)	0.10	<0.001 (0–3.02)	0.25
Dyslipidemia	0.47 (0.16–1.10)	0.086	0.61 (0.21–1.44)	0.28
Diabetes mellitus	0.17 (0.01–0.80)	0.020	0.25 (0.014–1.19)	0.090
% EWL 3>50[a]	2.20 (1.10–4.61)	0.020	2.04 (1.04–4.28)	0.037

HR hazard ratio, *CI* confidence interval, *EWL* excess weight loss

[a] More than 50% loss of excess weight at 3 months post Roux-en-Y gastric bypass

identified weight loss at 3 months post-LRYGBP of more than 50% of excess weight as the sole significant independent predictor of symptomatic gallbladder disease post-LRYGBP. No preoperative risk factors associated with post-LRYGBP symptomatic gallstone disease could be identified. In a recent study, Li et al. also found a weight loss of more than 25% of original weight to be associated with symptomatic gallstone formation.[23]Their patients underwent LRYGBP or a restrictive bariatric procedure (laparoscopic gastic banding or laparscopic sleeve gastrectomy). In our study group consisting only of patients who underwent LRYGBP, only weight loss at 3 months post-LRYGBP of more than 50% of excess weight was the sole significant independent predictor. There is controversy regarding the approach of performing prophylactic CCE together with RYGBP. Although some reports advocate prophylactic CCE, to date there is no clear consensus since other authors advise a conservative approach.[8,12,24–30]Based on our findings, we also recommend a conservative approach since only 6.9% of our patients develop symptomatic gallbladder disease after LRYGBP. A routine CCE in our study group would have exposed 582 patients to an unnecessary procedure. An advantage of delayed CCE is that the procedure might be technically easier to perform as a consequence of the reduced intra-abdominal fat and liver size after LRYGBP.[7]In a nationwide study, Livingston et al. showed that obesity was one of the major predictors for conversion from laparoscopic to open CCE.[31]Furthermore, since all LRYGBP procedures in the current series were performed laparoscopically only minimal adhesions are to be expected.

In summary, the frequency of gallstone disease was higher in our obese population compared to the general European population. No preoperative risk factors associated with postoperative symptomatic gallstone disease were found.

Symptomatic gallstone disease occurred only in 6.9% of patients post-LRYGBP. Multivariate analysis identified weight loss at 3 months post-LRYGBP of more than 50% of excess weight as the sole significant independent predictor of delayed symptomatic cholecystolithiasis in our study cohort. Routine prophylactic CCE should not be recommended at the time of LRYGBP.

Disclosures None

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ORIGINAL ARTICLE

Peptide Absorption After Massive Proximal Small Bowel Resection: Mechanisms of Ileal Adaptation

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Abstract
Background Protein absorption occurs as di- and tri-peptides via H^+/peptide co-transporter-1 (PepT1).
Aim The aim of this study is to identify mechanisms of ileal adaptation after massive proximal enterectomy.
Hypothesis Ileal adaptation in uptake of peptides is mediated through upregulation of PepT1 gene expression.
Study Design Rats underwent 70% jejunoileal resection. Total mucosal cellular levels of messenger RNA (mRNA) and protein and transporter-mediated uptake per centimeter of the di-peptide glycyl-sarcosine (Gly-Sar) were compared in remnant ileum 1 and 4 weeks postoperatively to control and to 1-week sham laparotomy rats. Histomorphology, food consumption, and weights of rats were monitored.
Results After 70% resection, although mRNA per cell for PepT1 decreased at 1 week ($p=0.002$), expression of mRNA at 4 weeks and protein at 1 and 4 weeks in remnant ileum were unchanged ($p>0.1$). Ileal Gly-Sar uptake (V_{max}—nanomoles per centimeter per minute, i.e., number of transporters per centimeter) increased at 1 and 4 weeks compared to control and 1-week sham ($p<0.05$ each); K_m (i.e., transporter function) was unchanged. Villous heights (millimeters) in remnant ileum increased at 1- and 4-week time points over controls (0.45 and 0.57 vs 0.21, resp; $p<0.001$).
Conclusions Ileal adaptation to proximal resection for peptide absorption occurs through cellular proliferation (hyperplasia) and not through cellular upregulation of PepT1 mRNA or protein per enterocyte.

Keywords Peptide absorption · PepT1 · Short bowel syndrome · Small bowel resection · Adaptation

Abbreviations

cDNA	Complementary DNA
GAPDH	Glyceraldehyde-6-phosphate dehydrogenase
GLUT2	Glucose transporter 2
Gly-Sar	Glycyl-sarcosine
IQR	Interquartile range
mRNA	Messenger RNA
NC	Naïve control rats
PepT1	Peptide transporter 1
RT-PCR	Real-time polymerase chain reaction
RXN	Resection (used in illustrations only)
SBS	Short bowel syndrome
TBS-T	Tris-buffered saline with Tween

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Introduction

Extensive operative resection of the small intestine can be imperative in the management of several pathologic conditions but may result in the devastating malabsorptive state of "short bowel syndrome (SBS)".[1, 2] A similar state

can follow traumatic loss of intestinal surface area, also leading to SBS. Despite recent advances in treatment options for patients with short bowel, current therapies still carry substantial risk of morbidity and mortality.[3–6]

Several studies conducted in laboratory animals as well as human subjects have shown that after massive, proximal-based, small bowel resection, the remnant ileum undergoes morphologic and functional adaptive changes in an attempt to maintain nutritional health by increasing net ileal absorption of enteral nutrients.[7–12] This inherent capability of the ileum to "adapt" has created interest in studying the cellular mechanisms responsible for ileal adaptation to formulate novel therapies for treatment of these patients. In our laboratory, we investigated previously the mechanisms of ileal adaptation for glucose absorption after extensive resection of the proximal intestine in a rat model.[13] Recently, we examined the response of the proximal jejunum to a massive, mid-small bowel resection by evaluating gene expression of the peptide transporter PepT1 and peptide absorption,[14] but to the best of our knowledge, this current study is the first comprehensive investigation of the effect of massive, proximal, jejunoileal resection on ileal gene expression of peptide transporter-1 (PepT1), the exclusive peptide transporter in the brush border of enterocytes and peptide uptake. Colonic gene expression of PepT1 was also investigated in this study before and after massive proximal small bowel resection.

The physiologic and clinical importance of intestinal PepT1 has been well recognized only recently; PepT1 mediates the uptake of essentially all di- and tri-peptides (the major protein digestion products) in addition to several peptide-like drugs from the intestinal lumen.[15–19] Indeed, PepT1 appears to account for the majority of protein absorbed. Absorption of short peptides and peptide-like substances under normal conditions occurs predominantly in the proximal intestine (primarily the jejunum), with the ileum absorbing a substantially lesser amount of peptides. Colonic absorption of peptides in the normal physiologic state is insignificant.[17–21] Our aim in this study was to identify the mechanisms of ileal adaptation (and colonic adaptation, if any) for peptide absorption after massive, proximal small bowel resection. We hypothesized that intestinal adaptation occurs via increased enterocyte expression of messenger RNA (mRNA) and protein for PepT1 in the enterocytes, resulting in increased absorption of small peptides.

Methods

After approval from our Institutional Animal Care and Use Committee and in accordance with the NIH guidelines for the humane use and care of laboratory animals, 30 male, Lewis rats weighing 200–250 g (Harlan, Indianapolis, IN) were maintained in a 12-h light/dark room (lights on from 6 AM to 6 PM). Twelve rats underwent a 70%, proximal-based, small bowel resection (see below, Small Intestinal Resection); these rats were then studied at 1 or 4 weeks postoperatively (n=6, each group). An additional group of six rats were studied 1 week after sham celiotomy to control for anesthesia and other postoperative changes; a group of six control rats (naïve control rats (NC)) housed in similar conditions were studied after 1 week. In addition, six unoperated rats were observed for 4 weeks to monitor weight gain and food consumption throughout the entire period of study (no tissue was harvested from this monitor group). All rats were housed in the 12-h photoperiod facility and allowed free access to water and standard rat chow (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO). Daily weights of the rat and the chow consumed (per light/dark cycle) were tabulated until the time of harvest. Because gene expression and transport function of PepT1 are known to vary diurnally,[18–21] a set time point of the light/dark cycle (3 PM) was chosen to harvest the intestinal tissue; we selected this time point, because we have shown previously that gene expression and transport function of PepT1 peak 3 h before the dark cycle.[20, 21]

Small Intestinal Resection

Rats were anesthetized initially using 2% inhaled isoflurane followed by intraperitoneal injection of 50 mg/kg sodium pentobarbital. A short celiotomy (1 cm) was performed, and the small bowel was exteriorized. The proximal 70% of the small intestine starting from the ligament of Treitz was resected after ligating the mesenteric blood supply which left about 15 cm of distal ileum. An end-to-end, single layer anastomosis was then performed using running 7–0 polypropylene sutures. The intestine was then returned into the peritoneal cavity, and the abdominal wall was closed in two layers with running 5–0 polyglactin suture. Sham celiotomy was performed under similar anesthesia using a short celiotomy with exteriorization of the entire small bowel. The intestine was manipulated manually for 5 min to simulate the resection procedure prior to reduction back into the abdomen. Abdominal closure was performed as above. Rats were deprived of food but not water for 24 h before any procedure. Postoperatively, all animals were maintained on water containing acetaminophen for 24 h after which they were allowed free access to chow.

Tissue Harvest

All tissue was harvested at a fixed time of the day due to known diurnal patterns in expression and function of

PepT1.[20, 21] At the time of tissue harvest, rats were anesthetized with inhaled isoflurane and intraperitoneal pentobarbital. After celiotomy, the duodenum was cannulated just distal to pylorus, and the bowel was flushed with cold (4°C) Ringer's solution (in mm: 128 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 20 $NaHCO_3$; pH 7.3–7.4; 290 mOsm). The remnant ileum and the proximal 5 cm of colon were harvested. In NC and sham rats, the distal 15 cm of ileum was harvested which corresponded to the same segment of ileum harvested from the resection group. The harvested tissue was placed immediately in cold (4°C), oxygenated (95% O_2/5% CO_2) Ringer's solution. The distal portion of remnant ileum was used for measuring peptide uptake using the everted sleeve technique (see below, Uptake Function), and the proximal segment of remnant ileum was used for mRNA and protein measurements. There was not enough ileal length to evaluate differences in the proximal and distal aspects of remnant ileum. Only PepT1 expression was studied in the colonic mucosal samples; colonic uptake was not evaluated, because our prior study[14] showed virtually no colonic absorption of the model dipeptide. The mucosa was scraped bluntly using a glass slide into cold, phosphate-buffered saline; although the mucosal scrapings include some non-enterocytes in the sample, PepT1 is expressed almost exclusively in the enterocyte only. Samples for mRNA analysis were placed in RNA stabilization buffer (RNA-Later, Qiagen, Valencia, CA) and stored immediately at −80°C. The samples for protein analysis were collected separately, placed in cold RIPA buffer containing protease inhibitors (Halt protease inhibitor cocktail, Pierce, Rockford, IL) and phenylmethane sulfonyl fluoride solution (PMSF; Sigma Aldrich, St. Louis, MO), and stored at −80°C. For histomorphometry, 0.5-cm portions were excised from the middle of remnant ileum, pinned out carefully on a support, and fixed in 10% buffered formalin.

mRNA Measurement

Real-time polymerase chain reaction (RT-PCR) was used to quantitate mRNA levels of PepT1 as described previously.[20, 21] Mucosal samples stored in RNA stabilization buffer were thawed on ice, and homogenized RNA was isolated using the RNeasy Midi kit (Qiagen). RNA was then reverse transcribed into complementary DNA (cDNA) using the Super Script III kit (Invitrogen, Carlsbad, CA) and random hexamer primers. The cDNA levels of PepT1 and the stably expressed housekeeping gene glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were then determined quantitatively using RT-PCR in a 7500 Thermocycler using Taqman® chemistries with primers and fluorescently labeled probes in assay mixes (Applied Biosystems, San Francisco, CA). Standard curves from serial dilutions of known copy numbers were used to calculate copy numbers of cDNA for each sample. All samples were run as duplicates with 2 μl of cDNA added to 23 μl of master mix. PCR was carried out at 50°C for 2 min, then 95°C for 10 min, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C during which fluorescence measurements were made. Transporter copy numbers were normalized to copy numbers of GAPDH from each sample in an attempt to determine relative expression per enterocyte.

Protein Measurement

Levels of total cellular protein for PepT1 were measured semiquantitatively using our well-characterized technique with Western blots.[14, 20, 21] Tissue samples stored in RIPA buffer containing Halt protease inhibitors and PMSF were thawed on ice; the presence of protease inhibitors was used to minimize protein degradation. Samples were homogenized using a Kontes Pellet Pestle (Fisher Scientific, Pittsburg, PA), and the protein-containing supernatant was separated by centrifugation at 5,000×*g* for 15 min. Protein concentrations were measured by the bicinchoninic acid method (Pierce); 200 μg of protein was resolved on a 10% SDS-PAGE gel (Bio-Rad, Hercules, CA) and transferred electrically to a PVDF membrane (Millipore, Bedford, MA). Membranes were blocked using 5% milk in Tris-buffered saline with Tween (TBS-T). GAPDH was used as a stably expressed "housekeeping" protein. Membranes were incubated overnight at 4°C with primary antibody for PepT1 (Santa Cruz Biotechnology, Santa Cruz, CA), and GAPDH antibody (US Biological, Swampscott, MA). After incubation with primary antibody, membranes were rinsed three times with TBS-T and incubated with secondary antibody in TBS-T containing 5% milk using horseradish peroxidase-conjugated, goat anti-rabbit IgG for PepT1 and anti-mouse IgG for GAPDH (Sigma). Protein bands were visualized by a colorimetric reaction using Opti-4CN substrate kits (Bio-Rad) for GADPH and amplified with Opti-4CN for PepT1. Scion Image (Scion Corp, Frederick, MA) was used for semiquantitative measurements based on band densitometry. Protein measurements were normalized to GAPDH as a technique designed to estimate relative amount of PepT1 per enterocyte.

Uptake Function

We measured transporter-mediated uptake of the di-peptide glycyl-sarcosine (Gly-Sar), a non-hydrolysable substrate for PepT1,[19] using a modified everted sleeve technique as we described previously.[14, 20, 21] Ileal segments (1 cm) were everted over a pre-grooved steel rod and secured with silk ties, thereby exposing the mucosal surface externally. Colonic uptake studies were not performed, because our prior work showed very little if any colonic uptake of

dipeptides.[14] The intestinal "sleeves" were kept in cold (4°C) Ringer's solution bubbled with 95% O_2/5% CO_2. The sleeves were transferred into 8 ml of warmed (38°C), Gly-Sar-free incubation medium (in millimolars: 129 NaCl, 5.1 KCL, 1.4 $CaCl_2$, 1.3 NaH_2PO_4, and 1.3 Na_2HPO_4; pH 6.0)[14, 19, 20] bubbled with 95%O_2/5% CO_2 and stirred at 1,200 rpm for 5 min. Then, the sleeves were placed in 8 ml of 38°C incubation medium containing 0.2, 1, 3, 20, or 40 mM Gly-Sar maintaining iso-osmotic conditions by replacement with appropriate amounts of NaCl in the test solutions. One microcurie of ^{14}C-Gly-Sar was included in the test solution to measure total uptake of Gly-Sar from which the transporter-mediated uptake by PepT1 was calculated (see below). After 1-min incubation, sleeves were removed, rinsed in 30 ml of ice-cold (Gly-Sar-free) incubation medium stirred at 1,200 rpm for 20 s, placed in glass scintillation vials, and solubilized in 1 ml of tissue solubilizer (PerkinElmer, Boston, MA) at 50°C in a water bath for 3 h. After complete solubilization, 15 ml of scintillation counting cocktail (Opti-Flour, PerkinElmer, Waltham, MA) was added, and disintegrations per minute of ^{14}C were determined using liquid β scintillation counting.

Transporter vs. Non-transporter-Mediated Uptake The method of estimating transporter vs. non-transporter-mediated uptake of Gly-Sar was described previously.[20, 21] To calculate transporter-mediated uptake, total uptake has to be corrected for passive diffusion and mucosal adherence (non-transporter-mediated "uptake"). Non-transporter-mediated uptake at lesser concentrations can be estimated from observed uptake at markedly greater concentrations.[20–22] As the substrate concentration increases, non-transporter-mediated passive uptake increases linearly before and after the transporter is saturated; thus, the linear increase in total uptake after the transporter is saturated is attributed only to non-transporter-mediated "uptake", i.e., passive diffusion and mucosal adherence. We used 20- and 40-mM concentrations of Gly-Sar (at which a linear increase in total uptake was observed) to estimate non-transporter-mediated "uptake" at the lesser concentrations (0.2, 1, and 3 mM). Subtraction of the estimated, non-transporter-mediated uptake from observed total uptake allowed estimation of the transporter-mediated uptake.

Villous Height

The formalin-fixed ileal tissues from all groups were embedded in paraffin, sectioned parallel to the villous axis, and stained with hematoxylin and eosin. Maximum villous height was measured from above the crypt to the tip of the villous at ×10 magnification using an optical reticule with a micrometer. A minimum of six sections were studied from each specimen with at least three measurements made per section.

Statistical Analysis

PepT1 mRNA and protein levels were expressed as the ratio of PepT1 to the housekeeping gene (GAPDH) in an attempt to estimate gene expression per enterocyte. Transporter-mediated uptake of Gly-Sar was measured in nanomoles per centimeter per minute with Lineweaver–Burke plots used to calculate V_{max} and K_m. Data are reported as median±interquartile range (IQR) unless otherwise specified. Kruskal–Wallis analysis was used to compare non-parametric data across multiple groups; Wilcoxon rank sums were used for direct comparisons between individual groups. p Values were corrected according to the Bonferroni method, only corrected p values of <0.05 were considered significant, and n values are number of rats.

Results

Food Intake/Weight Gain

Rats in both NC and sham celiotomy groups followed a nocturnal-based feeding pattern; greater than 70% of ingestion of chow occurred between 6 PM and 6 AM ($p<0.001$). The non-resected rats consumed similar amounts of food and had similar weight gain by the end of the 1-week study period (Fig. 1a). In contrast, rats in both resection groups (1 and 4 weeks) ingested significantly less chow compared to NC and sham rats; in addition, diurnal rhythmicity in the feeding pattern was disrupted during the first week postoperatively ($p<0.05$; Fig. 1a). Starting from the second week postoperatively, however, rats in the 4-week resection group followed a nocturnal-based feeding pattern and consumed daily amounts of food similar to the non-operated control rats monitored for 4 weeks (data not shown).

Resected rats had a net weight loss at 1 week postoperatively compared to NC and sham rats (Fig. 1b; $p<0.001$); nevertheless, rats in the 4-week resection group started to gain weight by the second week postoperatively until the time of tissue harvest in a rate similar to the non-operated, monitored control group (Fig. 1b). By the end of the 4-week period, rats that had undergone resection had gained less weight (median gain 44 g; IQR, 36–65 g) compared to monitor group (median gain 91 g; IQR, 84–98 g); this difference in total weight gain occurred due to the initial weight loss in resected rats during the first week postoperatively.

None of the rats (in all groups) developed diarrhea or other postoperative complications that required exclusion from the

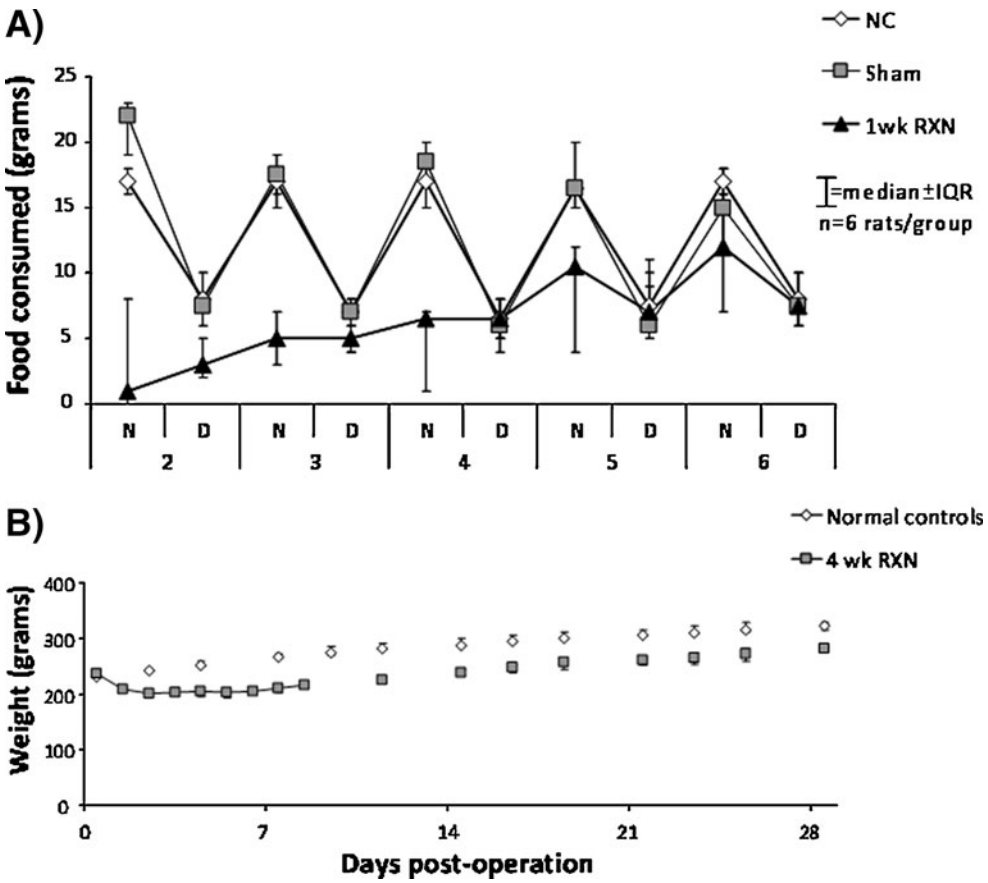

Fig. 1 **a** Patterns of food consumption over 1 week in non-resected, normal control (*NC*) rats, rats that underwent sham celiotomy, and rats that underwent 70% proximal jejunoileal resection (1 week resection (*RXN*)). NC rats and sham rats followed a predominately nocturnal feeding pattern; however, resected rats lost diurnal rhythmicity in feeding pattern and consumed less chow during the first week postoperatively. Nevertheless, both resected and non-resected rats had a similar feeding pattern and weight gain, starting from the second week postoperatively. **b** Weight changes over 4 weeks in monitored control rats and rats that underwent resection (4 weeks RXN). Resected rats had a net weight loss at 1 week postoperatively compared to NC and sham rats

study. Although total water intake and lean body mass were not measured in this study, the matched daily food intake and weight gain between non-resected and resected rats after the first week postoperatively suggest that there were no differences in total water intake or total body water.

mRNA Expression

Ileum There was no change in relative expression of PepT1 mRNA after sham celiotomy. At 1 week after 70% proximal small bowel resection, cellular mRNA levels of PepT1 in the remnant ileum decreased (about threefold decrease in median relative expression compared to NC; $p \leq 0.002$; Fig. 2a). In the 4-week post-resection group, however, ileal mRNA expression increased back toward "pre-resection" levels with no difference compared to NC and sham groups ($p > 0.05$).

Colon Cellular mRNA expression of colonic PepT1 remained unchanged both at 1 and 4 weeks after massive small bowel resection compared to NC and sham rats ($p > 0.5$; Fig. 2b). Furthermore, in each individual group, we compared relative cellular expression of PepT1 mRNA in colon versus remnant ileum. As expected, mRNA expression in colonic enterocytes was much less compared to remnant ileum in all four groups (about 1,000-fold difference; $p < 0.0001$).

Protein Expression

Ileum Despite the decreases in mRNA expression for ileal PepT1 at 1-week post-resection, relative protein expression by Western blotting for total cellular PepT1 did not differ among all four groups ($p > 0.6$; Fig. 3a).

Colon Similarly, there was no change in colonic protein expression per enterocyte after sham celiotomy or massive small bowel resection (both at 1 and 4 weeks) compared to control levels ($p > 0.4$; Fig. 3b). Moreover, we compared total cellular expression for PepT1 protein in colon versus remnant ileum. Unexpectedly, although mRNA expression in colon was 1,000-fold less than in ileum, there was no difference between colonic and ileal enterocytes in total protein expression of PepT1 across all four groups ($p > 0.05$).

Transporter-Mediated Uptake of Gly-Sar

Uptake Values Ileal uptake of Gly-Sar in all four groups demonstrated saturation kinetics consistent with transporter-mediated uptake. Transporter-mediated uptake of Gly-Sar at all three concentrations (0.2, 1, and 3 mM) was not different between NC and sham groups; however, uptake increased markedly in the remnant ileum at 1 and 4 weeks

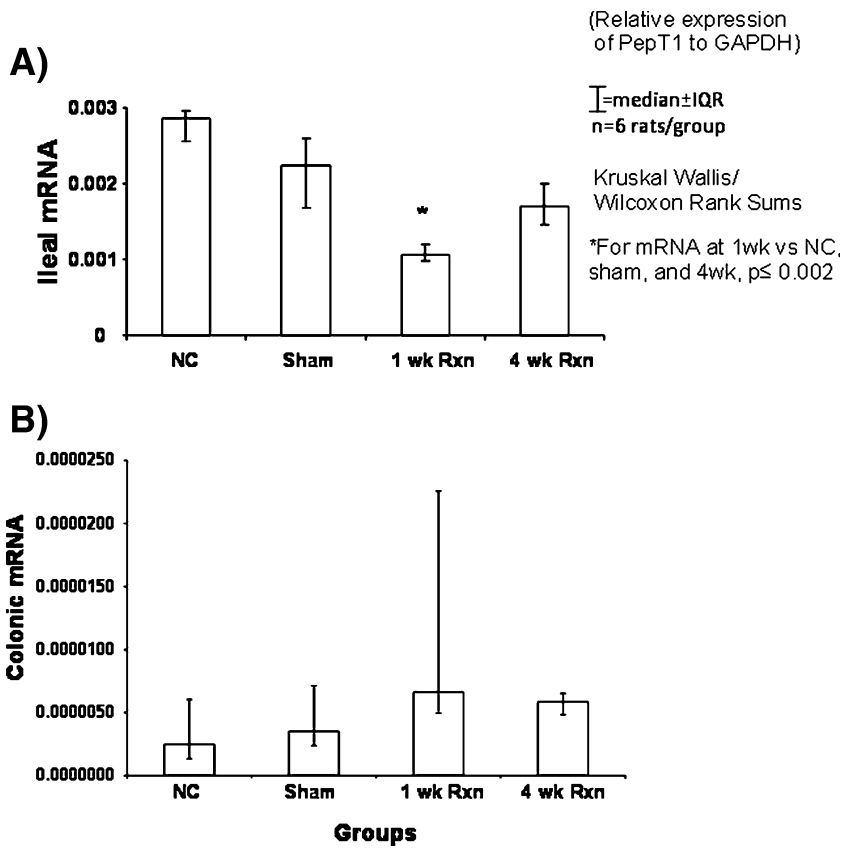

Fig. 2 Relative expression levels of PepT1 mRNA in **a** ileum and **b** colon for four study groups (NC, sham, 1 week RXN, and 4 weeks RXN). Although mRNA levels per enterocyte decreased in the remnant ileum at 1-week post-resection, expression of ileal mRNA at 4 weeks and colonic mRNA at 1 and 4 weeks after resection were unchanged compared to NC and sham rats

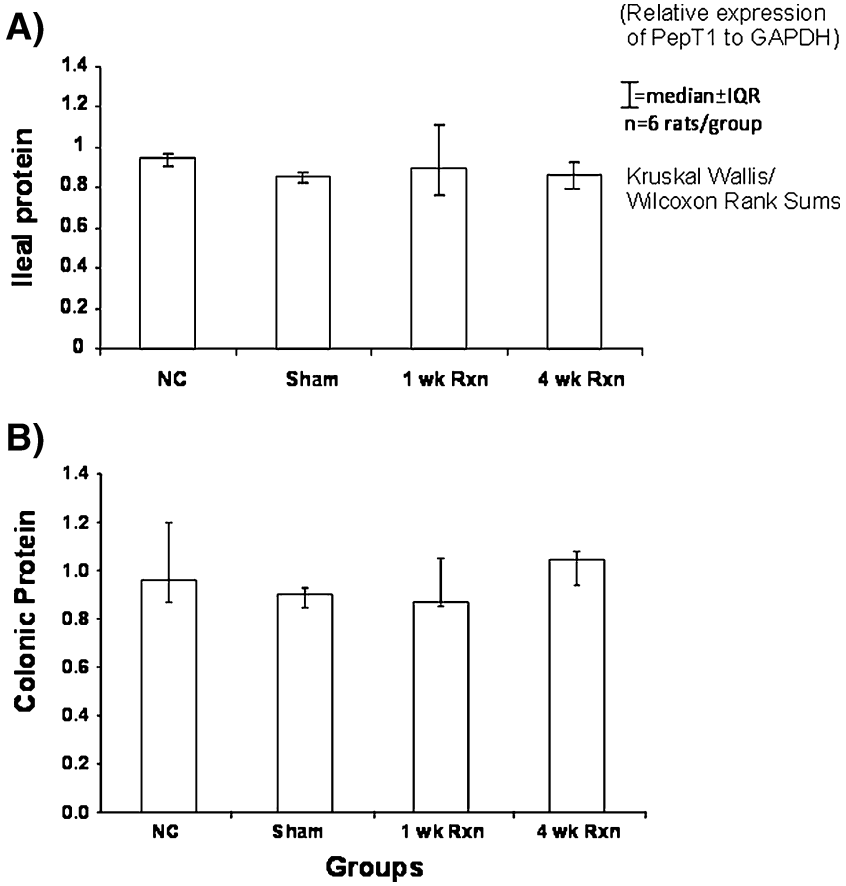

Fig. 3 Relative expression levels of PepT1 protein in **a** ileum and **b** colon for four study groups (NC, sham, 1 week RXN, and 4 weeks RXN). There were no differences among all four groups in their ileal or colonic protein expression for PepT1 per enterocyte

after 70% proximal small bowel resection ($p<0.05$; Fig. 4). There was no further increase in transporter-mediated uptake of Gly-Sar from 1 to 4 weeks after resection ($p>0.2$).

Uptake Kinetics Similar to uptake values, the calculated V_{max} (nanomoles per centimeter per minute, a function of the number of apical transporters participating actively in uptake) remained unchanged after sham celiotomy compared to NC; however, in the 1- and 4-week post-resection groups, the median V_{max} increased markedly; there was no difference between the 1- and 4-week groups (1 and 4 weeks vs NC and sham, 44 and 52 vs 22 and 23; $p<0.05$, Fig. 5a). K_m (millimolars), a function of transporter affinity to its substrate did not differ among all the groups ($p>0.05$; Fig. 5b).

Villous Height and Intestinal Length

In the ileal segment, median villous height (millimeters) was markedly greater at 1 and 4 weeks after resection compared to NC and sham rats (0.45 and 0.57 vs 0.21 and 0.24; $p \leq 0.01$; Fig. 6). There was no difference between NC and sham ($p>0.8$). Additionally, there was no difference in villous height in rats at 1 and 4 weeks after resection ($p>0.1$). Changes in villous height were compared to changes in V_{max} among all groups. At 1 week after resection, median villous height in remnant ileum increased by 114% compared to NC (0.45 vs 0.21 mm), while median V_{max} increased by 100% (44 vs 22 nmol/cm/min). At 4 weeks time point, median villous height increased by 171% compared to NC (0.57 vs 0.21 mm), while median V_{max} increased by 136% (52 vs 22 nmol/cm/min).

All resected animals were left with a remnant ileum of 15 cm in length at the time of resection. One week after resection, this length appeared to have shortened to a median 13.5 cm (range, 12.5–14.5 cm; $p<0.01$); after 4 weeks, the intestinal length appeared to have increased to a median of 17 cm (range, 14.5–19.5 cm; $p<0.01$). In addition to intestinal length, gross luminal diameter also increased notably over time, and while actual measurements could not be measured reliably, the everted sleeve technique necessitated larger caliber steel rods for eversion (4 mm in NC and shams, 5 mm 1 week after resection, and 6 mm 4 weeks after resection).

Discussion

Ileal adaptation after massive proximal small bowel resection has been the focus of increasing interest in recent years, especially as it pertains to exploring potential therapies for short bowel patients. Despite several treatment options, such as long-term parenteral nutrition, intestinal rehabilitation, various intestinal lengthening procedures, and, more recently, small bowel transplantation, the outcomes with current therapies are still not optimal for patients with SBS.[1–4] Preserving the distal ileum with the ileocecal junction in patients undergoing massive small bowel resection is associated with better surgical outcomes; however, the adaptive mechanisms occurring in the distal ileum for improving nutrient absorption, and especially for proteins as peptides, has not been characterized. Our laboratory has focused recently on the early adaptive changes after massive intestinal resections. Our studies have been designed to improve our understanding of the potential cellular mechanisms underlying the ileal adaptive response by studying gene expression and transport function of major nutrient transporters, including the recently recognized and important transporter PepT1. This topic has direct implications in our understanding of the pathophysiology of SBS and may lead potentially to improvements in our management of this condition.

Our data confirm that the ileum, which in the normal, intact gut does not account for much of the peptide absorption from the lumen, is highly adaptable and can increase its peptide transport after a proximal-based, 70% small bowel resection. This ileal adaptation was associated with a rapid increase in the villous height (i.e., hyperplasia) with increased numbers of enterocytes per centimeter in the

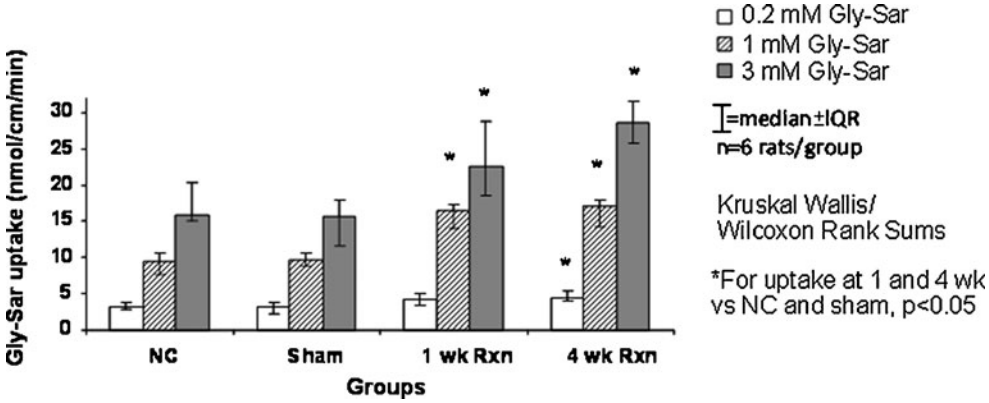

Fig. 4 Transporter-mediated uptake of Gly-Sar at three concentrations of Gly-Sar (0.2, 1, and 3 mM) in the ileum of four study groups (NC, sham, 1 week RXN, and 4 weeks RXN). Ileal uptake of Gly-Sar increased significantly at 1 and 4 weeks after resection compared to NC and sham groups. There was no further difference in Gly-Sar uptake between 1 and 4 weeks RXN groups

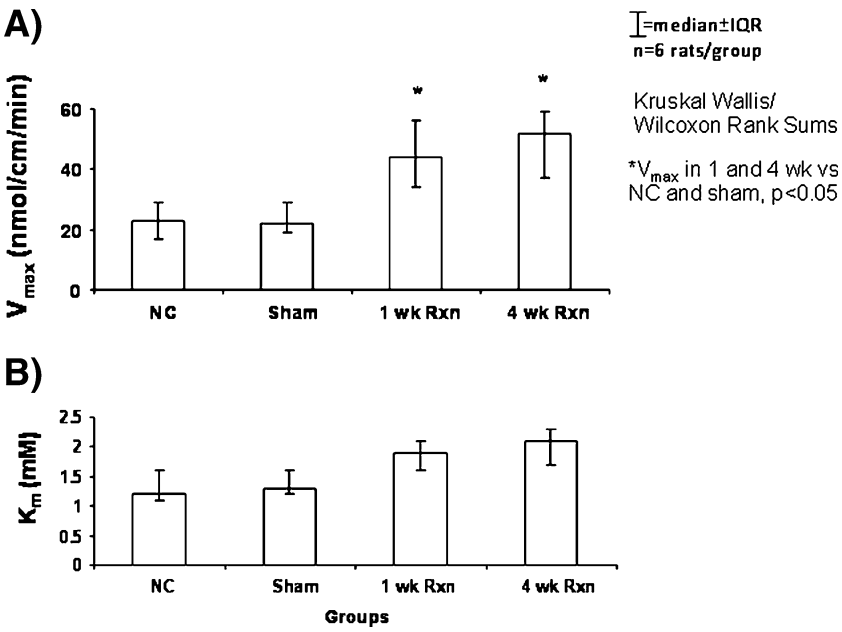

Fig. 5 Values for **a** V_{max} (in nanomoles per centimeter per minute) and **b** K_m (in millimolars) of Gly-Sar in the ileum of four study groups (NC, sham, 1 week RXN, and 4 weeks RXN). Similar to uptake values, the calculated V_{max} increased markedly at 1- and 4-week post-resection compared to NC and sham groups; **b** K_m values did not differ among all groups

remnant ileum in the resected groups. This adaptive response occurred as early as 1 week postoperatively in this rat model and also was sustained 4 weeks after resection. Interestingly, however, was that there was no cellular upregulation of mRNA and total protein expression for PepT1 in the ileal or colonic enterocytes after massive proximal small bowel resection at either time point (1 and 4 weeks post-resection) despite the marked increase in peptide uptake per centimeter of bowel. These data are consistent with our previous findings of marked, mid-ileal adaptation for glucose absorption and gene expression of hexose transporters as well as the response of the proximal jejunum to a 70% mid-small bowel resection for gene expression and transport function of PepT1;[14] in contrast, they differ from the response of the distal-most ileum which underwent hyperplasia but did not increase its absorption of dipeptides per enterocyte.[14]

PepT1 has emerged as a very important transporter in the gut mucosa. Not only is PepT1 the primary transport pathway for absorption of the majority of ingested proteins (via transport of short peptides after luminal protein hydrolysis), but PepT1 also is involved in the absorption of multiple therapeutic pharmacologic agents, including certain antibiotics, antiviral agents, antihypertensives, and other drugs as well. Indeed, much of the prior research on function and control of expression of PepT1 has been in the field of pharmacology.

Our interest focused on the control of PepT1 gene expression as an adaptive response to an acute massive loss of intestinal absorptive surface area. Intestinal adaptation to states of massive loss of absorptive area by disease or intestinal loss is well known to occur by a marked increase in absorptive capacity per unit length of bowel. This adaptation occurs in part secondary to a marked cellular hyperplasia (increased height of villi) and intestinal dilation, but whether the enterocyte itself "upregulates" gene expression (cellular levels of mRNA and/or protein), modifies post-transcriptional or post-translational processing, or alters the membrane levels of the transporter(s) (and thus V_{max}) remains poorly understood.

Our hypothesis was that ileal adaptation to a proximal, 70% jejunoileal resection would occur both by marked

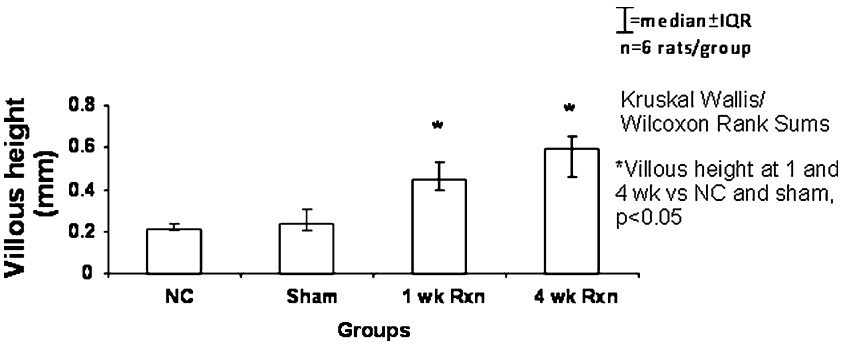

Fig. 6 Villous heights (millimeters) in the ileum of four study groups (NC, sham, 1 week RXN, and 4 weeks RXN). Median villous height was markedly greater at 1 and 4 weeks after resection compared to NC and sham rats. There were no differences between NC and sham rats ($p>0.2$) or between 1 week and 4 weeks RXN

hyperplasia and intestinal dilation as well as by cellular upregulation of both mRNA and protein expression of PepT1. Our findings did not completely confirm our pre-experiment hypothesis. While hyperplasia (increased villous height) and intestinal dilation did occur and uptake of the dipeptide was markedly increased, cellular levels of mRNA measured by quantitative real-time RT-PCR and protein by semiquantitative Western blot (corrected by relative expression against the constant expression of the housekeeping gene GAPDH) remained unchanged at 4 weeks post-resection. Moreover, although hyperplasia and increased uptake were evident even at 1-week post-resection, cellular mRNA levels in the ileal enterocyte decreased threefold at 1-week post-resection, while cellular protein levels remained unchanged. This decrease in gene expression of PepT1 despite the strong catabolic stress with a 70% proximal small bowel resection might be related to some counter-regulatory hormones (such as epidermal growth factor and triiodothyronine) or other negative regulators of PepT1 gene expression as has been suggested by other in vitro studies (in cell cultures);[23, 24] however, we have no objective data to support this statement. Similar findings of a decrease in jejunal mRNA for PepT1 were reported after enterectomy in rabbits, but no protein or uptake studies were carried out.[25]

Our data showed that the increase in uptake of dipeptides and V_{max} were proportionately less when compared with the changes in total absorptive surface area (villous heights and luminal caliber). This observation might suggest differential distribution of the population of PepT1 transporters along the crypt-villous axis in the remnant ileum. In fact, differences in distribution of PepT1 along the villi were reported previously in rat jejunum via measurements of immunohistochemistry showing a relative increase in expression toward the tip of villous.[26]

These combined observations suggest that either the increased uptake of dipeptide was related solely to the ileal mucosal hyperplasia and an increase in absorptive area or that the enterocyte regulated the apical levels of this transporter via still poorly understood intracellular mechanisms of PepT1 translocation without changing total cellular levels of PepT1. Indeed, there are good in vivo data[27–29] as well as our preliminary data in cell culture [30–32] supporting the concept of cellular regulation of translocation of membrane transporters such as glucose transporter 2 (GLUT2). The experimental data for PepT1 is suggestive of a similar cellular translocation of PepT1 from intracellular stores of pre-formed PepT1, but the lack of stereospecificity of the transporter and reliable techniques of quantitating selectively the apical levels of PepT1 protein has hindered progress in this field. Because we measured total cellular levels of PepT1 protein, our experimental design and techniques were unable to differentiate cellular from apical levels of PepT1 protein. We are actively investigating this form of cellular regulation of PepT1 currently.

Although these findings did not support our hypothesis, there are similar precedent data. We have studied previously the regulation of gene expression of the hexose transporters sodium-glucose co-transporter 1 and GLUT2 after this same model of proximal 70% jejunoileal resection with similar findings of increased uptake of glucose associated with increased villous height and a lack of alteration in gene expression of the hexose transporters.[13] In contrast, we studied recently the regulation of gene expression of PepT1 after a much different form of resection (70% mid-small bowel resection) with some different findings in the distal remnant ileum.[14] That recent work demonstrated a decrease in gene expression (mRNA and protein) of PepT1 after massive mid-small bowel resection but did not show any increase in dipeptide uptake in the very short remnant ileum despite modest increase, although less dramatic when compared with our current model, in villous height. In contrast, that same study showed a significant increase in the uptake of dipeptides in the remnant proximal jejunum (associated with increased villous height) without any changes in gene expression of mRNA or protein at either 1- or 4-week post-resection. Those differences in the response of remnant jejunum and ileum to proximal versus mid jejunoileal resection might be related to the presence of the proximal jejunum which has more capacity for peptide absorption and could decrease potentially the amount of dietary peptides reaching the terminal ileum and thereby alter ileal adaptation. Another possibility is that the distal ileum responds differently than does the mid ileum; unfortunately, there was not enough ileum to compare the proximal and distal segments of the remnant ileum in this current study of massive proximal-based jejunoileal resection. This constellation of findings suggests that the small bowel adapts to loss of surface area primarily by hyperplasia and dilation to increase absorption per unit length of bowel by increasing absorptive area and not by increasing net uptake per enterocyte via regulating levels of functional PepT1 in the apical membrane. These findings may have important implications in the attempt to augment the absorptive adaptation of the small intestine during the various conditions of the short gut syndrome, at least in the early postoperative period after massive loss of small bowel.

In addition, our two studies of massive intestinal resection suggest potentially important regional differences in the adaptive response of the rat enterocyte in the gene expression of PepT1. The jejunum appears to respond by hyperplasia without any change in cellular (enterocyte) levels of PepT1 mRNA or protein to increase peptide uptake per unit length of jejunum. In contrast, the remnant ileum, after massive proximal jejunoileal resection,

responds by a similar hyperplasia and increase in peptide uptake per unit length of bowel despite an overall paradoxic decrease in cellular levels of mRNA with no subsequent change in cellular levels of PepT1 protein. Our data are inadequate to determine whether there is a decrease in PepT1 transcription, a change in post-transcriptional processing, a more efficient synthesis of PepT1, or a change in PepT1 translocation to the apical membrane of the ileal enterocyte. Moreover, although mRNA expression in ileum was about 1,000-fold more than in colon, there was, unexpectedly, no difference between ileal and colonic enterocytes in total protein expression of PepT1 among resected and non-resected rats. These findings might be explained by differences in post-transcriptional processing or stability of mRNA for PepT1 in ileal versus colonic enterocytes. Although total cellular protein levels were similar between these two segments, our experimental design and techniques were unable to differentiate intracellular from apical levels of PepT1 protein. Subsequent studies, both in vivo and in vitro (such as cell cultures), are needed in the future to further characterize the cellular mechanisms of intestinal adaptation for peptide absorption after massive proximal jejunoileal resection.

Conclusion

The ileum appears to be highly adaptable in its ability to increase dipeptide absorption via PepT1 after massive proximal-based, small bowel resection, but this increase in absorptive capacity is due to cellular proliferation by villous hyperplasia and intestinal dilation to increase absorptive surface area as opposed to gene upregulation of PepT1 within the enterocyte. These findings might have important implications in the management of SBS as well as in the field of pharmacology.

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ORIGINAL ARTICLE

Clostridium difficile Colitis: Factors Associated with Outcome and Assessment of Mortality at a National Level

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Abstract
Background Previous descriptions of *Clostridium difficile* colitis (CDC) epidemics may overestimate cost and mortality of CDC.
Methods An analysis of the 2007 Nationwide Inpatient Sample was performed. Patients with CDC (N=41,207) were compared to a propensity score-matched cohort of patients without CDC.
Results Average length of stay was longer for CDC patients by 5 days ($p<0.001$). Mortality was higher for the CDC cohort (9.4% vs. 8.6%; $p<0.001$) though the absolute difference was small. Mean hospital costs were 56% higher for CDC patients ($p<0.001$). Higher odds of death with CDC were associated with small hospitals and self-pay patients. Chronic renal failure and diabetes were associated with lower hospital costs and lower odds of death in the CDC cohort.
Conclusions CDC is not as deadly of a disease as it may be perceived to be at larger hospitals, and mortality was actually unaffected by certain serious comorbidities. CDC is expensive due to a longer hospital stay.

Keywords *C. difficile* · Colitis · Outcomes · Mortality · Cost · Length of stay

Introduction

Clostridium difficile colitis (CDC) has evolved over the last 15 years from a disease of relative obscurity to an emerging, international epidemic. Over this time period, the accumulated epidemiologic evidence from population-based studies[1–3] from several countries, including the USA,[4] has demonstrated an increase in the incidence of CDC. Whereas in the past, CDC primarily affected immunocompromised patients, the disease now places at risk a much broader range of patients, including those who are younger, immunocompetent, and those undergoing elective surgery.[5] *C. difficile* strains with increased toxin production[6] and greater virulence,[7,8] referred to as epidemic or "hypervirulent" strains, have recently been identified, and the disease course is often resistant to many commonly used antibiotics, such as metronidazole,[9] though whether this represents antibiotic resistance versus a fulminant disease course that does not allow time for antibiotics to take effect is unclear. What is certain is that the incidence of severe colitis from *C. difficile* has increased at an alarming rate,[10,11] and has resulted in the need for many patients to undergo an emergency total colectomy[12] due to the failure of nonoperative management.

Despite recent reports from large referral centers indicating a greater incidence, virulence, and mortality with CDC,[13] the question of whether these excesses represent a national trend as opposed to events which are specific to

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certain types of hospital settings has not been adequately delineated. Much of the literature regarding CDC is based on single institution experiences and is retrospective. As these reports are largely based on the experience of tertiary referral centers, the description of the disease course of CDC provided in these studies may not be applicable to the general CDC patient population, and may not provide an accurate description of patient outcomes in care settings dissimilar to those of previous studies. Additionally, the true health-care cost of developing CDC is unclear, as many reports on CDC focus only on patients who contracted *C. difficile* as a secondary diagnosis while critically ill for other reasons, making the contribution of CDC to health care expense more difficult to differentiate from other factors.

The present study represents an analysis of a US-derived, nationwide, population-based database of inpatients. The aim is to provide a more accurate description of the additional cost and mortality associated with CDC in the general patient population, including both larger and smaller hospital settings and including a broad demographic of patients for comparison. More specifically, factors associated with excess attributable cost and mortality in CDC patients were identified, as was the relationship between patient factors and excess hospital length of stay (LOS) as a measure of disease severity and a contributing factor toward overall cost of care.

Methods

This was an IRB-approved, retrospective cohort study and included inpatients who developed CDC and inpatients who did not develop CDC. Potential confounders were controlled using both propensity score matching and multivariate analysis. Data came from the 2007 Healthcare Cost and Utilization Project Nationwide Inpatient Sample (NIS). The NIS is the largest publically available, all-payer inpatient care database in the USA, representing a 20% stratified sample of patients in US hospitals (http://www.hcup-us.ahrq.gov).

The presence of CDC was defined using the International Classification of Diseases, Ninth Revision, Clinical Modification code (008.45) for *C. difficile*, pseudomembranous colitis. The presence of this code in any of the 15 diagnoses in the discharge record was counted as CDC.

Three outcomes were studied: excess attributable costs, excess attributable hospital days, and excess attributable mortality. Costs were obtained by multiplying charges by institution-specific cost-to-charge ratios. Analyses controlled for several patient level variables, including demographics (age, gender, and race/ethnicity), payer status (Medicare, Medicaid, commercial, self-pay, other), and type of admission (elective, urgent, and emergent). The study also controlled for region of the country (northeast, midwest, south, west) and size of the hospital (small, medium, and large). The study controlled for patient disease severity using indicators for All Patient Refined Diagnosis-Related Groups. Comorbidities were controlled using the Agency for Health Care Research and Quality (AHRQ) comorbidity indicators.[14] DRG groupings using indicators for major diagnostic categories were controlled for in order to control for resource intensity.

Statistical Methods

The statistical analysis was designed to estimate the effect of CDC on hospital costs, LOS, and mortality, controlling for important potential confounders. In making univariate comparisons between patients with and without CDC, Chi-square tests for binary and categorical variables were used, while *t* tests were utilized for continuous variables. The outcomes were subsequently fit to multivariate models, controlling for a host of covariates. Cost and LOS were fit to linear regression models; mortality was fit to a logistic regression model.

It was recognized that if a significant imbalance in covariates between patients with and without CDC were to be present, then a regression model may not adequately control for covariates. Therefore, a propensity score matching analysis that dealt with potential covariate imbalance was performed, estimating the average treatment effect on the treated. This analysis first estimated the probability (propensity) that a patient would develop CDC. CDC patients were then matched one to one to patients without CDC based on the estimated predicted probability. The propensity score model was a logistic regression model, with covariates as described above, and matches were selected as the nearest neighbor.

Results

Demographic and summary information for the study patients is provided in Table 1, with no statistically significant difference ($p>0.05$) between the two cohorts. CDC and matched, non-CDC cohorts were very similar, including with regard to age (mean, 70 years), gender (female, 58%), and race (Caucasian, 76%). Using the definition of hospital size defined by HCUP,[15] hospitals were designated as being large- (60%), medium- (25%), or small-sized (14%) facilities. Approximately 72% of both cohorts were Medicare patients. A similar percentage of CDC patients (15.8%) and non-CDC patients (14.8%) were admitted to the hospital to undergo surgery, as opposed to

Table 1 Summary statistics for CDC and non-CDC matched cohorts

Variable	Non-CDC (N=4,032,865)	CDC (N=41,207)	Matched, non-CDC (N=41,207)
Age	56.6	70.3	70.7
Gender (%)			
Female	60.5	57.9	58.5
Male	39.5	42.1	41.5
Race (%)			
Caucasian	69.5	76.6	76.8
Black	15.5	12.7	12.8
Hispanic	9.3	5.9	5.8
Asian	2.7	2.1	2.0
Other	3.0	2.7	2.6
Hospital size (%)			
Small	14.0	14.6	14.1
Medium	25.4	25.2	25.4
Large	60.6	60.2	60.5
Payer (%)			
Medicare	44.7	72.1	72.6
Medicaid	14.0	6.6	6.7
Self-pay	6.0	2.0	1.9
No charge	0.8	0.4	0.3
Pay other	3.3	1.7	1.7
Unknown	31.2	17.2	16.8
Region (%)			
Midwest	15.6	17.6	17.6
South	48.2	41.1	41.3
West	7.2	6.1	6.1
Northeast	29	35.2	35.0
Reason for admission (%)			
Surgical	24.6	15.8	14.8
Nonsurgical	75.4	84.2	85.2
Admission type (%)			
Elective	27.7	12.9	12.4
Urgent	19.3	15.3	15.1
Emergent	53.0	71.8	72.5

patients admitted for nonsurgical indications. A comparison of both cohorts based on major diagnostic categories was also performed. Similar comorbidities were observed between CDC and non-CDC matched cohorts based on AHRQ comorbidity measures. A similar distribution of comorbidities existed between the CDC and non-CDC cohorts, with the most frequently associated illnesses related to the digestive system (39%), infectious diseases (13%), and respiratory diseases (11%).

Table 2 provides a summary of the mean values for cost of hospitalization, length of hospital stay, and mortality for CDC and matched, non-CDC cohorts, including the results of a comparison using a Chi-square analysis. The differences in these measures between the two groups were highly significant ($p<0.0001$). The mean cost of hospitalization was 56% higher in the CDC cohort (US $23,344 versus US $14,918). The mean length of hospital stay was noted to be longer for the CDC group (13±14 days versus 7.9± 9 days) by an average of 5 days, representing an important contributing factor to the excess cost associated with the CDC group. Though a statistically significant difference in mortality rates existed between the two cohorts (9.4% for CDC and 8.6% for non-CDC), the absolute difference between these mortality rates was quite small, representing a statistically significant difference of small clinical magnitude.

Linear regression was used to identify patient factors significantly ($p<0.0001$) associated with excess attributable hospital costs within the CDC cohort (Table 3). Using patients ≤25 years of age as a reference group, patients 26–50 years old accrued the highest excess charges (US $657 per day), while those >70 years of age had US $2,113 less in daily charges. Excess costs were greater for male gender compared to females, were higher for large hospitals compared to smaller facilities, were higher in the northeastern USA compared to other regions, and were higher for Medicaid patients compared to all other payer types, including Medicare and commercial insurance types.

Table 3 also lists hospital costs attributable to comorbidities among CDC patients. Statistically significant ($p<0.05$) higher hospital costs per hospitalization were associated with congestive heart failure (US $1,644), coagulopathy (US $8,279), cardiopulmonary disease (US $2,625), valvular heart disease (US $1,556), and significant unintentional weight loss (US $6,800). Paradoxically, lower hospital costs were noted in those CDC patients who also had chronic renal failure (US $2,765; $p<0.001$) as well as diabetes without (US $1,119; $p<0.001$) and diabetes with chronic complications (US $2,362; $p<0.001$), compared to CDC patients without these specific comorbidities.

Linear regression was also performed to identify significant ($p<0.0001$) factors associated with length of hospital stay (Table 4) among patients with CDC. Based on age strata, though the differences were statistically significant, the actual differences in length of stay were small between age groups (less than 1 day), as was also the case when ethnicity, gender, payer type, size of the hospital, urgency of admission for either elective or emergent indications, and region of the country where treatment occurred were analyzed. Based on comorbidities, only unintended weight loss (4.2 days) and paraplegia (2.1 days) contributed to a statistically significant and clinically relevant excess LOS.

Based on logistic regression modeling (Table 5), a significantly ($p<0.05$) increased odds ratio (OR) for death among CDC patients was associated with age groups older than 25 years (OR range, 1.1–2.4). Female gender was

Table 2 Summary of mean cost of hospitalization, hospital length of stay and mortality for CDC and matched, non-CDC cohorts

Variable	No CDC (*N*=4,032,865)	CDC (*N*=41,207)	Matches, no CDC (*N*=41,207)	*p* value
Mean cost of hospitalization	$9,247.67	$23,344.33	$14,918.06	<0.0001
LOS (days)	5	13.0±14	7.9±9	<0.0001
Mortality	2.2%	9.4%	8.6%	<0.0001

Dollars in US currency

associated with slightly greater odds of death (OR, 1.05; confidence interval (CI), 1.03–1.07), with a higher OR for Asian ethnicity (OR, 1.11; CI, 1.06–1.16) compared to other races. Higher odds of death was associated with small hospitals (OR, 1.09; CI, 1.06–1.11), self-pay status (OR, 1.25; CI, 1.20–1.31), and hospital care in the northeastern USA (OR, 1.17–1.40). The comorbidities significantly associated with higher odds of death included congestive heart failure, coagulopathy, and liver disease. Chronic renal disease (OR, 0.93; CI, 0.91–0.95) and diabetes both without (OR, 0.84; CI, 0.82–0.85) and with chronic complications (OR, 0.65; CI, 0.63–0.68) were unexpectedly associated with a lower odds of death in the CDC cohort, which was a strongly statistically significant finding.

Discussion

CDC is an infectious disease in transition, having originally represented a more rare form of infectious colitis with a niche opportunism, to more recently become an infectious complication that can affect any adult patient, even those patients without significant comorbidities or obvious risk factors for infection. The initial reports[7] describing the emergence of more virulent strains of *C. difficile* were heavily focused on the experience of university hospitals, and this trend in reporting has continued[16,17] to the present. To a certain extent, this reporting bias is a reflection of the nature of academic and teaching institutions, which participate in scientific investigations as part of their mission, as opposed to community hospitals who may not report their experience. However, the different patient populations that occupy a tertiary referral center versus a small- to medium-sized community hospital can lead to concepts regarding CDC disease course that may exaggerate the incidence of adverse outcomes if the results of larger academic centers were applied broadly. The experience of university-based hospitals with CDC, in the context of epidemics involving hypervirulent strains, may misrepresent the mortality rates associated with CDC in general as being higher than they actually are for the majority of patients who contract this infection. Many of these reports described the in-hospital mortality of CDC as being between 30% and 80%.[18–20] In contradistinction, the data from the NIS demonstrate that although there was a statistically significant difference in mortality rates between CDC patients and a matched control group without CDC, there was not a clinically relevant increase in mortality rates between these two groups, with a less than 1% absolute difference in mortality in this study. Length of hospital stay, however, was significantly longer in the CDC cohort than in non-CDC patients, and this would help to explain the significantly increased hospital costs seen for patients with CDC in this study. These data suggest that at a national level, taking into account a larger sample of patients from a variety of regions and care settings, CDC is a serious disease from the standpoint of health care costs, which is a reflection of the longer length of hospital stay with CDC patients. Without large series of patients who undergo bacterial strain identification and toxinotyping, it is unknown if the findings of this present study indicate a less virulent strain of *C. difficile* as the more common strain in the USA, or if other factors explain the disparity in results between this study and reports of CDC epidemics. Detailed bacteriologic information is needed to further investigate this issue, which would be best achieved through institutional *C. difficile* tissue banks that combine their results by region.

A question that remains unanswered is whether the cases of fulminant CDC associated with hypervirulent strains, which are associated with higher colectomy and mortality rates, are due in part to resistance to metronidazole and/or vancomycin. This question has received much speculation, but has, up to the present, not been answered in a scientifically rigorous manner, and therefore, it is still uncertain whether the virulent strains of *C. difficile* are resistant to antibiotics, or whether certain strains have an exuberant toxin production or specific toxinotype which creates fulminant disease and thus does not allow for adequate time for medical therapy to be effective. One of the best reviews[21] of the bacterial factors related to CDC of various severities was recently published in a nationwide Canadian study performed in 2005 by the Canada Nosocomial Infection Surveillance Program (CNISP). This study analyzed 1,008 patients from the CNISP database, which included information regarding the infecting strain of *C. difficile*. A total of 31% of patients were found to be NAP1 positive, with 12.5% of these cases experiencing a severe outcome as defined as the need for colectomy, intensive care unit admission, or a CDC-related death. This was in

Table 3 Results of linear regression modeling for determinants of health care costs in CDC patients

Variable	Coefficient	95% Confidence		*p* value
		Lower	Upper	
CDC	$8,179.86	$8,060.85	$8,298.87	<0.0001
Age				
<=25	Reference			
26–50	−$78.64	−$123.41	−$33.86	0.0010
51–70	$657.18	$604.50	$709.87	<0.0001
>70	−$2,113.99	$2,174.16	−$2,053.82	<0.0001
Gender				
Male	Reference			
Female	−$693.53	−$720.41	−$666.64	<0.0001
Race/ethnicity				
White	Reference			
Black	$811.42	$776.74	$846.10	<0.0001
Asian	$1,192.87	$1,118.58	$1,267.16	<0.0001
Hispanic	$999.59	$956.55	$1,042.64	<0.0001
Other	$1,648.86	$1,579.42	$1,718.30	<0.0001
Hospital size				
Large	Reference			
Medium	−$767.83	−$795.61	−$740.05	<0.0001
Small	−$324.83	−$359.95	−$289.71	<0.0001
Insurance				
Commercial	Reference			
Medicare	−$379.96	−$416.48	−$343.44	<0.0001
Medicaid	$407.41	$366.71	$448.11	<0.0001
Self-pay	−$566.78	−$621.10	−$512.47	<0.0001
No charge	$1,416.20	$1,284.35	$1,548.05	<0.0001
Other	−$407.01	−$475.41	−$338.61	<0.0001
Region				
Northeast	Reference			
Midwest	−$2,622.96	−$2,660.48	−$2,585.43	<0.0001
South	−$2,561.42	−$2,589.49	−$2,533.34	<0.0001
West	−$1,730.17	−$1,779.86	−$1,680.49	<0.0001
Comorbidities				
AIDS	$677.40	$449.75	$905.05	<0.0001
Alcohol	−$544.56	−$610.40	−$478.71	<0.0001
Anemia	$521.43	$485.01	$557.86	<0.0001
Arthritis	−$236.43	−$319.05	−$153.82	<0.0001
Blood loss	$1,139.60	$1,064.51	$1,214.69	<0.0001
CHF	$398.14	$348.69	$447.60	<0.0001
Chronic lung disease	$104.61	$71.95	$137.27	<0.0001
Coagulopathy	$5,009.11	$4,937.78	$5,080.44	<0.0001
Depression	−$291.45	−$335.02	−$247.87	<0.0001
Diabetes	−$291.16	−$324.70	−$257.62	<0.0001
Diabetes with complications	−$8.60	−$75.45	$58.26	<0.0001
Drug	$58.97	−$11.32	$129.26	0.1000
Hypertension	−$12.61	−$40.34	$15.13	<0.0001
Hypothyroidism	−$351.17	−$394.21	−$308.13	<0.0001
Liver disease	−$302.45	−$388.93	−$215.97	<0.0001
Lymphoma	$1,113.86	$969.64	$1,258.09	<0.0001
Electrolyte disorders	$1,014.30	$980.45	$1,048.15	<0.0001
Metastatic cancer	$208.56	$122.52	$294.60	<0.0001
Neuropathy	−$243.76	−$293.54	−$193.98	<0.0001
Obesity	$541.23	$493.61	$588.84	<0.0001
Paraplegia	$1,919.38	$1,831.07	$2,007.69	<0.0001
Perivascular	$874.12	$818.06	$930.18	<0.0001
Psychoses	$337.05	$270.18	$403.93	<0.0001
Pulmonary circulation	$1,877.57	$1,769.85	$1,985.29	<0.0001
Renal failure	−$1,350.39	−$1,396.98	−$1,303.79	<0.0001
Tumor	−$1,314.19	−$1,403.95	−$1,224.42	<0.0001
Ulcer	$2,208.52	$1,603.74	$2,813.31	<0.0001
Valvular disease	$324.75	$259.08	$390.42	<0.0001
Weight loss	$6,556.98	$6,479.86	$6,634.09	<0.0001
Admission status				
Elective	Reference			
Urgent	−$1,459.44	−$1,495.49	−$1,423.40	<0.0001
Emergent	−$2,655.32	−$2,687.20	−$2,623.45	<0.0001

Dollars in US currency

contradistinction to only 5.9% of patients with other infecting strains of CDC who experienced a severe outcome ($p<0.001$). Of particular interest was the study's information on in vitro antimicrobial susceptibility for *C. difficile* isolates. No statistically significant difference between 50% minimum inhibitory concentrations for metronidazole or vancomycin was seen in a comparison of NAP1 and non-NAP1 strains, suggesting that differences in clinical outcome between these two types of CDC may not be related to antibiotic resistance. Since CNISP hospitals were more likely to be university-affiliated, this would also suggest, but not prove, that this lack of antibiotic resistance in *C. difficile* might not be true for the general Canadian CDC population. This study goes on to correctly point out that in spite of previous studies[4,13] that have demonstrated exuberant toxin production in NAP1 strains, the association between this strain and severe colitis has not been explained mechanistically, and it is not clear that volume of toxin production is the sine qua non of fulminant disease.

It is of interest to observe how little influence was exerted on CDC patient mortality rates by certain comorbidities in this study. Even well-known predictors of in-hospital

Table 4 Linear regression results for determinants of length of hospital stay in CDC patients

Variable	Coefficient	95% Confidence		*p* value
		Lower	Upper	
Intercept	4.95	4.90	5.01	<0.0001
Age				
<=25	Reference			
26–50	0.16	0.13	0.18	0.0000
51–70	0.48	0.45	0.50	0.0000
>70	−0.09	−0.12	−0.06	<0.0001
Gender				
Male	Reference			
Female	−0.04	−0.06	−0.03	0.0000
Race/ethnicity				
White	Reference			
Black	0.60	0.59	0.62	<0.0001
Asian	0.63	0.59	0.66	<0.0001
Hispanic	0.30	0.28	0.32	<0.0001
Other	0.63	0.60	0.67	<0.0001
Hospital size				
Large	Reference			
Medium	−0.32	−0.34	−0.31	<0.0001
Small	−0.23	−0.25	−0.21	0.0000
Insurance				
Commercial	Reference			
Medicare	0.24	0.23	0.26	0.0000
Medicaid	0.61	0.59	0.63	<0.0001
Self-pay	−0.05	−0.08	−0.02	0.0000
No charge	0.83	0.77	0.90	0.0000
Other	0.16	0.12	0.19	0.0000
Region				
Northeast	Reference			
Midwest	−1.04	−1.06	−1.02	<0.0001
South	−0.79	−0.80	−0.78	<0.0001
West	−1.05	−1.08	−1.03	<0.0001
Comorbidities				
AIDS	0.29	0.18	0.40	<0.0001
Alcohol	−0.12	−0.15	−0.08	0.0000
Anemia	0.57	0.55	0.59	0.0000
Arthritis	−0.03	−0.07	0.01	0.2010
Blood loss	0.68	0.64	0.71	<0.0001
CHF	0.51	0.49	0.54	<0.0001
Chronic lung disease	0.20	0.18	0.21	0.0000
Coagulopathy	1.28	1.24	1.31	<0.0001
Depression	0.16	0.14	0.18	0.0000
Diabetes	−0.09	−0.11	−0.07	<0.0001
Diabetes with complications	0.67	0.63	0.70	0.0000
Drug	−0.18	−0.21	−0.14	0.0000
Hypertension	−0.15	−0.17	−0.14	<0.0001
Hypothyroidism	−0.08	−0.10	−0.06	0.0000
Liver disease	−0.08	−0.12	−0.04	0.0000
Lymphoma	0.11	0.04	0.18	0.0020
Electrolyte disorders	0.76	0.74	0.77	0.0000
Metastatic cancer	0.02	−0.02	0.06	<0.0001
Neuropathy	0.22	0.20	0.25	0.0000
Obesity	0.28	0.25	0.30	0.0000
Paraplegia	2.17	2.12	2.21	<0.0001
Perivascular	0.25	0.22	0.28	0.0000
Psychoses	0.76	0.73	0.79	<0.0001
Pulmonary circulation	0.80	0.75	0.86	0.0000
Renal failure	−0.08	−0.11	−0.06	0.0000
Tumor	−0.48	−0.52	−0.43	<0.0001
Ulcer	0.96	0.66	1.25	0.0000
Valvular disease	0.11	0.08	0.14	0.0000
Weight loss	4.21	4.17	4.25	<0.0001
Admission status				
Elective	Reference			
Urgent	−0.03	−0.05	−0.02	<0.0001
Emergent	−0.45	−0.46	−0.43	<0.0001

morbidity and mortality in general, such as diabetes and chronic renal failure, did not result in excess mortality in the CDC cohort compared to matched non-CDC patients. The reasons for lower odds of death in CDC patients with medical problems such as chronic renal failure and diabetes are surprising, but were, nonetheless, strongly statistically significant. It may be that the type of CDC encountered in many hospitals is mild enough such that even in the presence of other serious health care problems, and with timely care of CDC, mortality rates are not appreciably higher for the average CDC patient than for non-CDC patients with the same comorbidities. One weakness in the NIS and other large, national databases of this type is the inability to follow particular patients over time, as well as the lack of data regarding laboratory values, radiographic findings, vital signs, and other details of any single patient's course. It may be the case that particular subgroups of patients with comorbidities like diabetes and chronic renal failure would harbor higher mortality rates than non-CDC patients depending on the strain and toxin type that their *C. difficile* manifests. These additional bacterial factors could either mitigate or potentiate the effect of a patient's comorbidities.

The principal rationale for this study was the concern that the typical outcomes of CDC patients at a tertiary referral center, such as where the authors work, may not

Table 5 Logistic regression results for determinants for mortality in CDC patients

Variable	Odds ratio	95% Confidence		*p* value
		Lower	Upper	
CDC	1.11	1.07	1.16	<0.0001
Age				
<=25	Reference			
26–50	1.17	1.10	1.24	<0.0001
51–70	1.81	1.71	1.92	<0.0001
>70	2.45	2.31	2.61	<0.0001
Gender				
Male	Reference			
Female	1.05	1.03	1.07	<0.0001
Race/ethnicity				
White	Reference			
Black	0.94	0.92	0.97	<0.0001
Asian	1.11	1.06	1.16	<0.0001
Hispanic	0.87	0.84	0.90	<0.0001
Other	1.01	0.96	1.06	0.7390
Hospital size				
Large	Reference			
Medium	0.99	0.97	1.01	0.1690
Small	1.09	1.06	1.11	<0.0001
Insurance				
Commercial	Reference			
Medicare	0.79	0.77	0.81	<0.0001
Medicaid	0.99	0.96	1.03	0.6630
Self-pay	1.25	1.20	1.31	<0.0001
No charge	1.01	0.91	1.13	0.8260
Other	1.61	1.54	1.69	<0.0001
Region				
Northeast	Reference			
Midwest	0.83	0.81	0.85	<0.0001
South	0.89	0.87	0.91	<0.0001
West	0.60	0.58	0.62	<0.0001
Comorbidities				
AIDS	1.21	1.07	1.37	0.0020
Alcohol	0.83	0.79	0.86	<0.0001
Anemia	0.70	0.69	0.71	<0.0001
Arthritis	0.87	0.83	0.91	<0.0001
Blood loss	0.72	0.68	0.75	<0.0001
CHF	1.12	1.10	1.14	<0.0001
Chronic lung disease	0.94	0.92	0.95	<0.0001
Coagulopathy	1.44	1.41	1.48	<0.0001
Depression	0.78	0.75	0.80	<0.0001
Diabetes	0.84	0.82	0.85	<0.0001
Diabetes with complications	0.65	0.63	0.68	<0.0001
Drug	0.69	0.65	0.74	<0.0001
Hypertension	0.74	0.73	0.75	<0.0001
Hypothyroidism	0.88	0.86	0.90	<0.0001
Liver disease	1.32	1.27	1.37	<0.0001
Lymphoma	1.20	1.14	1.27	<0.0001
Electrolyte disorders	1.01	0.99	1.02	0.3350
Metastatic cancer	1.50	1.46	1.54	<0.0001
Neuropathy	1.09	1.06	1.12	<0.0001
Obesity	0.67	0.65	0.70	<0.0001
Paraplegia	0.85	0.81	0.88	<0.0001
Perivascular	1.02	0.99	1.05	0.1780
Psychoses	0.72	0.69	0.76	<0.0001
Pulmonary circulation	0.95	0.92	0.99	0.0120
Renal failure	0.93	0.91	0.95	<0.0001
Tumor	1.11	1.07	1.15	<0.0001
Ulcer	0.88	0.63	1.22	0.4360
Valvular disease	0.83	0.80	0.85	<0.0001
Weight loss	1.08	1.05	1.11	<0.0001
Admission status				
Elective	Reference			
Urgent	1.21	1.18	1.25	<0.0001
Emergent	1.09	1.06	1.11	<0.0001

represent the average CDC patient more commonly encountered in other hospital settings. The severity of CDC and the number of patients who require a life-saving emergent colectomy at a referral center may misrepresent the more common course of this infection. There has been little in the surgical or medical literature describing the typical mortality, cost, and length of stay for CDC patients on average. When background comorbidities were taken into consideration, the CDC cohort of this study did not fare much worse than the non-CDC matched cohort. We suggest that this indicates that when CDC is not fulminant, that even when certain, otherwise serious chronic comorbidities are present, that outcomes are comparable to non-CDC matched patients with similar background medical problems. This may not hold true with (1) the acute onset of these comorbidities, such as with acute renal failure as opposed to chronic kidney disease or (2) a fulminant episode of CDC. In the case of the latter, fulminant cases of CDC may still on the whole be uncommon enough that in a large study such as this, CDC and matched non-CDC patients have almost equivalent outcomes and manifest a more common, milder form of CDC. The lower costs associated with these comorbidities in this study may be related to different levels of care provided to those patients who may be perceived by physicians as being sicker and perhaps not warranting the most aggressive care.

The increased mortality associated with CDC patients clustered in the northeastern USA was a strongly significant finding. It may be the case that various strains of *C. difficile* cluster in particular regions, or that antibiotic conservation or other aspects of patient care may differ in the northeast from other regions of the USA. To the authors' knowledge, this is the first study that has shown a regional variation in CDC mortality rates in the USA.

Conclusion

Drawing from population-based data from the NIS, CDC is not as mortal of a diagnosis as it has been described in previous studies focused on its epidemical form as observed in larger hospitals. It is an expensive infection primarily due to a longer hospital stay.

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ORIGINAL PAPER

Elevated Serum IgG4 is Associated with Chronic Antibiotic-Refractory Pouchitis

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Abstract
Background and aim We recently reported mucosal infiltration of IgG4-expressing plasma cells in a patient with chronic antibiotic-refractory pouchitis (CARP). The role of serum IgG4 in the pathogenesis and clinical course of ileal pouch disorders has not been investigated. We hypothesized that IgG4-mediated autoimmunity may be a contributing factor for CARP. The aims of the study were to investigate the prevalence of elevated serum IgG4 in symptomatic patients with ileal pouches and to characterize clinical features of pouch disorders in these patients.
Methods A total of 124 consecutive symptomatic patients with ileal pouches from our subspecialty Pouchitis Clinic were enrolled in the study from January to October 2010. Serum IgG4 was measured at the time of presentation. Demographic, clinical, and laboratory characteristics were compared between the study (with serum IgG4 ≥112 mg/dl) and control (with serum IgG4 <112 mg/dl) groups.
Results There were ten patients (8.0%) with high serum IgG4 in the study group, while the remaining 114 (92%) patients were in the control group. The prevalence of elevated serum IgG4 in this series was 8%. None of the patients had a confirmed diagnosis of autoimmune pancreatitis. The median serum IgG4 in the study group was 144.5 vs. 14 mg/dl in the control group. The mean age of patients in the study and control groups was 35.5±14.5 and 42.0±13.2 years, respectively (p=0.137). Two patients in the study group (20.0%) had concurrent autoimmune disorders as compared to 19 patients (16.7%) in the control group (p=0.788). Three (30.0%) patients in the study group had coexisting primary sclerosing cholangitis (PSC) in contrast to 15 (13.2%) in the control group (p=0.147). Among the study group patients, five (50.0%) had CARP and one (10%) had Crohn's disease (CD) of the pouch, while in the control group, 23 (20.2%) had CARP and 24 (21.1%) patients had CD of the pouch (p=0.273). CARP was more commonly seen in patients with high serum IgG4 than patients with a normal IgG4 (50.0% vs. 20.2%, p=0.03).
Conclusions Approximately 8% of pouch patients presenting with symptoms of pouch dysfunction to our clinic had elevated serum IgG4. Patients with elevated serum IgG4 were more likely to have CARP.

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Keywords Autoimmune · IgG4 · Pouchitis · Primary sclerosing cholangitis · Ulcerative colitis

Abbreviations

AID	Autoimmune disorders
AIP	Autoimmune pancreatitis
CD	Crohn's disease
CARP	Chronic antibiotic refractory pouchitis
IBD	Inflammatory bowel disease
IPS	Irritable pouch syndrome
IPAA	Ileal pouch-anal anastomosis
NSAID	Non-steroidal anti-inflammatory drug
PSC	Primary sclerosing cholangitis

PDAI Pouchitis Disease Activity Index
UC Ulcerative colitis

Introduction

Elevation of serum IgG4 is considered to be one of the biomarkers for autoimmune pancreatitis (AIP). At the tissue level, pancreas along with other involved organs has characteristic infiltration with abundant IgG4-positive plasma cells termed as IgG4-related sclerosing disease.[1–6] While hypergammaglobulinemia was reported in 37–76% of individuals with AIP or IgG4-related sclerosing disease, elevated serum IgG4 appears to be more sensitive and specific for its diagnosis.[7] Recent studies suggest that IgG4 may play a role in the disease process of inflammatory bowel disease (IBD) in patients with or without a history of pancreatic disease.[3]

Idiopathic pouchitis is believed to be associated with dysbiosis, as the majority of patients respond favorably to antibiotic therapy. However, some patients do not respond to routine 2-week antibiotic therapy, which is termed chronic antibiotic-refractory pouchitis (CARP).[8] While multiple factors may contribute to the refractory course of pouchitis, autoimmunity may play a role in its pathogenesis. Our previous study showed that the presence of AID was associated with a 2-fold increase in the risk for CARP.[9] In addition, we recently reported a patient with CARP and multiple autoimmune disorders (AID) who had histologic evidence of IgG4-positive plasma cell infiltration in pouch biopsy without IgG4-repressing plasma cell infiltration of the ileum prior to colectomy surgery.[9] These findings lead us to embark on our current project with the hypothesis that IgG4-mediated autoimmunity may contribute to the disease process of CARP. The aims of the study were to investigate the prevalence of elevated serum IgG4 in symptomatic patients with ileal pouches and to characterize clinical features of pouch disorders in these patients.

Patients and Methods

Patients

This study was approved by our Institutional Review Board (IRB). The study involved consecutive eligible symptomatic patients presenting to the Pouchitis Clinic from January to October 2010. Patient's demographic and clinical data were retrieved from the IRB approved, prospectively maintained database. Patients were divided into two groups: those with serum IgG4 greater than 112 mg/dl (the study group) and those with no elevation of serum IgG4 (<112 mg/dl) (the control group). The cut-off level was set by institutional laboratory normal range.

Inclusion and Exclusion Criteria

Inclusion criteria were patients with ileal pouch-anal anastomosis (IPAA) for underlying ulcerative colitis (UC) with symptoms of frequency, urgency, pelvic discomfort, and abdominal cramps. Exclusion criteria were IPAA patients with a preoperative diagnosis of familial adenomatous polyposis, and patients with surgical complications of IPAA, including pouch sinus, abscess, and pelvic sepsis.

Clinical, Endoscopic, Laboratory, and Histologic Evaluation

Demographic, clinical, endoscopic, and histologic data were reviewed. As part of our routine clinical practice, all symptomatic patients underwent an outpatient pouch endoscopy with biopsy. Segmental evaluation and biopsies of the afferent limb, pouch, and anal transitional zone (the rectal cuff) were routinely performed during pouch endoscopy. The endoscopic features were documented and biopsies from each site separately labeled and submitted. Examination under anesthesia, contrast pouchography, computed tomography enterography, or magnetic resonance imaging of the pelvis was performed when Crohn's disease (CD) of the pouch was suspected. The modified Pouchitis Disease Activity Index (mPDAI) scores (range 0–12 points) were used to quantify symptom and endoscopy inflammation.[10,11]

An elevated concentration of serum IgG4 was defined by values greater than 112 mg/dl according to the reference standard used at our institution.

Definitions of Variables

CARP was defined as pouchitis (mPDAI ≥5 points) that did not respond to a 4-week antibiotic course of a single antibiotic (metronidazole [20 mg kg^{-1} day^{-1}] or ciprofloxacin [500 mg bid]).[12] The diagnosis of CD of the pouch was defined by ulcerated lesions of the small bowel or afferent limb without diffuse pouchitis (excluding backwash pouchitis) that persisted after ≥4 weeks of antibiotic therapy or by ulcerated strictures in the distal small bowel or pouch inlet with concurrent ulcers or inflammation of the afferent limb.[13] Those criteria were applied after the exclusion of non-steroidal anti-inflammatory drug (NSAID) use at the time of diagnosis. Irritable pouch syndrome (IPS) was defined as the presence of abdominal pain, pelvic discomfort, and diarrhea with no inflammation of the afferent limb, pouch, or cuff on endoscopy. Any other associated features for AIP, including primary sclerosing cholangitis (PSC)-like biliary changes and retroperitoneal fibrosis, were evaluated.

Demographic and clinical variables were defined as follows: "smoking": ever consumption of ≥7 cigarettes per week since the surgery; "family history of inflammatory bowel IBD": CD or UC in first-degree relatives; "duration of UC": the time interval between UC diagnosis and pouch construction; "duration of pouch": the time interval between completion of IPAA with ileostomy closure and entry into the study; "extensive colitis": endoscopic, macroscopic, or microscopic disease extending proximal to the splenic flexure; "indeterminate colitis": a histopathological diagnosis on proctocolectomy specimens that defied a clear distinction between CD and UC; "indication for colectomy": the primary reason for the surgery based on clinical presentation and preoperative diagnostic studies; "use of NSAID": regular use of NSAID more often than weekly at the entry into the current study; "PSC": the presence of intra- or extrahepatic bile duct abnormalities documented on endoscopic retrograde cholangiopancreatography and/or magnetic resonance cholangiopancreatography. Patients with PSC may or may not undergo orthotopic liver transplantation; "autoimmune mediated disorders": including adult-onset asthma, psoriasis, type 1 diabetes, rheumatoid arthritis, ankylosing spondylitis, autoimmune thyroid disease, systemic lupus erythematosus, celiac disease, pernicious anemia, and AIP.

Statistical Analysis

Descriptive statistics were computed for all factors in both the study and the control group. This included mean and percentiles for continuous factors and frequencies for categorical factors. Associations with categorical variables were done by Fisher's exact test or χ^2 test. Associations with quantitative and ordinal variables were performed by Student's *t*-test or Wilcoxon's rank sum test as appropriate.

Results

Demographic and Clinical Characteristics

The basic demographic and clinical information including NSAID use at the time of pouch endoscopy, duration of the pouch, type of pouch, preoperative and postoperative use of biologics and immunomodulators, the presence of concomitant autoimmune disorders, co morbidities and duration of IBD are summarized in Table 1.

There were ten patients (prevalence = 8.0%) with high serum IgG4 (the study group), while the remaining 114 (92%) patients had normal IgG4 (the control group). None of the patients had a confirmed diagnosis of AIP. The median serum IgG4 in the study group was 144.5 vs. 14 mg/dl in the control group. The mean age of patients in the study and control groups was 35.5± 14.5 and 42.0±13.2 years, respectively (p=0.137). Two patients in the study group (20.0%) had concurrent autoimmune disorders as compared to 19 patients (16.7%) in the control group (p=0.788). Three (30.0%) patients in the study group had coexisting PSC in contrast to 15 (13.2%) in the control group (p=0.147). Of the ten patients in the study group, five (50%) had CARP and one (10%) had CD of the pouch, while in the control group, 23 (20.2%) had CARP and 24 (21.1%) patients had CD of the pouch (p=0.273). CARP was significantly more common in the study group than in the control group IgG4 (50% vs. 20.2%, p=0.03).

Endoscopic Evaluation

Endoscopic inflammation of the afferent limb and pouch was documented. There was a significant difference in the PDAI symptom subscores between the study and control groups (3.88±0.9 vs. 2.88±1.5, p=0.042). However, there was no statistically significant difference in the PDAI endoscopic subscores in the afferent limb and pouch body between the patients in the study and control groups (Table 2).

Discussion

In this study, we investigated the possible role of serum IgG4 in pouchitis. Pouchitis likely represents a spectrum of disease processes, ranging from antibiotic-responsive, antibiotic-dependent, to antibiotic-refractory phenotypes. From etiological and pathogenetic perspectives, a variety of factors may contribute to the initiation, development, and progression of the disease process. These factors include genetic predisposition, dysbiosis, altered mucosal immunity, and colonic metaplasia due to fecal stasis. Immune-mediated factors likely play a major role in the pathogenesis of pouchitis, particularly in CARP and CD of the pouch. Our study showed that 8% of symptomatic patients at our Pouchitis Clinic had high serum IgG4. In addition, we found that patients with high serum IgG4 were more likely to have CARP. Also we found higher PDAI symptom subscores in patients with elevated serum IgG4. These findings suggest that immune-mediated mechanisms through IgG4 play a role in the disease process or pathogenesis in a subset of patients with pouchitis.

AIP is an increasingly recognized chronic inflammatory disease and criteria (the HISORt criteria) for the diagnosis of AIP were proposed.[14] Association between AIP with

Table 1 Demographic and clinical data

Factor	High serum IgG4 group (n=10)	Normal serum IgG4 group (n=114)	p Value
Mean age (years)	35.5±14.5	42.0±13.2	0.137
Mean duration of IBD before pouch (years)	8±5.4	7.7±7.5	0.905
Mean duration of pouch (years)	6.3±4.9	9.9±6.5	0.089
Male gender	6 (60.0%)	62 (54.4%)	1.000
Caucasian race	10 (100%)	113 (99%)	1.000
Tobacco consumption			
Active	2 (66.7%)	5 (27.8%)	0.247
Past	1 (33.3%)	13 (72.2%)	
Regular NSAID use	1 (10%)	8 (7%)	0.543
Family history of IBD	0 (0%)	26 (22.8%)	0.119
J pouch	9 (90%)	106 (93%)	0.543
Stage of pouch surgery			
1	1 (10%)	4 (3.5%)	0.623
2	8 (80%)	87 (76.3%)	
3	1 (10%)	16 (14%)	
4 or redo pouch	0 (0%)	7 (6.1%)	
Colectomy for refractory IBD	6 (60%)	98 (85.9%)	0.055
Extensive colitis	9 (90%)	111 (97.4)	0.289
Toxic megacolon	0 (0%)	7 (6.1%)	1.000
Pre-op diagnosis	9 (90%)	104 (91.2)	1.000
Ulcerative colitis	1 (10%)	10 (8.8%)	
Indeterminate colitis or Crohn's colitis			
Post-operative immunomodulator use	2 (20%)	14 (12.4%)	0.618
Post-operative biologic use	0 (0%)	16 (14.2%)	0.357
Extraintestinal manifestations			
None	6 (75%)	68 (67.3%)	1.000
Yes	2 (25%)	33 (32.7%)	
Primary sclerosing cholangitis	3 (30%)	15 (13.2%)	0.160
Concurrent autoimmune disorders	2 (20%)	19 (16.7%)	0.79

other autoimmune conditions such as PSC, retroperitoneal fibrosis, and sclerosing sialadenitis has been described.[4] One of the serology markers for AIP is an elevated serum IgG4. IgG4 is the rarest of the IgG subclasses and normally accounts for only 3% to 6% of the total IgG in the serum.[15] The serological criteria dictate that serum IgG4 need to be higher than 112 mg/dl in order to be considered for the diagnosis of IgG4-associated disease. A previous study reported that in patients with IBD and AIP, serum IgG4 was elevated in 50% of patients.[3] That study included all patients with a confirmed diagnosis of AIP which would explain the high prevalence of elevated IgG4. We found an elevation in serum IgG4 in 8% of patients in the study group. Similar elevations in IgG4 have been reported in 7–10% of patients with pancreatic cancer and 9% of patients with PSC.[16] In this study, we

Table 2 Symptomatic, endoscopic, and laboratory data

Factor	High serum IgG4 group (n=10)	Normal serum IgG4 group (n=114)	p Value
Mean PDAI symptom subscore	3.9±0.9	2.9±1.5	0.042*
Mean PDAI endoscopy subscore in the pouch	2.4±1.2	1.6±1.9	0.181
Mean PDAI endoscopy scores in the afferent limb	1.4±1.4	0.9±1.5	0.286
Chronic pouchitis	5 (50%)	23 (20.2%)	0.03*
Crohn's disease of the pouch	1 (10%)	24 (21.1%)	0.27
Concurrent autoimmune disorders	2 (20%)	19 (16.7%)	0.79

*p<0.05, significant

observed elevated IgG4 in three patients with PSC in the study group. It would be very hard to discern whether the elevation of serum IgG4 was related to PSC or CARP or was instead just an "innocent bystander" phenomenon.

The etiology and association error of elevated IgG4 with pouchitis are not clear. The current study showed that CARP was more common in patients with high serum IgG4. However, we did not see significant difference in the prevalence of AID between the study and the control group. This might have resulted from a type II error. The relationship between serum IgG4 and CARP warrants further investigation. Since IgG4-associated cholangiopathy has also been reported in two HLA identical siblings with UC there could be a role for IgG4 in IBD pathogenesis in patients even in the absence of AIP.[17]

The findings of the current study have several clinical implications. Patients with elevated IgG4 may represent a distinct subtype of pouchitis. Alterations in T-cell immunity with imbalance between proinflammatory and immunoregulatory cytokines have been described in pouchitis patients.[13] The findings of this study suggest a role of alterations in B cell immunity in the pathogenesis of pouchitis in some patients, as further evidenced by their role in IgG4 sclerosing disease.[18] The lymphoplasmacytic infiltrates seen in these patients include polyclonal B cells, plasma cells, and T cells. B lymphocytes can affect the inflammatory response by interaction with regulatory T cells.[19] Rituximab, a monoclonal antibody against CD20 has been used in the management of IgG4 related sclerosing disease.[20] Similarly, in patients with elevated IgG4 in pouchitis, treatment with other options including anti-B cell therapies can be explored. By definition, patients CARP were refractory to traditional antibiotic therapy. Thus some patients with CARP or even CD of the pouch with elevated serum IgG4 might be considered to be treated with B-cell targeted therapies. Glucocorticoids suppress cellular immunity and also humoral responses via inhibiting expression of IL-2 and IL-2 receptors on B cells to diminish B cell clone expansion and antibody synthesis. This property explains the efficacy of budesonide or prednisone in some patients with CARP from our clinical experience. Serum assay of IgG4 is routinely available in clinical labs. The finding of elevated serum IgG4 may help direct a proper therapy for the patients with pouchitis as well as exploration of concurrent hepatopancreaticobiliary disease in patients with IPAA.

This study has several limitations. The study population was recruited from a subspecialty Pouchitis Clinic. This might have had referral or selection biases and the findings would be difficult to be extrapolated to the general pouch population. Statistical significance was not achieved in certain other parameters including the coexistence of AID, which might have been from type II errors. We are continuing to recruit patients to generate a larger sample size for future multivariable analyses. We do not know whether elevated IgG4 is an epiphenomenon secondary to unrecognized antigens or whether it is a result of inflammation and disease process. We know that at least in patients with IgG4-sclerosing disease, a high level of serum IgG4 is not directly pathogenic as patients may have increase in IgG4 on treatment with clinical improvement.[21] The role of serum IgG4 in the pathogenesis of pouchitis in a subset of patients with IPAA warrants further study, which would help exploring treatment options for patients with refractory pouch disorders.

In conclusion, a subset of symptomatic pouch patients had elevated serum IgG4. It appears that pouch patients with an elevated serum IgG4 were more likely to have CARP. Future studies to investigate the role of IgG4 in pathogenesis of pouchitis and its clinical utility in the diagnosis, treatment, and prediction of prognosis are warranted.

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Conflict of interest The authors declared that they have no conflict of interest.

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ORIGINAL ARTICLE

Disease Characteristics of Inflammatory Bowel Disease (IBD)

Findings from a Tertiary Care Centre in South Asia

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Abstract
Background Inflammatory bowel diseases (IBD) include ulcerative colitis (UC) and Crohn's disease (CD), which are chronic inflammatory conditions affecting the gastrointestinal tract. There are only few published data on disease characteristics of IBD related to South Asia.
Objective To provide the disease characteristics of the IBD patients who presented to a tertiary care hospital in South Asia.
Methods Patients with an established diagnosis of IBD were identified after a review of their medical records and demographics, and disease characteristics and indications for surgical treatment were analyzed.
Results A total of 184 patients (women=101, 54.9%; UC=153, 83.2%) were included. Female preponderance was observed for UC (male/female ratio =1:1.5) and male for CD (male/female=2:1). Mean age at the time of diagnosis was 36.3 (range 7–71) years. CD was diagnosed at a significantly younger age than UC (27.35±10.22 vs. 38.14±13.05 years, $p<0.0001$). CD showed a peak age of onset in the third decade and that for UC was in the fourth decade. The mean duration of IBD was 8.17 (range 1–28) years. Presenting complaint of the majority (73.7%) of UC patients was blood and mucous diarrhea and that for CD (77.4%, 24/31) was left-sided abdominal pain. Only 9.5% ($n=18$) had at least one extra-intestinal manifestation. Among UC patients, 51.7% ($n=79$) had left-sided colitis and panproctocolitis was found in 18.3% ($n=28$). In IBD patients, 14.1% ($n=26$) underwent surgery. Only one patient developed malignancy.
Conclusions The majority of UC patients had left-sided colitis. CD compared to UC was diagnosed at a younger age. However, compared to data reported for some Western countries, extra-intestinal manifestations and malignancy rates were lower.

Keywords Ulcerative colitis · Crohn's disease · IBD · Extra-intestinal manifestations

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Introduction

Inflammatory bowel diseases (IBD), which include ulcerative colitis (UC) and Crohn's disease (CD), are a group of chronic disorders characterized by intestinal inflammation and sometimes extra-intestinal manifestations associated with periods of remission and unpredictable relapses. IBD occurs with varying incidence rates around the world. It is most common in certain Western countries like the UK and USA.[1,2] Several studies have been carried out regarding various aspects of IBD in Western countries. Many studies conducted previously have shown that IBD is rare, and prevalence is lower in the Asian region than in the West.[3–8] However, this may be due to under-diagnosis of cases as well as lack of awareness in the Asian region.

Recent studies from Japan and Korea have shown that the incidence of UC and CD are increasing in those countries.[4,5]At present, there is a paucity of data from countries in South Asia, and only few reports have been published from Sri lanka.[3,4,6,9]

Methodology

This study was conducted at the National Hospital of Sri Lanka, which is the premier tertiary-care center in the country. It accommodates referrals came from all parts of the country as it is the main center involved in managing a large volume of gastrointestinal (GI) work. The study was approved by the Ethics Review Committee of the hospital. Patients were enrolled prospectively over a period of 10 months. Before the interview, the patients were informed about the study and informed consent was obtained. All the patients who attended the outpatient clinics with an established diagnosis of either UC or CD were included in the study. Patients with unidentified colitis were excluded. Data including demographic aspects, symptoms, disease extent, duration, extra-intestinal manifestations and treatment aspects were collected from all patient records.

Extent of UC at first presentation was defined as follows: disease limited to rectum as proctitis, rectum and sigmoid colon as left-sided colitis, that up to the hepatic flexure as extensive colitis and involvement of whole colon as panproctocolitis.[10] Crohn's disease was classified according to the Montreal classification,[11] which classifies CD according to age at diagnosis (A1, ≤16 years; A2, 17–40 years; A3, >40 years), location of GI involvement (L1, ileal; L2, colonic; L3, ileocolonic; L4, isolated upper disease) and disease behavior (B1, no stricturing; B2, stricturing; B3, penetrating; P, perianal disease).

Patient Characteristics and Questionnaires

All IBD patients were interviewed via an interviewer-administered questionnaire which included personal details of the patients including socio-demographic data; disease characteristics and disease extent and management details. The findings were recorded in a structured data sheet. Formal education was categorized in to three groups: completed primary education, completed secondary education (more than 10 years formal education) and higher education (university/diploma).

Statistical Analysis

Statistical analysis was performed using SPSS (Version 15, SPSS, Chicago, IL). Measured values were expressed as mean ± SD. Significance level was set at $p<0.05$.

Results

A total of 184 patients (UC=153, 83.2%) were included in the study, of whom 136 (73.9%) were employed. Mean age of the study sample was 44.5 (range 20–78) years. There were 101 (54.9%) females. Female preponderance was observed for UC (male/female ratio=1:1.5 for UC) and a male preponderance for CD (male/female=2:1).None of our patients had a positive family history. Mean age at diagnosis for UC was 36.3 (range 7–71) years. Patients with CD were diagnosed at a significantly younger age than those with UC (27.35±10.22 vs. 38.14±13.05 years, $p<0.0001$). CD showed a peak age of onset in the third decade and that for UC was in the fourth decade (Table 1).

Disease Characteristics

Duration of the Disease

The majority had UC ($n=153$, 83.2%). Mean duration of IBD was 8.17 (range 1–28) years. The majority of the patients ($n=85$, 46.2%) had disease for less than 5 years, and only 20.1% ($n=37$) had the disease for 6- to 10-year duration. Nearly one-third ($n=62$, 33.7%) of the population had the disease for more than 10 years (Table 2). CD showed a peak age of onset in the third decade and for UC it was in the fourth decade (Fig. 1).

Clinical Presentation

Ulcerative Colitis Group Presenting complaint of the majority (73.7%, $n=135$) of UC patients was blood and mucous diarrhoea. Other symptoms in UC included abdominal pain in 46.8% ($n=86$), tenesmus in 34.2% ($n=63$) and loss of weight in 29.4% ($n=54$) (Table 2). Only 9.5% of the patients (UC: 6.8%, $n=13$; CD: 2.7%, $n=5$) had at least one extra-intestinal manifestation. None of the patients had sclerosing cholangitis. Colorectal cancer was seen in one (0.54%) patient with ulcerative colitis who had panproctocolitis. Two (1.3%) patients presented with lower GI bleeding.

Extent of the Colitis Based on histology of colonoscopic biopsies, the extent of the disease was proctitis in 21.6% ($n=33$), left-sided colitis in 51.7% ($n=79$), extensive colitis in 8.5% ($n=13$) and panproctocolitis in 18.3% ($n=28$).

Panproctocolitis Group Male-to-female ratio was 15:13. Mean age of the panproctocolitis group was 48.1 years (range 23–78).The majority ($n=18$, 64.3%) had a disease duration less than or equal to 10 years. Only ten had the disease for more than 10 years. One patient who had the

Table 1 Demographic characteristics of the study population

	Total IBD	UC	CD
Age at the diagnosis (years)			
≤10	2 (1.1%)	1 (0.7%)	1 (3.2%)
11–19	17 (9.2%)	13 (8.5%)	4 (12.9%)
20–29	42 (22.8%)	25 (16.3%)	17 (54.8%)
30–39	53 (28.8%)	49 (32%)	4 (12.9%)
40–49	39 (21.2%)	36 (23.5%)	3 (9.7%)
50–59	21 (11.4%)	19 (12.4%)	2 (6.5%)
60–69	8 (4.3%)	8 (5.2%)	–
70–79	2 (1.1%)	2 (1.3%)	–
Gender			
Male	83 (45.1%)	62 (40.5%)	21 (67.7%)
Female	101 (54.9%)	91 (59.5%)	10 (32.3%)
Education			
Primary (grades 1–5)	40 (21.7%)	35 (22.9%)	5 (16.1%)
Secondary (grades 6–13)	118 (64.1%)	101 (66%)	17 (54.8%)
Higher (university or higher)	26 (14.1%)	17 (11%)	9 (29%)
Employment			
None	72 (39.1%)	64 (41.8%)	8 (25.8%)
Student	11 (6%)	11 (7.2%)	–
Labourer	63 (34.2%)	50 (32.7%)	13 (41.9%)
Professional	38 (20.7%)	28 (18.3%)	10 (32.3%)

disease for more than 25 years was found to have an associated colonic cancer and underwent surgery.

Management of the UC A total of 14.1% (n=26) of the study population underwent surgical treatment. In the UC group, 8.5% (n=13) underwent surgical treatment (i.e., restorative proctocolectomy=12, colectomy with ileostomy =1). Indications for surgery are shown in Table 3.

Crohn's Disease Group In the CD group, the most common symptom at presentation was abdominal pain (77.4%, n=24) and other symptoms of presentation were blood and mucous

Table 2 Disease characteristics and endoscopic features of IBD

	Total IBD	UC	CD
Clinical presentation			
Watery diarrhoea	16 (8.7%)	11 (5.97%)	5 (2.7%)
Blood and mucous diarrhoea	146 (79.3%)	135 (73.4%)	11 (5.97%)
PR bleeding	65 (35.3%)	61 (33.1%)	4 (2.2%)
Tenesmus	72 (39.1%)	63 (34.2%)	9 (4.9%)
Abdominal pain	110 (59.8%)	86 (46.8%)	24 (13%)
Loss of weight	71 (38.6%)	54 (29.4%)	17 (9.2%)
Fever	23 (12.5%)	17 (9.2%)	6 (3.3%)
Extra-intestinal manifestations	18 (9.5%)	13 (6.8%)	5 (2.7%)
Duration of the IBD (years)			
1–5	85 (46.2%)	68 (44.4%)	17 (54.8%)
6–10	37 (20.1%)	29 (19%)	8 (25.8%)
11–15	31 (16.8%)	28 (18.3%)	3 (9.7%)
16–20	19 (10.3%)	16 (10.5%)	3 (9.7%)
21–25	4 (2.2%)	4 (2.6%)	–
26–30	8 (4.3%)	8 (5.2%)	–

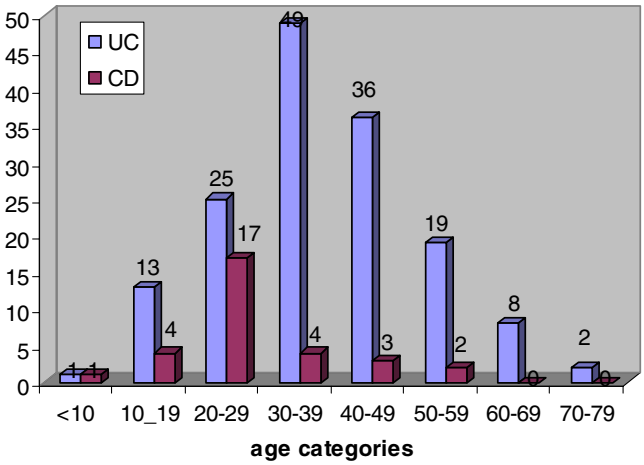

Fig. 1 Distribution of age at diagnosis of IBD

diarrhea (35.5%, n=11), weight loss (54.8%, n=17), per rectal bleeding (12.9%, n=4) and fever (19.3%, n=6). Other less common presentations included fistula in ano (3.2%, n=1), recurrent aphthous ulcers (3.2%, n=1) and extra-intestinal manifestations (2.7%, n=5, uveitis=1 sacroilitis=3, juvenile rheumatoid arthritis=1).

Disease Phenotypes in CD In 74.2% (n=23) of the patients, the location of the disease was colonic, followed by ileocolonic (12.9%, n=4), ileal (9.7%, n=3) and isolated upper GI (gastric antral stricture) (3.2%, n=1), respectively. Disease phenotype was non-stricturing and non-penetrating in 58.1% of the patients, stricturing in 19.4% and penetrating in 9.7%. Four patients had peri-anal disease including fistula in ano and peri-anal abscess formation. One patient (3.1%) underwent multiple strictureplasty surgery (disease characteristics according to the Montreal classification are shown in Table 4). Ten (32.2%) patients were found to have granuloma formation on histology and were differentiated from the tuberculosis by purified protein derivative test (mantoux) and PCR.

Treatments and Outcomes in CD Eighteen patients (58%) were on oral 5-aminosalicylates as maintenance treatment, 26 (83.9%) were on long-term azathioprine to maintain disease remission and 19 (61.3%) were on steroids during the time of analysis. The majority (80.6%) had one disease episode and others developed clinical relapses twice. At the time of data collection, the majority (58%) of patients were being managed on medical treatment alone, while 13

Table 3 Management of IBD

Medical		
	UC	CD
Sulphasalazine alone	67 (43.8%)	3 (9.7%)
Azathioprine alone	–	6 (19.4%)
Sulphasalazine and prednisolone	37 (24.2%)	2 (6.5%)
Sulphasalazine and prednisolone	15 (9.8%)	3 (9.7%)
Azathioprine and prednisolone	–	7 (22.6%)
Sulphasalazine, azathioprine + prednisolone	34 (22.2%)	10 (32.3%)
Surgical		
Procedure	Indication	*n*
UC		
Restorative proctocolectomy and ileoanal pouch	Steriod resistance, 7 atypia on histology, 4 Sigmoid colon cancer, 1	12 (7.8%)
Sigmoid colectomy	Stricture of sigmoid colon	1 (0.7%)
CD		
Drainage and fistulectomy	Perianal abscess and fistula	1 (3.2%)
Fistulectomy and repair	Recurrent enterocutaneous fistula	1 (3.2%)
Incision and drainage	R/Ischiorectal fossa abscess	1 (3.2%)
Repair of the fistula	Enrerocutaneous fistula	2 (6.4%)
R/Hemicolectomy and ileo transverse anastomosis	Strictures of the colon	4 (12.9%)
Total colectomy and ileostomy	Strictures of colon	2 (6.4%)
Repair of the fistula	Recto vaginal fistula	1 (3.2%)
Stricteroplasty, R/hemicolectomy and ileo transverse anastomosis	Two long segment narrowing —distal ileum multiple narrowing >10 in jejunum and proximal ileum and strictures of ascending colon	1 (3.2%)

Table 4 Disease characteristics according to the Montreal classification

Characteristics	No of patients
Age at the diagnosis (years)	
A1: ≤16	0
A2: 17–40	24 (77.4%)
A3: >40	7 (22.6%)
Disease location	
L1: Ileal	3 (9.7%)
L2: Colonic	23 (74.2%)
L3: Ileocolonic	4 (12.9%)
L4: Isolated upper disease	1 (3.2%)
Disease behavior	
B1: Non-stricturing, non-penetrating	18 (58.1%)
B2: Structuring	6 (19.4%)
B3: Penetrating	3 (9.7%)
P: Perianal disease	4 (12.9%)

(41.9%) underwent at least one operative procedure for the purpose of treating complications. None of our patients were on anti TNF treatment.

Six patients developed strictures of the colon as a complication of CD. Subsequently, four patients underwent right/hemicolectomy and ileo transverse anastomosis and other two underwent total colectomy and ileostomy creation (Table 3).

Discussion

Traditionally, IBD has been considered to be a rare disease in the Asia-Pacific region, but recent evidence indicate that both Crohn's disease and ulcerative colitis are becoming increasingly common in Asian populations.[4,5] In our study, the peak age of onset of UC is in the fourth decade (mean age, 38.14 years) (Fig. 1) and this is similar to other Asian countries like South Korea (i.e., 35 years)[4] and Singapore (i.e., 20–40 years).[12] In contrast to Japan, where the peak onset is 20–29 years for UC, our patients have a delayed onset of disease. In China,[13] UC presents at a mean age of 44 years, which is later than our population. Our observations showed that the peak age of onset for CD is earlier than UC, and it occurs in the third decade (mean age was 27.35± 10.22 years). When compared to other studies from China[14] (mean age, 37.2 years) and Hong Kong[8] (mean age, of 33.1 years), our patients presented at a slightly older age (mean age, 38.14 years). Our study also confirms the previous finding of Farrokhyar et al.,[15] who stated that 'there is no convincing evidence for a bimodal age of IBD onset in Asia,[7,8,16,17] unlike in western patients'. In the same study, Farrokhyar et al.[15] showed that UC tends to be slightly more common in men, whereas CD is marginally more common in women. But in our population, female preponderance was observed for UC (male/female=1:1.5 for UC) and male preponderance was seen for CD (male/female ratio=2:1). Our findings were identical to a Chinese study which showed that there are more males than females with CD.[18] In our study, none of the patients had a positive family history and others studies have also shown a very low incidence of family history (1.6%) in their patients.[19] Studies done in the West confirmed a higher incidence of CD than UC in both genders[20–22] but according to our observations UC is more common than CD. Our findings are also in keeping with other studies done in the Asian region.[3,6,12]

Differences and Similarities in the Extent of the Disease and Surgical Operation Rates

The majority (51.7%) of our patients had left-sided colitis, which was similar to other studies done in China (70.2%) and India (47.5%)[23,24] and some Western countries.[25]

UC is a disease which can be cured by surgery but also often responds well to medical treatment. In contrast, in CD, surgery is usually required especially for disease complications. In our study population, 29.1% (n=9) of CD patients had either stricturing or penetrating disease where surgery was indicated. In the UC group, 8.5% (n=13) underwent surgical treatment and that of CD was 41.9%. This is higher than that recorded in the study of Jiang et al.,[23] which involved 452 patients with IBD and where the operation rates of UC and CD were low (3% and 27%, respectively). However, when compared to the studies done in the West, in their study,[23] surgery was performed in 37.6% and 46% of patients with UC and CD, respectively.[26,27] Only 7.8 percent (n=12) patients with UC underwent pouch surgery; indications for surgery were atypia with high-grade dysplasia (n=4, 2.6%) on colonic biopsies, and only one had colonic adeno carcinoma (0.65%) (Table 3). All pouches were J-shaped and stapled ileal reservoirs with 20-cm limbs.

The current national crude annual incidence of colorectal cancer in Sri Lanka is 3.2 per 100,000 in women and 4.9 in men.[28] Taking into account the low rate of surgical treatment and colorectal cancer in our IBD patients, we think that this may be due to a milder form of disease compared to the western countries. The majority (58%, n=107) of patients in our study were from urban and suburban areas, whereas 42% (n=77) of patients were from rural areas. Although there was a significant number of patients from the rural areas, a previous study carried out in institution showed that our patients' disease-related knowledge of IBD was satisfactory when compared to other countries in the Asian region.[29]

In conclusion, IBD is an emerging disease in Sri Lanka, and records show that UC affects predominantly the young and middle-age female patients, whereas CD was observed to mainly affect young males. The most common clinical form of UC was left-sided colitis. Our patients had fewer extra-intestinal manifestations but had a considerably higher surgery rate compared to their Western counterparts. Although there are similarities between Asian and Western studies, considerable clinical differences exist. As our study is a hospital-based analysis, it may have underestimated the IBD complications. Therefore, population-based prospective studies are needed in Sri Lanka to assess the true incidence, prevalence and risk factors for IBD. In addition, we wish to state that the medical communities in South Asia should be aware of IBD as an emerging GI disease.

Authors' contribution All authors were involved in planning, data collection, analysis of data and writing the manuscript.

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ORIGINAL ARTICLE

Gender-Related Differences in Repopulation and Early Tumor Response to Preoperative Radiotherapy in Rectal Cancer Patients

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Abstract
Purpose Inhibition of tumor proliferation rate based on bromodeoxyuridine labelling index (BrdUrdLI), S-phase fraction (SPF) and MIB-1 labelling index (MIB-1 LI) as an early rectal cancer response to preoperative radiotherapy (RT).
Methods and materials A total of 122 patients qualified either for short RT (5 Gy/fraction/5 days) and surgery about 1 week after RT (schedule I) or for short RT and a 4-week interval before surgery (schedule II). Tumor samples were taken twice from each patient: before RT and at the time of surgery. In each sample, the BrdUrdLI, SPF and MIB-1 were calculated. Early tumor response was assessed by a biologist, a pathologist and surgeons.
Results Fifty-six patients were treated according to schedule I and 66 patients according to schedule II. Mean BrdUrdLI, SPF and MIB-1 LI before RT were 8.8%, 21.0% and 53.3%, respectively, and these values did not differ between the two compared groups. After RT, tumors showed statistically significant growth inhibition based on all assessed biological markers. As pretreatment assessed parameter was not predictive for early clinical and pathologic tumor response, prognostic role of the relative value (RV), that is, the ratio of assessed parameter after RT to before RT for each of the assessed markers, was considered. The ratios were calculated separately for fast and slowly proliferating tumors and separately for male and female patients. Fast proliferating tumors were more responsive. Differences with regard to sex were visible only in slowly proliferating tumors. Accelerated cell repopulation (4.8–28%/day) was noticed in female slowly proliferating tumors about 4 weeks after RT. Only for relative MIB-1 LI it was possible to show significant correlation with pathological tumor regression. Lack of such correlation for BrdUrdLI and SPF might reflect accelerated repopulation, particularly in slowly proliferating female tumors.
Conclusions Accelerated repopulation was noticed in slowly proliferating tumors in females about 4 weeks after RT.

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Keywords BrdUrdLI · MIBLI · SPF · Proliferation rate · Repopulation · Early tumor response · Rectal cancer · Preoperative radiotherapy

Introduction

In many institutions, preoperative radiotherapy (RT) for 5 days (25 Gy in five fractions) is considered as standard in the treatment of patients with resectable rectal cancer.[1] The RT schedule involves different intervals, a week or 4–8 weeks, before surgery[2] yet the optimal timing of surgery after preoperative radiotherapy in rectal cancer remains unknown. Recently, benefits from the short RT course have been questioned for the break between RT and surgery exceeding a week.[2] It has been shown that patients given a short course of RT who have undergone surgery 11–17 days after the start of RT, present higher a complication rate than those operated within 1 week after RT.[2] Thus, the rate of cancer cell proliferation seems to be a very important prognostic factor.

In our earlier study,[3] pretreatment bromodeoxyuridine labelling index (BrdUrdLI) was not predictive for early clinical and pathological tumor response although the after/before RT ratio (relative BrdUrdLI) revealed that response in tumors having different proliferating status varied. We showed that in short preoperative radiotherapy (5×5 Gy) the interval between RT and surgery longer than 2–3 weeks might cause accelerated repopulation, especially in slowly proliferating tumors. Now, we want to compare the prognostic significance of other proliferative markers in a larger group of patients. Therefore, the aim of this study is to evaluate relative BrdUrdLI, S-phase fraction (SPF) and Ki-67 labelling index (MIB-1 LI), as a measure of inhibition of tumor proliferation and prediction of tumor response to neoadjuvant RT in patients with rectal cancer. As recent studies have shown gender disparities in colorectal cancer patients' survival,[4–6] the aim of our study was also to check if there exists any gender-related difference in biological tumor response.

Methods and Materials

Patients

Between November 2003 and January 2006, we recruited 122 patients with resectable rectal carcinoma for whom curative surgery was planned. Patients were eligible for the trial if they had a histopathologically proved adenocarcinoma (T2/T3)[7] situated less than 12 cm from the verge of anus and gave informed consent to participation. The protocol was approved by the Ethics Committee of the Center of Oncology, and each patient had given written consent prior to participation in the study.

The criteria for exclusion were as follows: locally nonresectable tumor, plan to perform only local tumor excision, known metastatic disease, previous radiotherapy of the pelvic region, other malignant disease, patient's refusal.

Preoperative Radiotherapy

The patients assigned to preoperative radiotherapy received a total tumor dose of 25 Gy. The treatment was given in five fractions over 5 days, one posterior and two lateral wedged fields were irradiated with photons of maximum 6 MV energy. Using randomly blinded selection, surgery was performed either the following week (schedule I) or after a longer interval of 4 weeks (schedule II).

Surgery

Anterior resection of rectum or abdominoperineal excision was performed within a week or a month after the completion of RT. The type of surgery applied was resection of the rectum and lower sigmoid with involved adjacent tissue and regional lymph nodes up to or above the origin of inferior mesenteric artery. A minimal touch technique was used with high tight ligation of the inferior mesenteric artery.

Biological Assessment of Tumor Response

Tumor samples were taken twice: before radiotherapy (through a rectoscope) and during surgery, from the same place, i.e., at the lowest edge of the tumor mass. Each biopsy sample was divided into two parts: one was used for cytofluorymetric assessment (BrdUrd LI, SPF) and the second was used for immunohistochemical analysis (MIB-1 LI).

Assessment of Tumor Proliferation Markers

Flow Cytometric Analysis

Incorporation of BrdUrd in tumor samples from a biopsy (0.3–0.5 cm^3) was carried out in vitro via the high pressure oxygen method. The BrdUrd staining procedure and flow cytometry have been described in detail elsewhere.[8] The stained preparations were analysed with a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems, Sunnyvale, CA, USA) and 20×10^3 events were collected in each histogram. BrdUrdLI was calculated as a percentage of BrdUrd-labelled cells in a sample which incorporated BrdUrd during 1 h of incubation at 37°C (with

discrimination of diploid subpopulation in aneuploid tumors).

MIB −1 Labelling Index

Following rehydration, blocking the endogenous peroxidase, 5-μm sections were microwaved at 800 W in 10 mM sodium citrate buffer (pH=6.0) three times for 5 min. After 20 min, the sections were washed, flooded with normal swine serum for 20 min and incubated overnight at 4°C in a 1:200 dilution of mouse anti-Ki-67 monoclonal antibody (clone MIB-1; DAKO) in Tris-buffered saline (TBS, pH 7.4). After washing, slides were incubated for 1 h with DAKO En Vision visualisation system containing goat anti-mouse IgG. The sections were stained with diaminobenzidine (DAB), counterstained with hematoxylin, dehydrated and mounted. The MIB-1 labelling index (MIB-1 LI) was calculated as the percentage of positively immunostained nuclei (brown). About 500 to 1,000 cells were counted in several (four to six) areas of tissue sections.

Clinical Assessment of Tumor Response

Tumor size before RT was assessed basing on measures taken during rectoscopy and endorectal sonography. Tumor regression after RT was assessed by surgeons at the time of operation according to the following RECIST criteria[9] — complete response (CR): 100% disappearance; partial response (PR): 30–99% decrease; progression of disease (PD): 20% increase in sums of tumor longest diameters, stable disease (SD): neither CR, PR nor PD criteria met.

Pathological Assessment of Tumor Response

Tumor regression after RT was evaluated by a pathologist on the excised tumor mass. The following criteria of tumor regression assessed by Dworak et al.[10] were applied:

D0 no regression
D1 dominant tumor mass with obvious fibrosis and/or vasculopathy
D2 dominantly fibrotic changes with few tumor cells or groups
D3 very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance
D4 no tumor cells, only fibrotic mass (total regression or response).

Statistical Methods

Statistical analysis was performed with STATISTICA v.9. Intergroup differences in the ordinal data were tested with analysis of variance (ANOVA) test or Student's *t*-test. *P* values of less than 0.05 were considered as statistically significant. Tumors with values higher than the mean for all proliferation markers were considered as fast, and those with lower values than the mean were considered as slowly proliferating. For repopulation assessment in slowly proliferating tumors exponential regression functions have been estimated for relative BrdUrdLI and relative SPF for a break between RT and surgery longer than 28 days, separately for male and female patients.

Results

Patients

A total of 122 patients were included in the study. Fifteen (10.9%) out of 137 patients initially qualified to this study were excluded from the analysis due to discontinuation of treatment, metastatic tumor noticed at operation or no tumor samples taken for biological assessment during surgery. Mean age for the entire group of patients was 60.9 (range 30–82) years (Table 1). There were 85 males and 37 females. At the time of recruitment, no statistical differences between the two groups were found for prognostic factors such as sex, age, histological grade or tumor stage (Table 1).

In our series of patients, there were 33 stage T1 (27.0%), 75 stage T2 (61.5%) and 14 stage T3 (11.5%). Tumor cells were well differentiated in 32 patients (G1), moderately differentiated in 83 (G2), and poorly differentiated in five (G3) (Table 1). In two patients, tumor malignancy could not be assessed. Fifty-six patients received treatment according to schedule I in which time interval between end of irradiation and surgery averaged 9.5 days (range 2–16) (Table 1). In 66 patients, schedule II was applied in which mean break was 32.1 days (range 17–45). Because the interval between RT and surgery appeared to be longer than planned, mean break in the treatment lasted from 2 to 45 days (mean 21.7 days) (Table 1).

Clinical and Pathological Assessment of Tumor Response

In the clinical assessment of tumor mass resected during surgery, 46 (37.7%) tumors showed stable disease, 13 (10.6%) showed progressive disease, 57 (46.7%) showed partial response and six (4.9%) showed complete response. Partial and total tumor regression was observed in 49 (40.2%) tumors. The tumors were classified according to the World Health Organization classification of intestinal carcinoma[7] and staged according to the TNM classification.[11] Of the total 122, six (4.9%) patients had no tumor present (pTNM=0), 51 (41.8%) were pT1, 19

Table 1 Selected characteristics of patients and treatment parameters

Characteristics	Schedule				Total	
	I		II			
Age, mean (range) (years)						
F	14*	60.2 (44–82)	23*	60.2 (43–77)	122*	60.9 (30–82)
M	42*	60.0 (30–76)	43*	62.2 (45–74)		
Tumor stage						
TMN 1	11		20		33	
TMN 2	34		41		75	
TMN 3	8		6		14	
pTNM						
0	0		6		6	
1	23		28		51	
2	11		8		19	
3	21		21		42	
4	1		3		4	
Histological grade						
G1	9		23		32	
G2	41		42		83	
G3	5		0		5	
Interval between RT and surgery, mean (days)	9.5 (2.0–16.0)		32.1 (17.0–45.0)		21.7 (2.0–45.0)	
OTT mean (range) days	14.5 (7.0–21.0)		37.1 (22.0–50.0)		26.7 (7.0–50.0)	
Surgery						
Sphincter-preserving	30		42		72	
Abdominoperineal resection	25		25		50	

(15.6%) were pT2, 42 (34.4%) were pT3 and four (3.3%) were pT4.

Pathologic assessment of tumor regression after RT according to classification described by Dworak et al.[10] was performed in 121 out of 122 patients (for one patient, the assessment was impossible). The analysis showed no regression (D0) in 23 (19.0%) tumors, dominant tumor mass (D1) in 65 (53.7%), a few tumor cells with fibrotic mass (D2) in 23 (19.0%), single tumor cells (D3) in four (3.3%) and no tumor cells (D4) were observed in six (5.0%) of the examined tumors.

Biological Assessment of Tumor Response

Mean BrdUrd LI before RT was 8.8% (range 1.0–25.9%), SPF was 21.0% (range 3.8–49.9%) and MIB-1 LI 53.3% (range 21.5–84.8%), respectively. The mean values did not differ between the two schedules (Table 2). Tumor grade had no influence on proliferative marker values. After RT, tumors treated according to both schedules showed statistically significant growth inhibition (reduction of BrdUrd LI, percentage of SPF cells and MIB-1 LI) in comparison with the values obtained before RT (Table 2). Radiation induced inhibition of tumor proliferation was expressed as the percentage of the after RT to before RT marker value (e.g., relative value [RV]). This ratio ranged from 2.5% to 700% for BrdUrd LI, from 5.8% to 522.2% for SPF, and from 10.2% to 181.2% for MIB-1 LI. When we stratified patients into two groups according to their biological RT response — those radioresponsive with post-irradiation reduction of pretreatment values above 50% and those less responsive, with reduction below 50% — it turned out that the mean RVs for the more radioresponsive tumors were significantly lower than for less responsive tumors and their pretreatment values were higher. Therefore, we divided tumors into slowly and fast proliferating tumors based on the mean pretreatment marker values. Then the RVs were presented separately for fast (BrdUrd LI >8.8%, SPF >21.0%, MIB-1 LI >53.3%) and slowly (BrdUrd LI ≤8.8%, SPF ≤21.0%), MIB-1 LI ≤53.3%) proliferating tumors. Mean relative BrdUrdLI value for fast proliferating tumors (55 cases) showed statistically significant (P=0.007) reduced pretreatment percentage (44.6%) in comparison with slowly proliferating tumors (90.3%, 67 cases) (Fig. 1). The same was true for SPF (61.5%, 53 cases) and MIBLI (56.3%, 51 cases) of fast and slowly proliferating tumors (114.5%, 69; 75.6%, 45), respectively (P=0.000, P= 0.021). When patient's gender was taken into consideration, the difference between fast and slowly proliferating tumors

Table 2 Status of biological parameters before and after RT

Marker	Schedule I	Schedule II	Total
BrdUrdLI (%)			
Before RT	8.7[b] (1.1–24.2)	9.0[b] (1.0–25.9)	8.8[b] (1.0–25.9)
After RT	4.1 (0.8–14.0)	4.6 (0.4–18.3)	4.4 (0.4–18.3)
Relative BrdUrdLI[a] (%)	70.5 (3.5–700.0)	68.2 (2.5–514)	69.7 (2.5–700)
SPF (%)			
Before RT	20.4[b] (5.0–49.2)	21.5[b] (3.8–49.9)	21.0[b] (3.8,–49.9)
After RT	15.3 (1.5–47.9)	15.3 (2.6–46.5)	15.3 (1.5–47.9)
Relative SPF[a] (%)	101.3 (5.8–22.2)	83.2 (15.2–328.4)	91.5 (5.8–522.2)
MIB-1 LI (%)			
Before RT	52.9[b] (21.5–75.5)	53.7[b] (26.6–84.8)	53.3[b] (21.5–84.8)
After RT	31.6 (6.2–72.0)	35.6 (3.0–71.9)	33.8 (3.0–72.0)
Relative MIB-1 LI[a] (%)	63.4 (11.9–166.7)	68.5 (10.2–181.2)	66.4 (10.2–181.2)

[a] Value of the marker after RT/ before RT

[b] Significant difference ($P<$ 0.000) between the assessed parameter before and after RT

became obvious in males with respect to the three markers (P=0.020, P=0.007, P=0.001), while in females this was observed only with respect to relative SPF (P=0.009).

As the second determination of the markers occurred within 2–45 days after RT, we looked at tumor growth retardation kinetics after RT. For this purpose, we analysed the RVs of the markers separately for fast and slowly proliferating tumors and also separately for male and female patients (four subgroups), for five time points between RT and surgery, i.e., for the break shorter than 10 days (arithmetic mean, 5 days), lasting 11–19 days (mean, 15 days), 20–30 days (mean, 25 days), 31–35 days (mean, 33 days) and longer than 35 days (mean, 40 days). After RT, at each time point, greater decrease in the RVs was observed for fast than for slowly proliferating tumors, irrespective of the patients' sex, basing on relative BrdUrdLI (Table 3) and relative SPF (Fig. 2). For relative MIBLI, however, significant difference (P=0.002) between fast and slowly proliferating tumors was indicated only for males, while in female tumors, the difference was not observed.

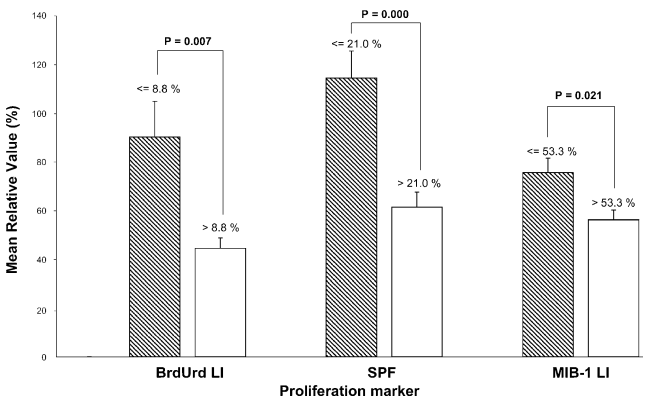

Fig. 1 Influence of tumor proliferative rate on growth retardation (mean relative value) of BrdUrdLI, S-phase fraction (*SPF*) and MIB-1 LI after preoperative RT of rectal cancer. Tumors were stratified into slowly (below the mean pretreatment value) and fast proliferating (above the mean pretreatment value) of the measured marker. Each bar represents the mean±SE

The RVs showed fluctuation in time (5 time points), which was similar for relative BrdUrdLI and relative SPF and slightly different for relative MIBLI. The RV for the last marker, for the first time point (5 days), was similarly low (40–60%) for all subgroups and increased about 15 days (reaching higher values for slowly proliferating tumors) with later decrease about 25 days after RT. Accelerated repopulation was observed about 35 days after RT, especially in female tumors. Because in 25 patients, MIBLI was not assessed (tumor sample was too small for analysis) and no data were available for slowly proliferating female tumors for the break of about 40 days, these data are not shown.

Preoperative RT with a dose of 25 Gy reduced the number of DNA synthesizing cells in fast proliferating tumors to about 40–60% of pretreatment values (RVs), independently of break and similarly in male and female tumors. However, the RVs for slowly proliferating tumors showed fluctuations with time, which differ between male and female patients. The ratios for female slowly proliferating tumors were higher than those for male tumors. This was particularly visible in two time points: 15 and 40 days after RT (Table 3, Fig. 2). The lowest RVs were observed about 25 days after RT, followed by repopulation starting from about 35 days, which was greater in female slowly proliferating tumors. About 40 days after RT, the highest RVs were visible only in slowly proliferating female tumors.

As in these tumors, the mean RVs for DNA synthesizing cells much exceeded pretreatment values, this may indicate accelerated repopulation of tumor cells surviving RT. Therefore, we tried to estimate the degree of accelerated repopulation basing on Relative BrdUrdLI and SPF values. For this purpose, exponential regression functions have

Table 3 Influence of tumor proliferation rate and patients sex on growth retardation (relative BrdUrdLI) after radiotherapy

Interval between RT and surgery	Males		Females	
	BrdUrdLI ≤8.8% *X*±SE	BrdUrdLI >8.8% *X*±SE	BrdUrdLI ≤8.8% *X*±SE	BrdUrdLI >8.8% *X*±SE
≤10 days	(17)[a] 91.4±23.0	(6) 54.5±13.1	(4) 50.4±9.1[§]	(6) 29.5±6.5
11–19 days	(9) 49.1[#]±9.7	(13) 33.9±6.4	(2) 390.0±310.0	(3) 43.6±14.6
20–30 days	(3) 76.6[†,**]±11.5	(2) 21.9±12.8	(4) 26.1[¶]±7.8	(1) 86.1
31–35 days	(15) 102.6±31.5	(9) 63.0±8.3	(6) 64.7±10.3	(5) 66.2±28.0
>35 days	(5) 43.7**±15.9	(6) 36.6±13.8	(2) 282.3±202.0	(4) 31.9±2.5
All	(49) 81.3[††]±12.9	(36) 44.4±4.8	(18) 113.2±42.4	(19) 44.8±8.4

Tumors were divided into subgroups based on pretreatment BrdUrdLI: ≤8.8%, slowly proliferating; >8.8%, fast proliferating

[a] Number of tumors

[†] P=0.054, [††] P=0.02, difference between slowly and fast proliferating tumors in males

[§] P=0.09, [¶] P=0.04, difference between slowly and fast proliferating tumors in females

[#] P=0.02, [**] P=0.08 difference in slowly proliferating tumors between males and females

been estimated for slowly proliferating tumors for the break longer than 28 days, separately for men and women (Fig. 3). For male tumors, repopulation appeared to be nonsignificant (P=0.939). For women, however, it was highly significant (P=0.042). From the estimated function, it was calculated that average repopulation occurring after the break longer than 28 days is equal to 28.4%/day basing on relative BrdUrdLI and 4.7%/day basing on relative SPF.

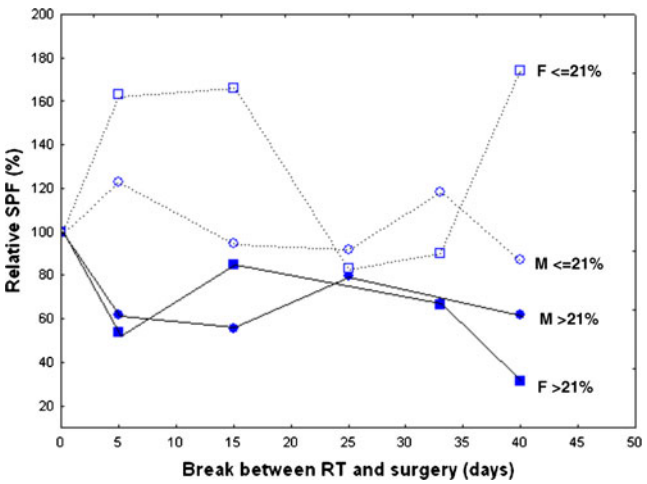

Fig. 2 Gender-related difference in tumor response after preoperative RT. Changes in relative SPF (e.g., after/before RT SPF) are shown during the break between RT and surgery. Data are presented for five time points (breaks) which represent arithmetic mean of time break: 5, 15, 15, 33, 40 days. Tumors were divided into four subgroups differing in proliferative rate (*open symbols* slowly proliferating, *closed symbols* fast proliferating) and patients gender (*F* females, *M* males). Each point represents mean of 2–11 tumors. Error bars are omitted for clarity

Correlation Between Biological, Clinical and Pathological Tumor Response

Pretreatment BrdUrdLI, SPF and MIB LI were not correlated with early clinical and pathologic tumor response. None of the relative proliferation markers were predictive for clinical response. Only the relative MIB-1 was predictive (P=0.005) for pathological tumor response (Fig. 4). However, the response was not uniform in tumors differing in gender. In slowly proliferating tumors of male patients, the correlation was nonsignificant (P=0.06), but in female tumors it was statistically significant (P=0.041).

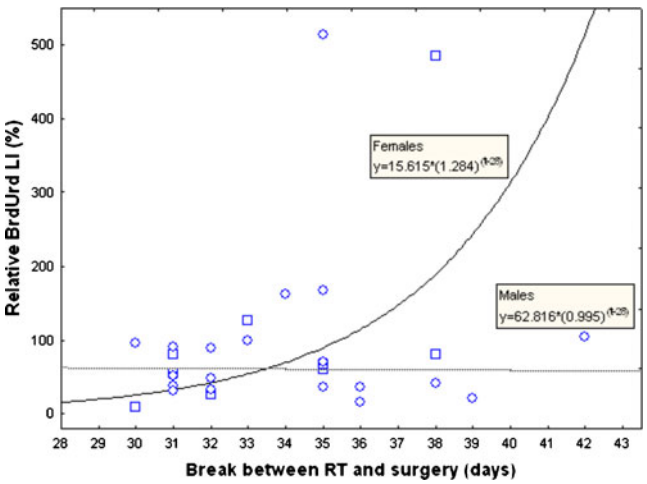

Fig. 3 Tumor cell repopulation in female (*open square*) and males (*open circle*) slowly proliferating tumors occurring 28–45 days after RT. From exponential regression functions, it has been estimated that about 5 weeks after RT accelerated repopulation in female tumors was significant (P=0.042) and equal to 28.4%/day, whereas in male tumors it was not significant (P=0.939)

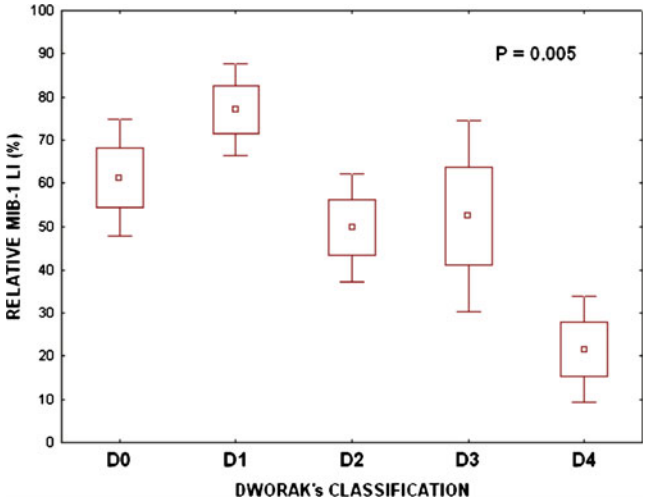

Fig. 4 Correlation between relative MIB-1 LI and pathological assessment (Dworak classification): *D0* no response, *D1* dominant tumor mass with obvious fibrosis, *D2* dominantly fibrotic changes with few tumor cells, *D3* very few (difficult to find microscopically) tumor cells in fibrotic tissue, *D4* no tumor cells. Box plot displays mean relative MIB-1 LI and SE

Similar correlation was indicated for fast proliferating tumors. In male tumors, it was not significant (P=0.500), but was predictive for pathological regression in female tumors (P=0.044).

Discussion

This study provides evidence of a clinically significant different tumor response after pre-operative radiotherapy in tumors varying in proliferating rate and patients' sex. In order to assess inhibition of tumor proliferation, three proliferation markers (BrdUrdLI, SPF and MIBLI) were studied before and after radiotherapy. The study showed differences in the pretreatment tumor proliferation rate, which did not vary significantly by tumor grade and patient gender. The pretreatment results are in agreement with those of other research groups.[12–15] After RT, the mean values of the analyzed markers significantly decreased.

Biological tumor response was measured by inhibition of cell proliferation and expressed by the RVof each marker, e.g., after/before RT ratio. The ratio appeared to be significantly higher for slowly than for fast proliferating tumors what indicates lower reduction of the number of DNA synthesizing or proliferating cells. Also gender difference in RVs of the markers was observed between fast and slowly proliferating tumors. This was shown for the first time. In males, the difference was more statistically significant (and based on three markers) than in females (based on Relative SPF). The reason for this discrepancy may be the fact that slowly proliferating female tumors reacted more heterogenously (Relative BrdUrdLI), or were incomplete (lack of MIB-1 LI for the longest break).

In order to perform cell kinetics analysis during the post-irradiation period, the mean RVs for each marker were analyzed for different (2 to 45 days) breaks and patients sex. Gender-related difference in cell kinetics was observed between fast and slowly proliferating tumors, which was more pronounced for BrdUrdLI and SPF than for MIBLI, as the first two markers are considered more sensitive for measuring cell proliferation. Within the examined breaks, cell fluctuation was observed, showing different kinetics of measured cell subpopulations, especially within the shortest break. At the first time point, relative MIBLI values were lower than RVs for other markers, indicating cell loss and proving that changes in growth fraction require more time for activation of many genes and cell recruitment. However, at the same time, DNA synthesizing cells which were already in the cell cycle at the time of irradiation, responded quickly and heterogenously. About 1 week after RT, decrease in the Relative SPF and BrdUrdLI was observed, but only in fast proliferating tumors (despite of sex difference). For slowly proliferating tumors, however, increased RVs were observed, which were most pronounced in female tumors. It is probable that slowly proliferating female tumors might have greater propensity to recruit cells into rapid cycle in response to treatment than fast proliferating which might have little reserve capacity for further accelerating their cell cycle.[16] Later (mean break, 25 days), RVs were below pretreatment value for all four analyzed subgroups. Starting from about 4–5 weeks, acceleration of cell proliferation was observed again, but only in slowly proliferating female tumors, reaching levels much higher than pretreatment values. This may indicate accelerated cell repopulation. It was calculated that accelerated repopulation is equal to 4.8–24.8%/day.

Our data confirm Withers et al.'s statement[17] that accelerated growth began, on average, at about 3 to 5 weeks after the start of treatment with a very short lag time.[18] Tumor regeneration is a response to depopulation, may be mediated by growth factors or hormones,[16,19] and is likely primarily the consequence of changes in stem cell daughter differentiation.[20] The data may also confirm the findings of the earlier study on head and neck tumors indicating that accelerated repopulation starts as soon as 2 weeks after irradiation.[21] Our results show that fast proliferating tumors are more radioresponsive, in agreement with numerous earlier studies.[22–24] To our knowledge, this is the first study to show sex-related differences in post-irradiation tumor response in slowly proliferating tumors. For many decades, fast proliferation of tumor cells was considered a negative prognostic factor in terms of local control and patients' survival,[25–27] and no attention has been given to sex differences.

Recently, clinical studies have been published showing gender difference in rectal cancer patients survival.[4–6,28,29] They clearly show better survival rates for younger female patients compared to male patients,[5,6] which suggests the protective effect of estrogen on colorectal cancer and results from hormonal differences between genders.

None of the assessed relative proliferation markers was predictive for early clinical tumor response. However, only the relative MIB-1 LI was predictive for early pathological tumor regression. This marker also revealed difference in tumor response between sexes. Lower relative MIBLI was predictive for pathological response in female and not in male tumors, independently of proliferating status. This may, however, be caused by lack of RVs of the marker for slowly proliferating female tumors for the longest break. Because RVs for other markers and longer breaks were relatively high, showing higher proliferation, this may indirectly prove the significant influence of this tumor subpopulation on tumor response.

Our observation is in agreement with recent clinical data showing high complication rate in patients if surgery is delayed beyond 10 days after the start of RT.[2] If late tumor response confirms that patients with slowly proliferating tumors treated with short RT schedule and longer break present more recurrences and lower survival rates, we need to obtain proof that long breaks should not be applied.

Conclusions

Relative marker values showed significantly higher inhibition of cell proliferation in fast than in slowly proliferating tumors. Accelerated repopulation was observed in slowly proliferating female tumors more than 4 weeks after irradiation. It was calculated to be 4.8–28.4%/day.

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Conflict of interest None.

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ORIGINAL PAPER

Prevalence and Clinical Implications of Positive Serum Anti-Microsomal Antibodies in Symptomatic Patients with Ileal Pouches

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Abstract
Background and aim Autoimmune disorders (AID) have been shown to be associated with chronic antibiotic-refractory pouchitis (CARP). The role of anti-microsomal antibodies in ileal pouch disorders has not been investigated. The aims of the study were to investigate the prevalence of positive anti-microsomal antibody in symptomatic patients with ileal pouches and to investigate its clinical implications.
Methods A total of 118 consecutive symptomatic patients with ileal pouches were included between January and October 2010. Anti-microsomal antibodies were measured at the time of presentation. Demographic, clinical, and laboratory characteristics were compared between patients with positive and negative anti-microsomal antibody.
Results There were 14 patients (11.9%) with positive serum anti-microsomal antibody. The mean age of patients in the antibody positive and negative groups were 41.8±14.4 and 42.0±14.0 years, respectively (p=0.189). All 14 patients in the antibody positive group (100%) had some form of AID, as compared to 20 patients (19.2%) in the antibody negative group (p<0.001). Four (28.6%) patients in the antibody positive group had at least one AID in addition to Hashimoto's thyroiditis in contrast to four (3.8%) in the antibody negative group (p=0.003). In addition, five (35.7%) patients had associated primary sclerosing cholangitis (PSC) in the antibody positive group compared to nine (8.7%) in the antibody negative group (p=0.012). Eleven patients (78.6%) in the antibody positive group required steroids for treatment of pouch related symptoms in contrast to 26/104 (25%) patients in the antibody negative group (p=0.002).
Conclusions Anti-microsomal antibodies were common in pouch patients presenting with symptoms. Patients with positive anti-microsomal antibodies were much more likely to have concurrent AID and PSC. These patients were more likely to require therapy with steroids.

Keywords Autoimmune · Anti-microsomal · Pouchitis · Primary sclerosing cholangitis

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Abbreviations

AID	Autoimmune disorders
AIP	Autoimmune pancreatitis
CD	Crohn's disease
CARP	Chronic antibiotic refractory pouchitis
IBD	Inflammatory bowel disease
IPAA	Ileal pouch-anal anastomosis
NSAID	Non-steroidal anti-inflammatory drug
PSC	Primary sclerosing cholangitis
PDAI	Pouchitis Disease Activity Index
TSH	Thyroid stimulating hormone
UC	Ulcerative colitis

Introduction

Autoimmune disorders (AID) have been shown to be more common in patients with inflammatory bowel disease (IBD) than in those without IBD, suggesting that they may share common etiopathogenetic factors.[1] Some patients with chronic pouchitis do not respond to routine antibiotic therapy, which is termed chronic antibiotic-refractory pouchitis (CARP).[2] The predominant theory on etiopathogenesis of pouchitis is dysbiosis and its associated abnormal mucosal immune response,[3] as the majority of patients with pouchitis respond favorably to antibiotic therapy.[2] Other factors, including autoimmunity, may contribute to refractory pouchitis. Our previous study showed that the presence of AID was associated with a 2-fold increase in the risk for CARP.[2]

Previous epidemiologic studies and case reports/series have reported an association between thyroid disorders and IBD.[4–6] The association appears to be stronger with ulcerative colitis (UC) than with Crohn's disease (CD). Other investigations have found alterations in thyroid physiology and anatomy in the form of thyroid enlargement by ultrasound in patients with IBD who did not have clinical signs or symptoms of thyroid dysfunction.[7] Similarly, in a previous study, increased iodide uptake and increased daily fractional turnover of thyroxine in IBD patients was seen as compared with controls.[8] However, the role of thyroid disorders in patients with ileal pouch-anal anastomosis (IPAA) is not clear.

In our clinical practice, we found that antimicrosomal antibody was often present in patients with concurrent AID and IPAA and patients with pouchitis in this setting frequently did not respond to traditional antibiotic therapy. These observations lead us to embark on our current project with the hypothesis that anti-microsomal antibody-mediated autoimmunity may contribute to the disease process in some patients with pouchitis. The aims of this study were to investigate the prevalence of positive serum anti-microsomal antibodies in symptomatic patients with ileal pouches and to characterize clinical features of pouch disorders in these patients.

Patients and Methods

Patients

The study involved consecutive symptomatic patients presenting to the Pouchitis Clinic from January to October 2010. Patient's demographic and clinical data were retrieved from the IRB approved, prospectively maintained database. Patients were divided into two groups: those with positive anti-microsomal antibody and those with negative anti-microsomal antibody.

Inclusion and Exclusion Criteria

Inclusion criteria were patients with IPAA for underlying UC with symptoms of frequency, urgency and abdominal cramps. Exclusion criteria were IPAA patients with a preoperative diagnosis of familial adenomatous polyposis and patients with pouch dysfunction secondary to structural abnormalities, surgical causes, and cuffitis.

Clinical, Endoscopic, Laboratory, and Histologic Evaluation

Demographic, clinical, endoscopic, and histologic data were reviewed. As a part of our routine clinical practice, all symptomatic patients underwent an outpatient pouch endoscopy with biopsy. Examination under anesthesia, contrast pouchography, computed tomography enterography, or magnetic resonance imaging of the pelvis was performed when CD of the pouch was suspected. The modified Pouchitis Disease Activity Index (mPDAI) scores (range 0–12 points) were calculated to define pouchitis.[9,10]

Other laboratory tests which were abstracted from the database were thyroid stimulating hormone (TSH). Anti-microsomal antibody was measured by immunoenzymatic assay and antibody level greater than 9 IU/ml was indicative of a positive test.

Definitions of Variables

CARP was defined as pouchitis (mPDAI ≥5 points) that does not respond to a 4-week antibiotic course of a single antibiotic (metronidazole 20 mg kg^{-1} day^{-1} or ciprofloxacin 500 mg bid).[11] The diagnosis of CD of the pouch was defined by ulcerated lesions of the small bowel or afferent limb without diffuse pouchitis (excluding backwash pouchitis) that persisted after ≥4 weeks of antibiotic therapy or by ulcerated strictures in the distal small bowel or pouch inlet with concurrent ulcers or inflammation of the afferent limb.[12] Those criteria were applied after the exclusion of non-steroidal anti-inflammatory drug (NSAID) use at the time of diagnosis.

Demographic and clinical variables were defined as follows: "smoking": ever consumption of ≥7 cigarettes per week since the surgery; "family history of inflammatory bowel IBD": CD or UC in first-degree relatives; "duration of UC": the time interval between UC diagnosis and pouch construction; "duration of pouch": the time interval between completion of IPAA with ileostomy closure and entry into the study; "extensive colitis": endoscopic, macroscopic, or microscopic disease extending proximal to the splenic flexure; "indeterminate colitis": a histopathological diagnosis on proctocolectomy specimens that defied a clear distinction between CD and UC; "indication for proctocolectomy": the primary reason for the surgery based on clinical presentation

and preoperative diagnostic studies; "use of NSAID": regular use of NSAID more often than weekly at the entry into the current study; "primary sclerosing cholangitis (PSC)": the presence of intra- or extrahepatic bile duct abnormalities documented on endoscopic retrograde cholangiopancreatography and/or magnetic resonance cholangiopancreatography (the patients with PSC may or may not undergo orthotopic liver transplantation); "autoimmune mediated disorders": including adult-onset asthma, psoriasis, rheumatoid arthritis, autoimmune thyroid disease, autoimmune pancreatitis (AIP) and vitiligo.

Statistical Analysis

Descriptive statistics were computed for all factors in both the study and the control group. This included mean and percentiles for continuous factors and frequencies for categorical factors. Associations with categorical variables were done by Fisher's exact test. Associations with quantitative and ordinal variables were performed by Student's *t*-test or Wilcoxon's rank sum test as appropriate.

Results

Demographic and Clinical Characteristics

The basic demographic and clinical information including duration of the pouch, type of pouch, preoperative and postoperative use of biologics and immunomodulators,

Table 1 Comparison of patients with and without microsomal antibody

Factor	Anti-microsomal antibody positive group (n=10)	Anti-microsomal antibody negative group (n=114)	p Value
Mean age (years)	41.8±14.4	42.0±14.0	0.19
Mean duration of IBD before pouch (years)	9.4±6.2	9.1±8.2	0.858
Mean duration of pouch (years)	8.4±7.7	9.0±6.8	0.763
Male gender	7 (50.0%)	54 (51.9%)	0.881
Caucasian race	13 (92.9%)	104 (100%)	0.118
Tobacco consumption			
Active	3 (21.4%)	9 (8.65%)	0.153
Past	0 (0%)	3 (2.88%)	1.000
Family history of IBD	3 (21.4%)	20 (19.2%)	1.000
J Pouch	13 (92.9%)	100 (96.2%)	0.474
Stage of pouch surgery			0.754
1	1 (7.1%)	2 (1.9%)	
2	11 (78.6 %)	73 (70.2%)	
3	1 (7.1%)	24 (23.1%)	
4 or redo pouch	1 (7.1%)	5 (4.8%)	
Colectomy for refractory IBD	10 (71.1%)	91 (87.5%)	
Extensive colitis	13 (92.9%)	99 (95.2%)	0.78
Toxic megacolon	1 (7.1%)	14 (13.5%)	1.000
Pre-op diagnosis			
Ulcerative colitis	14 (100%)	95 (91.4%)	0.596
Indeterminate colitis or Crohn's colitis	0 (0%)	9 (8.7%)	
Post-operative immunomodulator use	0 (0%)	8 (7.7%)	0.593
Post-operative biologic use	0 (0%)	4 (3.8%)	1.000
Post-operative steroid use	11 (78.6%)	26 (25.0%)	0.002
Pouchitis responded to steroids	11 (100%)	17 (65.8%)	0.03
Extraintestinal manifestations	8 (57.1%)	47 (45.2%)	0.569
Primary sclerosing cholangitis	5 (35.7%)	9 (8.7%)	0.012
Presence of autoimmune disorders in addition to Hashimoto thyroiditis	4 (28.6%)	4 (3.8%)	0.003
Antibiotic responsive pouchitis	3 (21.4%)	59 (56.7%)	0.02
Chronic antibiotic refractory pouchitis	7 (50.0%)	26 (25.0%)	0.06
Crohn's disease of the pouch	4 (28.6%)	19 (18.3%)	0.579

presence of concomitant AID, comorbidities, and duration of IBD are summarized in Table 1.

There were 14 patients (11.9%) with positive anti-microsomal antibody. Among the 14 patients in the antibody positive group, six patients had a previous diagnosis of hypothyroidism and Hashimoto thyroiditis and were on thyroxine supplement therapy at the time of their initial Pouch Clinic visit. The other eight patients were diagnosed with positive anti-microsomal antibodies after presenting to the Pouch Clinic with symptoms of pouch dysfunction, and were newly diagnosed with Hashimoto thyroiditis. Among the eight patients, three patients were euthyroid on further testing with free thyroxine and TSH; and five patients were diagnosed with hypothyroidism secondary to Hashimoto's thyroiditis and were started on thyroxine after the visit. In all, there were 11 patients with hypothyroidism and three patients with euthyroid status in patients in the antibody positive group.

The mean age of patients in the antibody positive and antibody negative groups were 41.8+14.4 and 42.0+14.0 years, respectively (p=0.189). There was no difference in the extent of colitis or the indication for colectomy prior to IPAA surgery between the two groups. All 14 patients in the antibody positive group (100%) had some form of concurrent AID including Hashimoto's thyroiditis as compared to 20 patients (19.2%) in the antibody negative group (p<0.001). Furthermore, four (28.6%) patients in the antibody group had at least one more concurrent AID in addition to Hashimoto's thyroiditis in contrast to four (3.8%) in the antibody negative group (p=0.003). The AID seen in these four patients were vitiligo in one, rheumatoid arthritis in one, psoriasis in one, and autoimmune pancreatitis in one. Moreover, five (35.7%) patients had concurrent PSC in the antibody positive group compared to nine (8.7%) in the antibody negative group (p=0.012). Seven patients (50%) in the antibody positive group had CARP vs. 26 (25.0%) in the antibody negative group (p=0.06). Four patients (28.6%) in the antibody positive group and 19 patients (18.3%) in the antibody negative group had CD of the pouch (p=0.579).

Treatment

Oral administration of topically active corticosteroids (i.e. budesonide) has routinely been used in treating refractory pouchitis and/or autoimmune-associated pouchitis. Among the patients with positive anti-microsomal antibody, 11 (78.6%) required budesonide for control of pouch-related symptoms, while in the control group, 26 (22.8%) patients required budesonide (p=0.002). Of the patients who required budesonide, all 11 patients (100%) responded clinically to budesonide and required them for maintenance therapy as compared to 17/26 (65.4%) who responded clinically and required budesonide for maintenance therapy (p=0.03).

Discussion

In this study, we investigated the prevalence and clinical implications of seropositive anti-microsomal antibody in patients with pouch dysfunction. A variety of factors may contribute the initiation, development, and progression of pouchitis including genetic predisposition, dysbiosis, altered mucosal immunity, and colonic metaplasia due to fecal stasis.[5] Autoimmune factors may play a role in the pathogenesis of pouchitis, particularly in CARP and CD of the pouch.[2] Our study showed that approximately 12% of symptomatic patients at our Pouch Clinic had seropositive anti-microsomal antibody. There was clustering of AID and PSC in patients with seropositive anti-microsomal antibody. Patients with positive anti-microsomal antibody with symptoms of pouchitis were much more likely to respond to budesonide than controls. We did not find significant association between the presence of the antibody and CARP which might have resulted from type II error.

Microsomal antibodies are directed against components of thyroid microsomes, in particular peroxidase. Thyroid peroxidase in fact accounts for virtually all of the antigenic determinants reacting with the autoantibodies commonly termed as anti-microsome.[13] Anti-microsomal antibodies are present in Hashimoto's thyroiditis, Graves' disease, hypothyroidism, atrophic thyroiditis, and are sometimes increased in the elderly. In addition to the above mentioned etiologies, the prevalence of autoantibodies for thyroid antigens, like anti-microsomal antibody, is as high as 30% in patients with other AID, such as Sjogren's syndrome and systemic lupus erythematosus.[14,15] Although anti-microsomal antibodies are elevated in a number of AIDs, they are the most useful measurement for detecting autoimmune thyroid diseases (Hashimoto thyroiditis).[16] In this study, 11/14 patients with positive antibody were hypothyroid and were on medications. The remaining three patients were euthyroid at the time of the study. These three patients did not show any evidence of Sjogren's syndrome or systemic lupus erythematosus. These patients did not have any coexisting AID and had a normal TSH. Although euthyroid at present, these patients are at risk of developing hypothyroidism on follow-up.[17] Development of overt hypothyroidism occurs at a rate of 4–5% per year in adults with elevated TSH and antithyroid antibodies, and a rate of 2% per year in patients with antithyroid antibodies alone.[17] None of our patients with anti-microsomal antibodies were hyperthyroid. Nevertheless, hyperthyroidism can present with diarrhea and can confound the picture with pouchitis. Hence screening for thyroid dysfunction in patients with

IPAA presenting with increased frequency of bowel movements is required.

A recent population-based study from England has highlighted the coexistence of AID in patients with either Graves' disease or Hashimoto's thyroiditis.[18] The frequency of another AID was 9.7% in Graves' disease and 14.3% in Hashimoto's thyroiditis index cases ($p=0.005$). There were higher prevalences of Addison's disease (10-fold higher) and pernicious anemia (3-fold higher) in those with Hashimoto's thyroiditis, than the subjects with Graves' disease. Rheumatoid arthritis was the most common coexisting AID.[16] Relative risks of almost all other autoimmune diseases in Graves' disease or Hashimoto's thyroiditis were significantly increased (pernicious anemia, systemic lupus erythematosus, Addison's disease, celiac disease, and vitiligo). IBD was also more common in female patients with Graves' disease, but did not reach significance in patients with Hashimoto's thyroiditis. Similarly we found a clustering of AID in patients with positive anti-microsomal antibody which points to the autoimmune nature of their pouchitis. There was also a trend towards increased prevalence of CARP in anti-microsomal antibody positive group. Rheumatoid arthritis was seen in 1/14 patients with anti-microsomal antibody. Patients in the antibody positive group were treated with budesonide rather than antibiotics and the response rate was also higher highlighting the role of autoimmunity in the pathogenesis of pouch dysfunction.

A study from Sweden revealed thyroid diseases in 8.4% of the 119 patients with PSC.[19] Similarly, in a study from the Mayo Clinic, the prevalence of thyroid dysfunction in PSC was 11% at initial evaluation.[20] In the study group, three patients had PSC. It would be very hard to discern whether the positive anti-microsomal antibodies were related to PSC or CARP or was just an "innocent bystander." PSC was more common in patients with a positive anti-microsomal antibody highlighting the role of autoimmunity. The relationship of CARP, PSC and thyroid antibodies points to the common pivotal role of autoimmunity in the pathogenesis of these diseases. The overlap of immune response in IBD and autoimmune thyroid disease has been previously studied. One study found the immune response of both autoimmune thyroid disease and IBD to be polyclonal by examining immunoglobulin and T cell antigen receptor gene rearrangement.[21] Autoimmune thyroiditis and UC are speculated to be Th2-mediated disease processes because both are associated with the production of autoantibodies. Alterations in T-cell immunity with imbalance between proinflammatory and immunoregulatory cytokines have been described in pouchitis patients.[5] Thus the relationship between anti-microsomal antibodies and pouchitis warrants further investigation.

The findings of this study have several clinical implications. Patients with positive anti-microsomal antibodies may develop a distinct subtype of pouchitis. Whether patients with IPAA are prone to develop autoimmune thyroid disorders is not known. We had reported a case of de novo celiac disease after IPAA surgery.[22] De novo AID has been reported in patients with bowel-anatomy-altering surgeries, such as in Whipple's procedure.[23] In fact, eight of 14 patients had newly diagnosed autoimmune thyroid disease after the evaluation at our Pouchitis Clinic. We speculated that altered bowel anatomy in IPAA patients may predispose them to development of AID. On the other hand, concurrent AID[2] and PSC[24] may impact the disease course of pouchitis. By definition, patients with CARP were refractory to traditional antibiotic therapy. Thus some patients with CARP or even CD of the pouch with positive anti-microsomal antibodies might be considered to be treated with targeted therapies including steroids or biologics. Serum assay of anti-microsomal antibodies is routinely available in clinical labs. The finding of positive anti-microsomal antibodies may help direct a proper therapy for the patients with pouchitis as well as exploration for concurrent thyroid disease in patients with IPAA. There was also a trend for CARP in microsomal antibody positive patients; however, it did not reach statistical significance.

This study has several limitations. The study population was recruited from a subspecialty Pouch Clinic. This might have had referral or selection biases with patients being refractory to routine treatment and the data would be difficult to be extrapolated to the general pouch population. Our study cohort did not have a long-term follow-up to see the natural course of the disease. Statistical significance was not achieved in certain parameters including the presence of CARP. The small sample size of the study group precluded meaningful multivariable analysis. We are continuing to recruit patients to generate a larger sample size for future multivariable analyses.

In summary, approximately 12% of pouch patients presenting with symptoms of pouch dysfunction to our clinic had positive anti-microsomal antibody. Patients with positive anti-microsomal antibodies were much more likely to have concurrent AID and PSC. Further study to investigate the usefulness of testing patients with pouch dysfunction for anti-microsomal antibody is required.

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Specific author contributions Study concept, data monitoring and paper preparation—Udayakumar Navaneethan.
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ORIGINAL ARTICLE

Synchronous Rectal and Hepatic Resection of Rectal Metastatic Disease

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Abstract
Background The objectives were to determine the feasibility of combined rectal and hepatic resections and analyze the disease-free survival and overall survival.
Study Design Sixty patients who underwent resection for metastatic rectal disease from 1991 to 2005 at Mayo Clinic were reviewed. Inclusion criteria were: rectal cancer with metastatic liver disease and resectability of metastases. The exclusion criteria were: metachronous resection ($n=15$). Kaplan–Meier Survival estimated overall survival (OS) and disease-free survival (DFS). Cox proportional hazard models examined the association between groups and survival.
Results The cohort comprised 22 men and 23 women, with median age of 63 years. Surgical management included: abdominoperineal resection, 13 patients (29%); low anterior resection, 29 (64%); local excision, one; total proctocolectomy, one; and pelvic exenteration, one. Major hepatic resection was performed in 22%. There was no mortality, but there were 26 postoperative complications. Disease-free survival from local recurrence at 1, 2, and 5 years was 92%, 86%, and 80%, respectively. Disease-free survival from distant recurrence at 1, 2, and 5 years was 62%, 43%, and 28%, respectively. Overall survival at 1, 2 and 5 years was 88%, 72%, and 32%, respectively.
Conclusions Combined rectal and hepatic resection is safe. Morbidity and mortality do not preclude concurrent resection. The DFS and OS are comparable to that of patients undergoing a staged procedure.

Keywords Rectal · Hepatic · Metastatic · Synchronous

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Introduction

Adenocarcinoma of the colon and rectum is the third most common malignancy and the second leading cause of cancer-related death in the USA. Approximately half of all colorectal carcinomas occur in the lower sigmoid region or rectum, and 20% of the rectal cancer patients at initial presentation have hepatic metastases. Stage IV rectal carcinoma carries a very high mortality rate with <10% of diagnosed patients alive at 5 years.[1] Not uncommonly, such patients will have distant metastatic disease limited only to the liver at presentation. Resection of isolated hepatic metastases from colorectal cancer remains the most effective form of treatment with survival rates approaching 58%.[2–6] Indeed, surgical resection of the primary carcinoma and hepatic metastases is the only therapy with the potential

for cure.[7] Due to the complex multimodality treatment regimens for patients with stage IV rectal cancer, specifically the role of neoadjuvant therapy, the optimal timing for surgical resection of the hepatic metastatic disease is debated and not clearly defined. In fact, resection of hepatic metastases without resection of the primary has been proposed to avoid perioperative risks which could affect adjuvant therapy. Specifically, data are limited regarding patients with synchronous resections for rectal cancer, including low anterior resection or abdominoperineal resection (APR). The primary objective of this study was to determine the procedural feasibility and safety of combined rectal resections and hepatic resections. The secondary objective of this study was to analyze the disease-free survival (DFS) and overall survival (OS) of this patient cohort.

Materials and Methods

A retrospective review was performed on 60 consecutive patients with stage IV rectal cancer who underwent synchronous or staged resections of primary rectal cancer and liver metastases between 1991 and 2005 and was approved by the Institutional Review Board at the Mayo Clinic, Rochester, MN. Inclusion criteria included age greater than 18 years, pathologically documented rectal cancer with metastatic rectal cancer to the liver, and resectability of hepatic metastases by surgical assessment. Exclusion criteria included patients undergoing a staged rectal and liver resection (n=15). Data elements abstracted from the patient chart included demographics, symptomatic presentation, preoperative imaging, TNM (tumor-node-metastasis) staging, operative and pathologic findings, chemoradiation regimen, postoperative complications, date of death, and date of disease recurrence.

Preoperative evaluation of patients with rectal cancer included abdominal computed tomography performed at our institution or prior to patient referral and subsequently evaluated by our institutions' radiologists and surgeons. Endorectal ultrasound was utilized for staging the primary rectal tumors and was performed by our experienced institutional gastroenterologists. Hepatic metastases were diagnosed by preoperative imaging and by intra-operative evaluation and ultrasound. Hepatic resections were typed by the Brisbane Classification.[8] Postoperative complications were classified by the Dindo–Clavien system.[9] Neoadjuvant therapy, if administered, consisted of 5-flurouracil-based infusional chemotherapy and external-beam radiation in the range of 45–54 Gy.

Descriptive statistics are reported as number (percent) and as mean (standard deviation, or median (range) as appropriate. Disease-free survival was calculated from time of rectal and hepatic resection to time of disease recurrence, while OS was calculated from time of rectal and hepatic resection to death or last follow-up, censoring at patient death when not due to disease progression. Overall survival and DFS survival were calculated by the method of Kaplan and Meier with the median survival reported as the point in time when the survival estimate reaches 50%. Cox proportional hazard regression was used to assess the association between specific variables, and overall and DFS. The hazard ratio (HR) and 95% confidence interval (CI) are reported. The association between recurrence and patient death was assessed considering the date of recurrence as a time-dependent covariate in the Cox model. The alpha-level was set at $p<0.05$ for statistical significance.

Results

Demographics and Presentation

Of the 60 eligible patients, 15 patients were excluded, leaving a study group of 45 patients, 22 males and 23 females. The median age of the overall study group at operative resection was 63 (range, 47–71 years). The median age of patients receiving or not receiving neoadjuvant chemoradiation was similar (64 versus 64.5 years, p = NS). Patients were followed a median of 5 years, to either death or last contact. Eighteen patients (40%) received neoadjuvant therapy at outside institutions prior to referral. The mean patient body mass index was 25 (22.7–27.6 kg/m^2). The American Society of Anesthesiology (ASA) score distribution was as follows: ASA I (one patient), ASA II (22 patients), ASA III (22 patients). Forty-one patients (91%) were symptomatic from the rectal cancer with bleeding (58%) and bowel movement changes (22%) constituting the majority of symptoms. The T and N stage grouping for the primary rectal cancer is shown in Table 1. The mean distance of the tumor from the anal verge was 7 cm (4.25–10 cm). The mean preoperative carcinoembryonic antigen (CEA) level was less than 200 ng/mL for 42 patients (93%; range 0–459 ng/mL).

Surgical Management

An 8-week interval from completion of neoadjuvant therapy to surgery was observed in all patients receiving neoadjuvant therapy. All patients had R0 resections of both the primary rectal cancer and the hepatic metastases. Thirteen patients (29%) underwent APR; 29 patients (64%) underwent low anterior resections; one patient underwent local excision; one patient underwent total proctocolectomy, and one patient underwent pelvic exen-

Table 1 Group characteristics

Characteristic	Number (%)
Gender	
Male	22 (49)
Female	23 (51)
Mean age (years)	63
Initial presentation	
Bleeding	26 (58)
Bowel movement changes	10 (22)
Pain	2 (5)
Anemia	1 (2)
Obstruction	1 (2)
Weight loss	1 (2)
Asymptomatic	4 (9)
Clinical uT stage	
Stage 1	1 (2)
Stage 2	3 (7)
Stage 3	38 (84)
Stage 4	3 (7)
Clinical uN stage	
Stage 0	15 (33)
Stage 1	25 (56)
Stage 2	5 (11)
Mean number of nodes taken	16.7
Mean number of nodes positive	4.8
Mean number of liver metastases	2
Mean size of largest liver metastases (cm)	3

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teration. Six patients (13%) received intra-operative radiation therapy. Thirteen patients (40%) who underwent low anterior resection were temporarily diverted. Types of hepatic resections are shown in Table 2. Three patients received radiofrequency ablation in addition to hepatic resection, and three patients had hepatic artery infusion pumps placed. The lymph node (LN) status of the primary rectal cancer was pathologically positive in 33 patients (73%). The average number of pelvic LNs excised was 16.7 with an average number of 4.8 nodes positive for metastatic cancer. The mean LN ratio in the 33 patients with a positive node removed was 0.3, with a median of 0.2. The rectal carcinomas were classified pathologically as moderately differentiated in 12 patients (27%), poorly differentiated in 31 patients (69%), and dedifferentiated in two patients (4%). Seven patients (15%) had liver metastases >5 cm in size; 13 patients (29%) had bilateral liver metastases, and eight patients (18%) had >4 total metastases. The average number of hepatic metastases was two, and the average size of resected hepatic metastases was 3 cm. Twenty-one patients (46%) required intra-operative transfusion with an average of 2.4 units of packed red blood cells.

Table 2 Hepatic operative procedures

Type of liver procedure	Number (%)
Wedge	20 (44)
Right hepatectomy	8 (19)
Segmentectomy	6 (14)
Subsegmentectomy	5 (11)
Segmentectomy + wedge	2 (4)
Extended right hepatectomy	1 (2)
Right hepatectomy + wedge	1 (2)
Left lateral sectorectomy + segmentectomy	1 (2)
Segmentectomy + subsegmentectomy	1 (2)

Neoadjuvant and Adjuvant Therapy

Eighteen patients (40%) received neoadjuvant chemotherapy consisting of 5-fluorouracil/leucovorin (5FU/LV). Twenty-two patients (49%) received radiation, with 18 of these patients (82%) receiving the radiation preoperatively. Of these 18 patients, 16 patients (89%) received both neoadjuvant chemotherapy and radiation. Thirty-three patients (73%) received adjuvant chemotherapy, with 52% receiving 5FU/LV, 42% receiving folinic acid, fluorouracil, and oxaliplatin (FOLFOX), and one patient receiving both FOLFOX and folinic acid, fluorouracil, and irinotecan (FOLFIRI). No patients experienced direct hepatic complications as a result of chemotherapy as defined by the surgical team.

Postoperative Morbidity and Mortality

There was no perioperative mortality. There were 26 postoperative complications in 21 patients (Table 3). None required reoperation. Only three patients required readmission due to surgical complications. These complications did not significantly delay adjuvant chemotherapy. There was no significant association between presence of a major complication (Clavien grade ≥3 compared with <3) and surgery type (APR compared with non-APR), $p=0.31$, nor with a hepatic resection complexity (minor <3 resections compared with major ≥3 resections), $p=1.0$.

Disease-Free Survival

Disease-free survival from local pelvic recurrence at 1, 2, and 5 years was 92%, 86%, and 80%, respectively. Disease-free survival from distant recurrence at 1, 2, and 5 years was 62%, 43%, and 28%, respectively (Fig. 1). Seven patients developed local pelvic recurrence (four anastomotic, one presacral, one pelvic cul de sac, and one at the ureter). Sixteen patients developed liver recurrence.

Table 3 Postoperative complications

Complication	Number
Anastomotic leak	1
Abscess	2
SBO/ileus	2
Wound infection	3
Urinary retention	4
Cardiac	2
Pulmonary	2
Renal failure (ATN and acute or chronic)	2
UTI	4
Pancreatitis	1
Bile leak	1
Seizures	1
Upper extremity thrombophlebitis	1

SBO small bowel obstruction, *ATN* acute tubular necrosis, *UTI* urinary tract infection

Other sites of metastases included: four patients with liver recurrence and lung metastases, three patients with lung metastases only, one patient with liver recurrence and bone metastases, two patients with bone metastases only, and one patient with peritoneal metastases. Three of the 18 patients who received neoadjuvant radiation developed a local recurrence while four of the 27 patients who did not receive neoadjuvant radiation developed a local recurrence (p=1.0, HR 1.0, 95% CI 0.2–4.6). Disease-free survival, local or distant, was not associated with nodal disease of the primary tumor (p=0.58, HR=0.81), the size of liver lesion ≥5 cm (p=0.31, HR=0.54), number of metastases (p=0.11, HR=1.2), or preoperative CEA≥200 (p=0.84, HR=0.82).

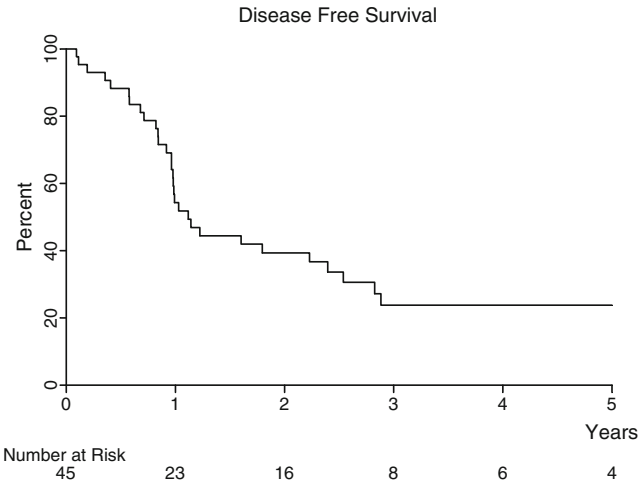

Fig. 1 Five-year distant disease-free survival

Overall Survival

Overall survival at 1, 2, and 5 years was 88%, 72%, and 32%, respectively (Fig. 2). At last contact, ten patients are alive without recurrence, while seven patients are alive with recurrence. Four patients died from causes unrelated to their rectal cancer, and 23 patients died due to their disease. Overall survival was not associated with nodal disease of the primary tumor (p=0.19, HR=1.8), the size of liver lesion ≥5 cm (p=0.96, HR=1.0), unilateral liver versus bilateral liver disease (p=0.74, HR=1.2), or preoperative CEA≥200 (p=0.76, HR=0.7).

Discussion

The results of our study reveal that combined rectal and hepatic resections are safe and feasible with no mortality and acceptable morbidity. The 5-year DFS and OS of our patient population with stage IV rectal cancer is similar to other reports on hepatic resection for stage IV disease.[3,4,6] Finally, our series, though relatively small, supports concurrent resection of the primary rectal cancer and hepatic metastases when both colorectal and hepatic surgical expertise is available. These data suggest that concurrent resection for stage IV rectal cancer with metastases to the liver proves safe as a standard for treatment. We feel that this paper is unique to reports of synchronous colon and hepatic resection due to the different entity of rectal operations. Rectal operations are technically more challenging and pose a much greater morbidity than colon procedures, especially when combining with a major hepatic resection.

Historically, surgical procedures for rectal cancer, specifically an APR, were associated with increased morbidity and mortality when compared with resections of the proximal colon. A recent systematic review by Paun et al.

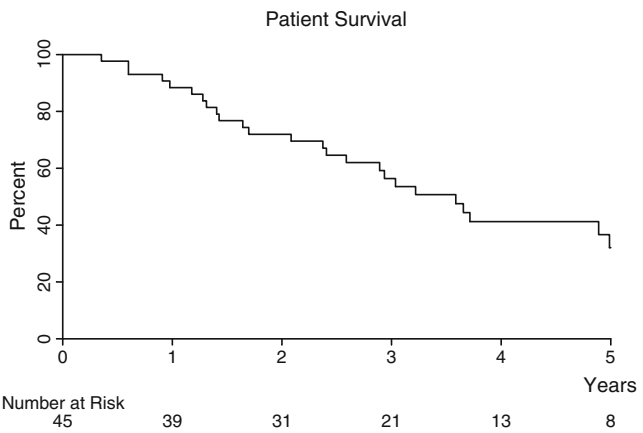

Fig. 2 Five-year overall survival

of 53 prospective cohort studies and 45 randomized controlled trials aimed at determining postoperative complication rates of radical surgery, specifically APR and anterior resection for rectal cancer, reported complications including anastomotic leak of 11%, pelvic sepsis of 12%, wound infection of 7%, and mortality of 2%.[10] Our own data of 237 patients undergoing low anterior resection after neoadjuvant therapy revealed an overall postoperative morbidity of 26% with 0% mortality.[11] Thus, our data do not suggest increased perioperative risk following resection of rectal cancer herein.

Whether the risk of concurrent resection of the primary colorectal cancer and hepatic resection of metastases differs from staged resection of the primary cancer and metastases is unclear. Historically, surgical management of the primary colorectal cancer with synchronous hepatic metastases prompted a staged approach with the hepatic resection occurring at least 2 months subsequent to the primary carcinoma resection. This approach has shifted in recent years, however. Multiple institutions have performed successful concurrent resections of colorectal carcinomas and hepatic metastases with acceptable morbidity and mortality. Moreover, oncologic outcomes after concurrent resections have been similar to those reported after staged resections.[12] Our mortality was zero. Although our overall morbidity rate was 57%, only 16% had major complications (Dindo–Clavien class 3 or 4). These complications did not affect length of hospital stay or outcome. Moreover, these complications did not significantly delay adjuvant chemotherapy in our patients. These findings are similar to other published data. Martin et al., at the Memorial Sloan-Kettering Cancer Center, prospectively reported on 240 patients who underwent synchronous resection of a primary colorectal carcinoma. However, the vast majority of these resections were in colon cancer (anal canal, 16 patients; sigmoid/rectum, 95 patients; right/transverse/descending colon, 129 patients). The complication rate was significantly lower for patients with synchronous resections compared with staged resections. In fact, multivariate analysis revealed that a staged resection was an independent predictor of overall complications, attributed to the need for two independent laparotomies. Thus, they concluded that synchronous resection was superior to a staged approach in experienced centers.[12] Similarly, our own retrospective experience of patients with synchronously resected colorectal cancers and hepatic metastases (27 colon, 32 rectal) compared with those who underwent a staged procedure (15 colon, 13 rectal) showed that morbidity was equally distributed between the two groups, thus allowing for the conclusion that combined resection is safe.[13] Morbidity following ileostomy takedown was not addressed in this series. However, in a large series at our institution of 237 patients, the postoperative leak rate was 0.5%; the rate of small bowel obstruction requiring operative intervention was 0.5%; the rate of wound infection was 3% and of developed ileus, 12%.[11]

No study, to our knowledge, has specifically addressed the combined resection of solely rectal cancers and hepatic metastases. Martin et al., as mentioned prior, reported rectal cancers in 38% of the patient population; however, 23% of these patients were in the staged procedure category and did not undergo simultaneous liver resection. Furthermore, they report that 37% of the simultaneous resected patients underwent wedge procedures while 72% of the major liver resections were reserved for the staged population.[12] Similarly, concurrent resection of rectal cancer and hepatic metastases was performed in 17% of patients in our prior series, and most hepatic resections were subsegmental.[13] Recently, Capussotti et al. reported 88 synchronous resections of colorectal cancers and hepatic resections. Low anterior resections for rectal carcinoma with synchronous major hepatectomy were performed in only nine patients, though morbidity was only 22%.[14] Our current study included only patients who had concurrent resections of primary rectal cancer and hepatic metastases. Major hepatic resection was undertaken in 22% of these patients. Regardless, our data showed that the risk of concurrent resection of the primary rectal cancer and hepatic metastases is not prohibitive.

Our study has several limitations. First, this non-randomized retrospective study is small and heterogeneous with regard to demographics, TNM stage, neoadjuvant and adjuvant therapy, and types of resections for both the primary cancers and their metastatic sites. Second, advanced stage cancers are more commonly referred to major cancer centers. Referral bias to tertiary centers may limit the generalizability to non-academic centers, without large volumes of stage IV rectal cancers, or centers without combined colorectal and hepatic surgical expertise. Third, concurrent resection of the primary rectal cancer and hepatic metastases was not compared with staged resections; therefore, we cannot conclude at this juncture whether subsets of patients with stage IV rectal cancer incur less morbidity by staged resections. Finally, evaluation of oncological approaches to optimize long-term survival was precluded. Although our outcomes were consistent with survival reported for resection of stage IV rectal cancers, the independent impact of concurrent resections on survival was precluded.

Conclusion

There are limited data regarding synchronous rectal and hepatic resection. Combined rectal and hepatic resections are safe and feasible with minimal morbidity and mortality.

The DFS and OS in this patient population are comparable to those of patients undergoing a staged procedure.

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ORIGINAL ARTICLE

Liver Segment IV Hypoplasia as a Risk Factor for Bile Duct Injury

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Abstract
Introduction Bile duct injury remains constant in the era of laparoscopic cholecystectomy and misidentification of structures remains one of the most common causes of such injuries. Abnormalities in liver segment IV, which is fully visible during laparoscopic cholecystectomy, may contribute to misidentification as proposed herein.
Methods We describe the case of a 36-year-old female who had a bile duct injury during a laparoscopic cholecystectomy where the surgeon noticed an unusually small distance between the gallbladder and the round ligament.
Results We define hypoplasia of liver segment IVas well as describe the variation of the biliary anatomy in the case. We also intend to fit it in a broader spectrum of developmental anomalies that have both hyopoplasia of some portion of the liver and variations in gallbladder and bile duct anatomy that may contribute to bile duct injury.
Discussion To our knowledge, hypoplasia of liver segment IV has not been suggested in the literature as a risk factor for bile duct injury except in the extreme case of a left-sided gallbladder. Surgeons should be vigilant during laparoscopic cholecystectomy when they become aware of an unusually small distance between the gallbladder bed and the round ligament prior to beginning their dissection, variations in the common bile duct and cystic duct should be expected.

Keywords Hypoplasia · Liver segment IV· Segment IV· Bile duct injury

Introduction

Liver segment IV is fully visible during laparoscopic cholecystectomy. It can be identified between the gallbladder and the round ligament with its base just above the hilar plate. To our knowledge, hypoplasia of liver segment IV has never been defined nor suggested as a risk factor for bile duct injury (BDI) except on the extreme case of a left sided gallbladder in one case report[1] which is quite a different scenario.

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Although BDI during cholecystectomy is multifactorial,[2] misidentification of biliary anatomy (present in 70–80% of cases) is one of the most contributory.[3] Anatomic variability of the common bile duct (CBD) and the cystic duct have been classified extensively reflecting their vital importance in promoting injury.[4,5]

Although rare, anomalies of the gallbladder and liver may also play a role in the genesis of BDI, both because they can lead to the misidentification of structures and because they may be associated with bile duct anomalies.[6] The extreme of this situation, seen in the literature, is represented by two case reports of bile duct injury where the gallbladder was located to the left of the round ligament and attached to liver segment III. Both of these cases presented a confluence medial to the umbilical fissure and one of them described a hypoplasic segment IV. The latter was found during a left hepatectomy and thus hardly replicates the case described herein.[1,7] Although not associated with BDI, well over 100 case reports of left-sided gallbladders have been reported in the literature, several describing an underdeveloped segment IVas well as

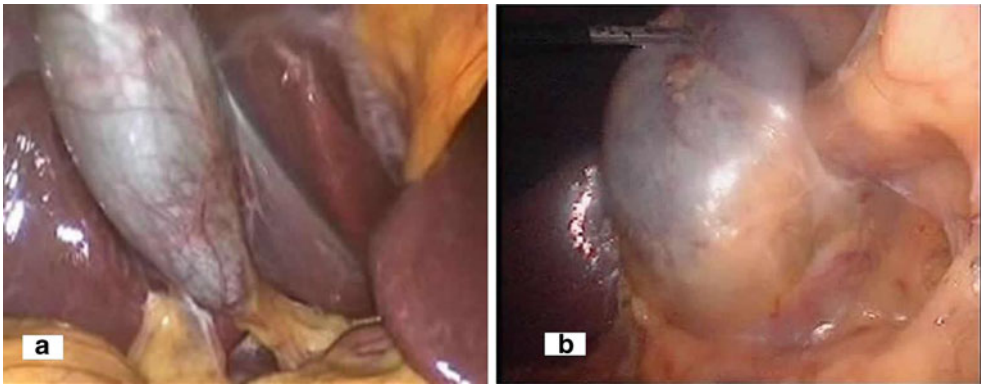

Fig. 1 **a** Laparoscopic view of a gallbladder with a normal segment IV (note the distance between the gallbladder and the round ligament.) **b** Laparoscopic view of a hypoplasic segment IV were the gallbladder is practically in contact with the round ligament

anomalies in the cystic duct, cystic artery, and the portal system.[8–10] The incidence of a true left-sided gallbladder is roughly calculated to be present in 0.3% of cholecystectomies.[11] These cases should not be confused with those with a right-sided round ligament which are also reported in the literature nor with cases with situs inversus.[12,13]

In our patient, the short width of this segment was accompanied by a bile duct variation that caused misidentification of structures that ultimately led to injury.

Case Report

A previously healthy 36-year-old female presented to an outside hospital with a 2-month history of biliary colic and nausea. Abdominal ultrasound showed gallstones and no evidence of acute cholecystitis. She had no other symptoms and had a negative history for abdominal surgery. Her blood work showed normal complete blood count, liver function tests, and coagulation tests. She underwent a laparoscopic cholecystectomy were it was noted that the gallbladder was unusually close to the round ligament (Fig. 1). After an erroneous dissection of the hepatoduodenal ligament (Fig. 2), a CBD injury was identified intraoperatively and the author was summoned for an acute repair (Fig. 3). A Strasberg E1 lesion was identified and a high side to side Roux-en-Y hepatojejunostomy was performed as has been previously described by our group.[14] The left duct was perceived as being considerably shorter than usual. The repair was performed during the same operation and thus no diagnostic imaging was needed.

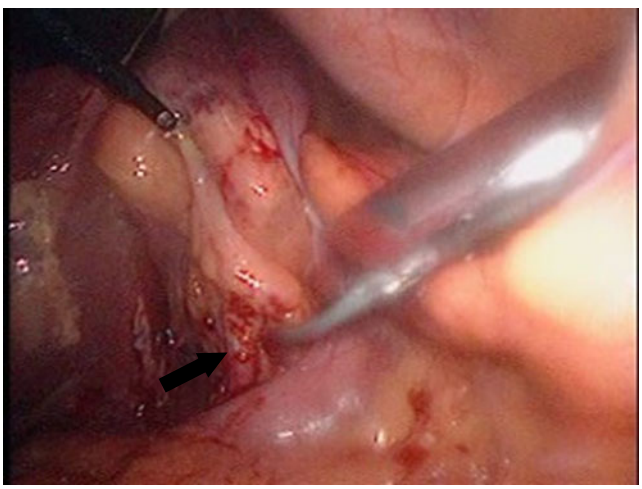

Fig. 2 Laparoscopic view of an erroneous dissection of the porta hepatis. *Arrow* indicates the common bile duct

Discussion

During organogenesis, between the third and seventh week of gestation, the close interaction between the septum transversum and the liver diverticulum enables the latter to divide into two separate buds. A solid cephalic portion that will eventually form the liver, and a hollow caudal portion which forms the gallbladder, bile duct and cystic duct.[1,15,16] This is why malposition of the gallbladder is often accompanied by abnormalities in the liver such as hypoplasia as described by Couinaud and cols.[1,16] These anomalies have been described together in cases where the gallbladder lies in the diaphragmatic surface of the right liver,[15] in cases with a left-sided gallbladder[11] and in cases with an absent left lobe and a floating gallbladder.[17] Although our case report is not as extreme, it is a milder and probably more prevalent example of this phenomenon.

Inexact terms such as a thin or underdeveloped segment IV have been used in the literature. An objective and measurable definition should be sought. We propose that the distance from the medial aspect of the gallbladder fossa to the umbilical fissure just above the hilar plate should be used to define a hypoplasic liver segment IV during laparoscopic cholecystectomy (before dissection and without traction of the gallbladder). Establishing normality in terms of this distance will have to be the first step towards defining hypoplasia.

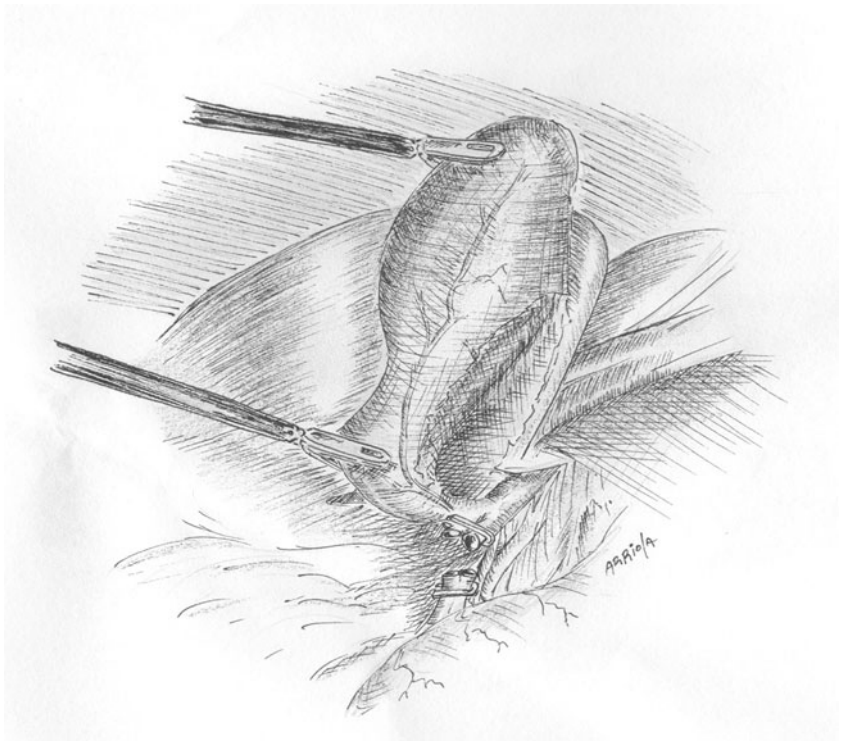

Fig. 3 Schematic representation of a video of the index operation of a bile duct injury with segment IV hipoplasia

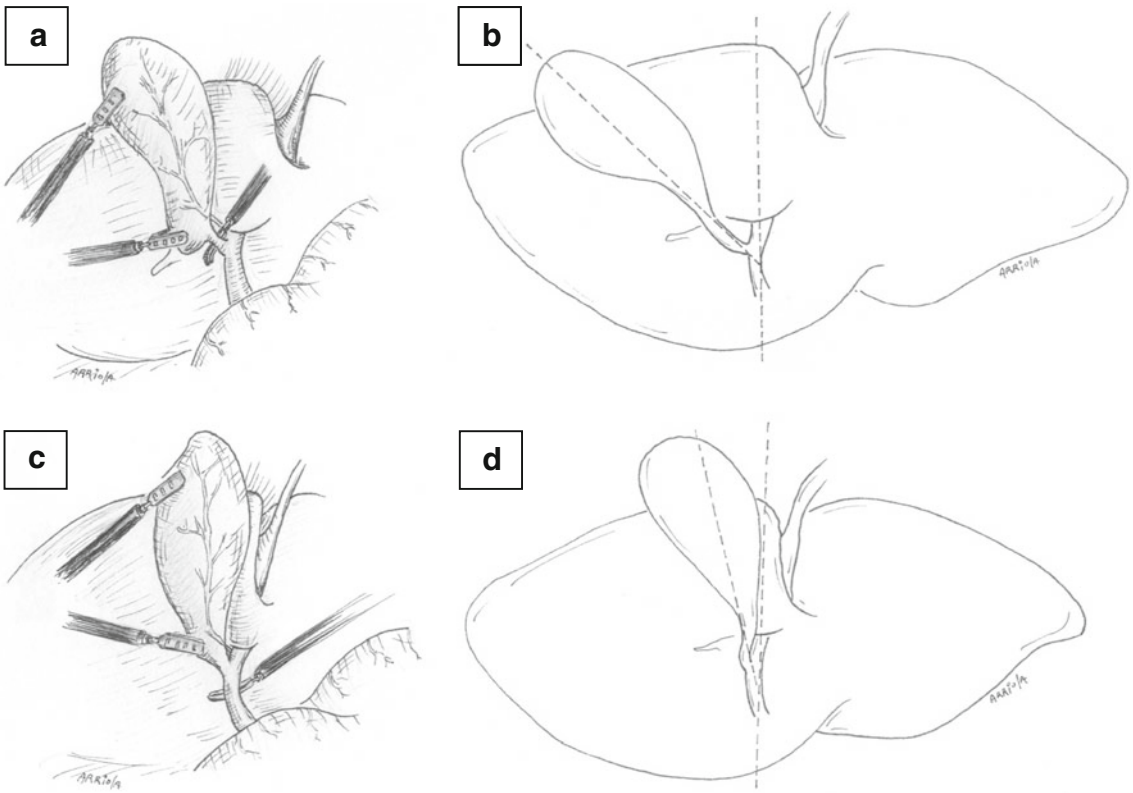

Fig. 4 Images **a** and **b** represent schematic views of a normal angle between the cystic duct and common bile duct (CBD) in cases without liver segment IV hipoplasia. Images **c** and **d** represent schematic views of hipoplasia of liver segment IV with a narrowed angle between de cystic duct and CBD

In the standard laparoscopic cholecystectomy, after retracting the gallbladder fundus superiorly and to the right shoulder, Calot's triangle (or hepatocystic triangle) is narrowed. After sectioning the peritoneum, the traction of Hartmann's pouch to the right broadens the hepatocystic triangle facilitating the critical view with a clear identification of the cystic artery and duct.[18] In a patient with a normal segment IV, the gallbladder and cystic duct are usually in a 45° angle with respect to the umbilical fissure superiorly and to the CBD inferiorly (Fig. 4a, b). When the distance between the gallbladder and the round ligament is very small (hypoplasia of liver segment IV), the angle of the gallbladder and cystic duct with respect to the common bile duct is narrowed, so that the cystic duct becomes almost parallel to the bile duct and the umbilical fissure (Fig. 4d). When the gallbladder is retracted upward and to the right shoulder, the cystic duct reinforces this abnormal position (Fig. 4c). Lateral traction of Hartmann's pouch produces traction of the common bile duct as well as the cystic duct instead of correcting this angle. If the CBD is thin it can be easily confused with the cystic duct and the surgeon might begin the dissection between the hepatic artery and the CBD, thus falling into a classic prelude to injury (Figs. 2, 3, and 4).

It is difficult to estimate the frequency of this abnormality and the amount of risk it confers in bile duct injuries. But if one classifies the position of the gallbladder in a continuum; from the few cases where it has been found on the diaphragmatic surface of the right liver (usually a hypoplasic right liver), to intraparenchymal, to a normal position, to a malpositioned gallbladder near the round ligament (but not to the left of it) with hypoplasia of segment IV (as reported in our case) to a left-sided gallbladder often accompanied by hypoplasia of liver segment IV to a floating gallbladder with absence of the left liver, it would make sense that the extreme variants, those that differ more from the usual anatomy would be the most infrequent (as is probably the case). It is likely that our patient's variation is more common than the extreme left-sided gallbladder estimated to be present roughly in 1:10,000 cholecystectomies.[7] Thus, a good number of surgeons and patients alike could benefit from the simple observation of the size of liver segment IV before initiating dissection of Calot's triangle during laparoscopic cholecystectomy.

Critical view exposure of the hepatocystic triangle proposed by Strasberg[5] plays a major role in the prevention of injuries. If complete dissection of Calot's triangle is achieved, the probability of having an injury is substantially diminished. If the surgeon is not able to complete the dissection to obtain the critical view, seeking advice of a more experienced surgeon or conversion to an open procedure are good alternatives. When the surgeon chooses cholangiography he must be sure that the catheter is introduced through the cystic duct. Introduction of the catheter in the main duct can cause injury in itself (usually a thin duct). In our opinion, the critical view is the best option to avoid injury in the presence of a hypoplasic segment IV.

To our knowledge, isolated observations on liver segment IV hypoplasia as a risk factor for BDI have not been reported in the English literature. This observation sets the background for prospective analysis to further understand the genesis of biliary injury during cholecystectomy.

In conclusion, hypoplasic liver segment IV produces an anatomic variant that promotes injury. The rectification in the position of the gallbladder modifies the hepatocystic triangle and its elements so that the artery and cystic duct have a more parallel position rather than a perpendicular position with regard to the CBD. Surgeons should be aware that if an unusually small distance is found between the gallbladder and the round ligament anatomic variants in the position of Calot's triangle are to be expected.

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ORIGINAL ARTICLE

Convergence Process of Volumetric Liver Regeneration After Living-Donor Hepatectomy

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Abstract
Background We investigated the long-term profiles of liver regeneration after living-donor hepatectomy.
Methods Thirty-three donors participated in the study. Preoperative and postoperative liver volume was calculated using computed tomography. Volume assessment was repeated at 1 week, 2 weeks, 1 month, 3 months, 6 months, 12 months, and 4 years postoperatively.
Results Donors were divided into the right (n=23; residual liver volume, 42%) and left (n=10; residual liver volume, 63%) groups according to the operative procedures. The restoration ratio to the preoperative liver volume (right vs. left groups) were 51%, 57%, 64%, 74%, 77%, 81%, and 88% vs. 69%, 72%, 76%, 79%, 83%, 84%, and 91% at 1 week, 2 weeks, 1 month, 3 months, 6 months, 12 months, and 4 years, respectively; the interindividual variation in the restoration ratio to the preoperative liver volume became narrower with time.
Conclusion Liver resection in humans resulted in rapid regeneration during the first 3 months, followed by a more moderate rate of regeneration thereafter, in proportion to the amount of liver mass resected. The volume of the regenerating liver appeared to converge towards the individual preoperative volume with time. However, the liver volume was not restored to the preoperative volume at 4 years after the resection.

Keywords CT volumetry · Donor hepatectomy · Liver regeneration · Total liver volume

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Abbreviations

ALB	Albumin
LDLT	Living-donor liver transplantation
TLV	Total liver volume
CT	Computed tomography
ICG	Indocyanine green
BMI	Body mass index
TB	Total bilirubin
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
PT-INR	Prothrombin time international normalized ratio
ICG R15	ICG retention rate at 15 min

Introduction

Although the knowledge that the liver can regenerate after being deprived of its mass dates back to the ancient Greek myth of Prometheus, a scientific description of

this process cannot be traced back prior to that by Higgins and Anderson in 1931. They reported that the weight of the residual liver after two thirds partial hepatectomy in rats increased to approximately 45% and 70% of the original liver weight by 24 and 72 h after the operation (early phase).[1] The rate of liver regeneration became slower during the subsequent late phase, but eventually, the original preoperative weight was reached by approximately 2 weeks after the operation.[1,2] Subsequently, the process of liver regeneration has been studied extensively. Although most studies were conducted using the rodent model of partial hepatectomy, the phenomenon of liver regeneration following the loss of liver mass is seen in all vertebrate organisms, from fish to humans, and that it is also triggered when the livers from small individuals, e.g., dogs, are transplanted into larger recipients of the same species.[3,4]

This regenerative phenomenon of the liver serves as the biological basis of living-donor liver transplantation (LDLT) in adults[5] as a safe and valid treatment option for end-stage liver diseases in the era of donor shortage;[6] it has been demonstrated that both a partial liver graft implanted into a large recipient and a paired residual donor liver show regenerative activity. In addition, the practice of LDLT provides a unique research opportunity, since the livers of living donors are supposed to follow a pattern of regeneration almost identical to the natural regenerative process of a normal liver, in contrast to the regenerative process in recipients influenced by multiple factors or that after hepatectomy in patients with a diseased liver.[7–9]

To date, several reports have investigated the liver regeneration process after donor hepatectomy.[10–18] The findings of these previous reports have been conflicting; while some reported almost complete liver regeneration within 2 weeks,[11] others documented that the donor livers did not return to their preoperative volume even by 6–12 months after the hepatectomy.[12–16,18] In addition, because most previous studies were conducted in donors undergoing right hemiliver resection, the regeneration responses to different extents of liver mass deprivation have not yet been precisely assessed. Moreover, the liver volumetric follow-up was carried out for no longer than 6–12 months in most previous studies. Thus, the long-term chronological profiles of volumetric regeneration of the donor liver remain largely unclear.

The question remains to be addressed whether each LDLT donor is able to finally achieve the full restoration of his/her original liver volume after the graft donation. Therefore, in the present study, we conducted an assessment of the pattern of liver regeneration in donors for LDLT serially until 4 years after the operation. The aim of the present study was to clarify, in detail, the chronological profiles of normal liver regeneration after different extents of major hepatic resection, paying particular attention to the long-term outcomes, and to investigate the clinical factors influencing the regenerative process.

Patients and Methods

Donors

Our criteria for potential living donors were as follows: healthy individuals between 20 and 65 year of age; ABO blood type, identical or compatible; no significant medical history; no underlying liver disease, including a history of viral hepatitis; candidates within three degrees of consanguinity or a spouse.[19,20] Deviation from these criteria, if any, was discussed on a case-by-case basis by both the transplant team and the institutional ethics board. Total liver volume (TLV), as well as the segmental liver volume, which corresponds to the scheduled graft volume of the donor, was estimated by contrast-enhanced computed tomography (CT).[21] The type of the graft is determined by balancing the safety of the donor and the adequacy of the graft volume, as previously described.[22] The indocyanine green (ICG) retention test is then performed to rule out the presence of liver disease and/or injury unidentified by the conventional liver function test. Liver biopsy is performed when a fatty liver is suspected, especially in donor candidates whose body mass index (BMI) exceeds 25.0. If the ratio of steatosis exceeds 10%, the candidate is requested to reduce his/her weight to improve the steatotic condition.

According to these criteria, we conducted 63 LDLTs between May 2001 and September 2002. Among these selected donors, 30 refused participation, and consequently, 33 were enrolled in the present study after providing written informed consent. The background characteristics of these 33 donors are shown in Table 1. The study protocol was approved by the local ethics committee of the Graduate School of Medicine, University of Tokyo and was conducted in accordance with the Declaration of Helsinki. The same donors had also participated in our previous study conducted to investigate the relationship between volumetric and functional liver regeneration over the short-term after donor hepatectomy; therefore, the liver volumetric data at 7 and 14 postoperative days overlap with those in the previous report.[23]

Donor Hepatectomy

The surgical techniques for various types of donor operation have been described in detail previously.[22,24–27]

Table 1 Donor characteristics

	All donors (n=33)	Right group (n=23)	Left group (n=10)	P value
Sex (male/female)	22:11	15:8	7:3	NS
Age	34.0 (18–61)	32.0 (18–61)	41.5 (19–59)	NS
BMI	20.9 (16.7–29.0)	19.8 (16.7–26.6)	25.0 (17.8–29.0)	<0.01
ICG R15 (%)	5.8 (2.8–12.0)	5.6 (3.0–10.2)	8.0 (2.8–12.0)	NS
Congestive area (present/absent)	12:21	2:21	10:0	<0.0001
Operation time (min)	514 (355–700)	514 (355–685)	515 (430–700)	NS
Blood loss (g)	500 (169–1,125)	470 (169–1,125)	530 (285–1,080)	NS
Peak TB (mg/dl)	1.9 (1.1–4.2)	2.0 (1.5–4.2)	1.6 (1.1–3.2)	0.02
Peak AST (IU/L)	191 (108–856)	177 (108–398)	235 (149–856)	0.006
Peak ALT (IU/L)	210 (97–623)	171 (97–429)	290.5 (125–623)	0.01
Peak PT-INR	1.59 (1.21–2.52)	1.67 (1.21–2.52)	1.42 (1.27–2.10)	NS
Complication (yes/no)	18 (55%):15 (45%)	15 (65%):8 (35%)	3 (30%):7 (70%)	NS

Values are expressed as median (range)

BMI body mass index, *ICG R15* indocyanine green retention rate at 15 min

P indicates the results of comparisons between Right and Left groups

Of the 33 donors in the present series, a right hemiliver graft with (n=2) or without (n=21) the middle hepatic vein was obtained from 23 donors, a left hemiliver graft with the middle and left hepatic veins and with the caudate lobe was obtained from 6 cases, and a right lateral sector graft with the right hepatic vein was obtained from 4 cases, respectively. We previously reported that the overall donor liver regeneration at 3 months after LDLT was not retarded in spite of the impaired regeneration in congested parts since the regeneration of the other noncongested parts showed compensatory augmentation.[28,29] Taking this into account, we classified the donors undergoing right hemiliver resection with and without the middle hepatic vein together into the right group (corresponding to resection of approximately two thirds of the TLV), while amalgamating donors of a left hemiliver graft and a right lateral sector graft was classified as the left group (corresponding to resection of about one third of the TLV).

Pringle's maneuver was applied during donor hepatectomy.[30] After the operations, the hepatic function was assessed by blood tests for the serum total bilirubin (TB), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) and the plasma prothrombin time international normalized ratio (PT-INR). All postoperative complications were recorded.[19]

Assessment of the Liver Volumetric Change

The residual liver volume just after the operation, i.e., the liver volume at zero time-point, was calculated by subtracting the graft weight from the preoperative TLV, assuming that the liver has the same density as water. Subsequent liver regeneration was assessed by CT conducted at 1 week, 2 weeks, 1 month, 3 months, 6 months, 12 months, and 4 years after the operation. Serial abdominal transverse CT scans taken at 0.5-cm intervals were used. The boundary of each liver slice was traced manually by one of the authors (T.A.), and the encircled areas were calculated using Photoshop® software. The degree of liver regeneration at the respective time-points was then expressed as a percentage of the liver volume to the preoperative TLV and was designated as "restoration ratio to the preoperative TLV." In addition, the rate of change in the restoration ratio to the preoperative TLV from the previous time-point was calculated and was expressed as the percent change per month.

Assessment of Biochemical Parameters After Donor Hepatectomy

Biochemical parameters were assessed preoperatively and at 3 and 12 months after the hepatectomy. The parameters assessed were AST, ALT, TB, ALB, and PT-INR.

Statistics

The chronological changes in the donor liver volume in the right and left groups, as well as the rate of change in the restoration ratio to the preoperative TLV, were analyzed by two-way analysis of variance with repeated measures, followed by Bonferroni–Holm corrected post hoc t tests.[31] Multiple comparisons of postoperative values of the biochemical parameters with those measured before surgery were conducted using the Bonferroni correction. The significance of the correlation of the potential clinical factors with the regeneration process was analyzed using

the multiple regression models. The stepwise method with a P value of ≤0.15 for the variable elimination was used to select the variables.[32]

Results

Donor Characteristics in the Right and Left Groups

The characteristics of the donors in the right and left groups were compared and are shown in Table 1. The BMI was significantly higher in the left group compared with that in the right group ($P<0.01$). The ratio of the residual liver volume relative to the preoperative TLV just after the donor hepatectomy was 42.3±5.5% (range, 28.8–49.6%) in the right group vs. 63.4±3.5% (range, 57.5–66.4%) in the left group ($P<0.01$).

Time Course of Liver Regeneration After Donor Hepatectomy

The regeneration of the donor liver (restoration ratio to the preoperative TLV) in the right and left groups is shown in Fig. 1a, b. Volumetric data for the regenerating livers were available for 25 donors (17 in the right group and 8 in the left group) at 1 year after surgery and for 13 donors (8 in the right group and 5 in the left group) at 4 years after surgery. The rate of liver regeneration was rapid during the first 3 months (early phase; Fig. 1b). The restoration ratio to the preoperative TLV in the right group was 51.5±4.8%, 57.4±5.7%, 63.5±7.6%, and 73.6±7.8% at 1 week, 2 weeks, 1 month, and 3 months, respectively, and that in the left group at the corresponding time-points was 69.0±6.1%, 71.9±5.1%, 75.7±5.9%, and 79.3±5.3%, respectively. Therefore, the liver regained approximately one third and one half of the resected liver mass by 1 and 3 months postoperatively, respectively, irrespective of the extent of the liver resection. Thereafter, after 3 months, the rate of liver regeneration decreased (late phase). The restoration ratio to the preoperative TLV in the right group was 76.9±6.7%, 80.9±6.7%, and 88.2±5.7% at 6 months, 12 months, and 4 years, respectively, and that in the left group at the corresponding time-points was 83.3±7.0%, 84.5±6.7%, and 91.1±5.7%, respectively.

Meanwhile, the rate of change in the restoration ratio to the preoperative TLV from the previous time-point in the right group was 36.4±3.6%/month, 23.8±18.3%/month, 12.8±10.3%/month, 5.2±2.7%/month, 1.1±1.1%/month, 0.6±0.6%/month, and 0.2±0.1%/month at 1 week, 2 weeks, 1 month, 3 months, 6 months, 12 months, and 4 years, respectively, while that in the left group at the corresponding time-points was 22.4±20.1%/month, 11.6±8.8%/month, 7.6±9.5%/month, 2.4±2.7%/month, 1.4±1.1%/month, 0.2±0.2%/month, and 0.1±0.2%/month, respectively.

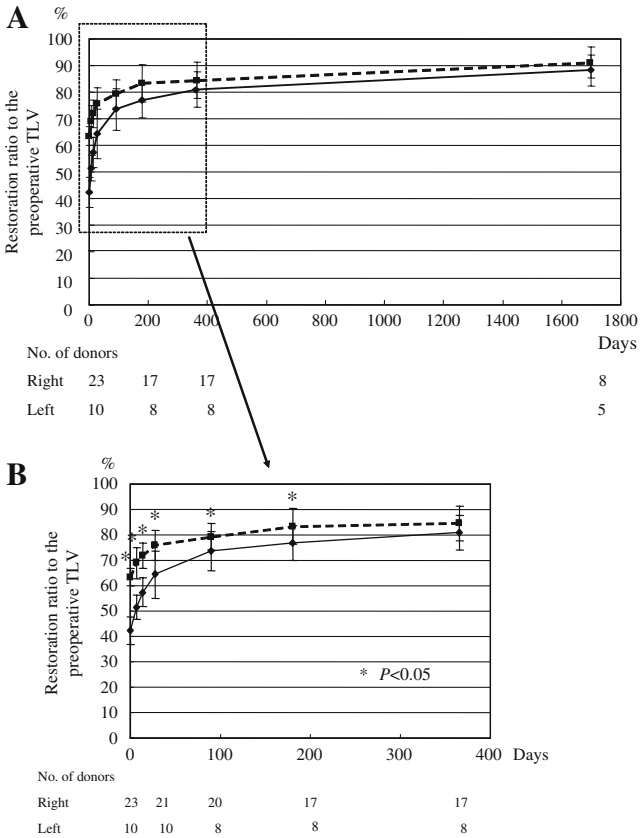

Fig. 1 Chronological profiles of liver regeneration in the right (*solid line*) and left (*dotted line*) groups as assessed by estimation of the restoration ratio to the preoperative TLV. **a** Degree of regeneration by the end of 4 years postoperatively. Restoration ratio at each time-point, when compared with that at the previous time-point, showed statistical significance throughout the study period until 4 years, e.g., $P=0.0004$ for 12 months vs. 4 years. **b** Magnified view of the regenerating liver at the end of 12 months postoperatively. Comparison of the restoration ratio in the right and left groups revealed significant differences at 1 week, 2 weeks, 1 month, 3 months, and 6 months after the operation (*$P<0.05$); however, the difference was not significant at 12 months and 4 years post surgery. *TLV* total liver volume

Comparison of the restoration ratio to the preoperative TLV at each time-point with that at the previous time-point showed statistical significance throughout the study period until 4 years (Fig. 1a). Nevertheless, only 1 out of the 25 donors at 1 year and 1 out of the 13 donors at 4 years who underwent postoperative volumetric examination showed full (more than 95%) restoration to the preoperative TLV, and both of these donors belonged to the left group.

Comparison of the restoration ratio to the preoperative TLV between the right and left groups revealed significant intergroup differences at 1 week, 2 weeks, 1 month, 3 months, and 6 months after the operation; however, the difference was not significant at 12 months and 4 years post surgery. On the other hand, the rate of change in the

restoration ratio to the preoperative TLV from the previous time-point tended to be greater in the right group than in the left group at 2 weeks (P=0.05), 3 months (P=0.02), and 12 months (P=0.07), suggesting that it took about 12 months for the right group to compensate for the difference in the extent of the donor hepatectomy, although the livers in the right group regenerated faster than those in the left group.

The interindividual differences in the liver regeneration process following liver resection are summarized in Table 2; this is illustrated by a histogram of the restoration ratio to the preoperative TLV at time 0 (post operation) and at 6 months, 12 months, and 4 years after the surgery (Fig. 2).

Factors Influencing Liver Regeneration

The following 13 variables were entered into the regression analyses: sex, age, BMI, ICG retention rate at 15 min (ICG R15), operative procedures (right vs. left), presence of areas of congestion (i.e., disturbance of venous outflow) in the remnant liver, operative time, operative blood loss, postoperative peak values of TB, AST, ALT, and PT-INR, and occurrence of postoperative complications. Out of the variables examined, sex, the operative procedure (right vs. left), and the postoperative peak ALT value were shown to affect the rate of change in the restoration ratio to the preoperative TLV during the first 3 months, but none of the variables were significantly associated with the rate of change in the restoration ratio to the preoperative TLV from 3 to 12 months postoperatively (Table 3).

Assessment of Biochemical Parameters After Donor Hepatectomy

The values of the various biochemical parameters at 3 and 12 months after the operation were available for 28 and 15 donors, respectively (Table 4). The moderate degree of derangement in some of these parameters observed at 3 months was almost normalized at 12 months postoperatively. Although the ALB was lower at 12 months, compared with the preoperative value, its absolute value remained within the normal range. Moreover, no correlation was observed between the ALB at 12 months after the operation and the restoration ratio to the preoperative TLV measured at this time-point (P=0.67).

Discussion

Although human liver regeneration after major hepatic resection was reported anecdotally in cases of liver tumors,[8,9] regeneration process in normal human liver could be examined with the advent of adult LDLT and several groups have investigated the pattern of regeneration after right hemiliver resection for LDLT to date.[11–18] There appears to be agreement that liver regeneration in humans under these circumstances follows the pattern observed in rodent models, which consists of an early phase of rapid regeneration (until 72 h in the rat and until 3 months in humans), followed by a subsequent phase (late phase) of regeneration at a slower rate (3–14 days in the rat and after 3 months in humans). However, the reported speed of regeneration varies widely, especially in relation to the early phase, and the reported follow-up period in most reported studies is between 6 and 12 months postoperatively, at which time-point complete regeneration may not yet have been achieved. Bearing this in mind, we conducted a detailed evaluation of the chronological pattern of liver regeneration for 4 years following donor hepatectomy (right or left hemiliver resection) for LDLT.

The early phase of liver regeneration after donor hepatectomy for LDLT was first investigated by Marcos et al.[11] They reported that, after right hemiliver resection that resulted in a residual liver volume of 41%, the liver mass doubled by 7 days and returned to the original preoperative volume by 60 days postoperatively. Serial examinations of the early phase of donor liver regeneration were conducted subsequently by three other investigators, and all reported a much slower regenerative processes.[12,13,17] They reported that, after donor hepatectomy resulting in a residual liver volume of 41–49%, the percent regeneration to the preoperative TLV was 60–64% at 1 week and 65–70% at 1 month postoperatively. Therefore, it is considered that the

Table 2 Distribution of the percent regeneration to the preoperative TLV

Standard	Timing	Number	Average (%)	SD (%)	Interquartile range (%)	Range (%)
Preoperative TLV	6 months	25	78.9	7.30	10.6	26.5
	12 months	25	82.1	6.81	8.5	27.4
	4 year	13	89.3	5.70	6.3	21.7

Volumetric data were available for 25, 25, and 13 donors at 6 months, 12 months, and 4 years after the operation, respectively

SD standard deviation, *TLV* total liver volume

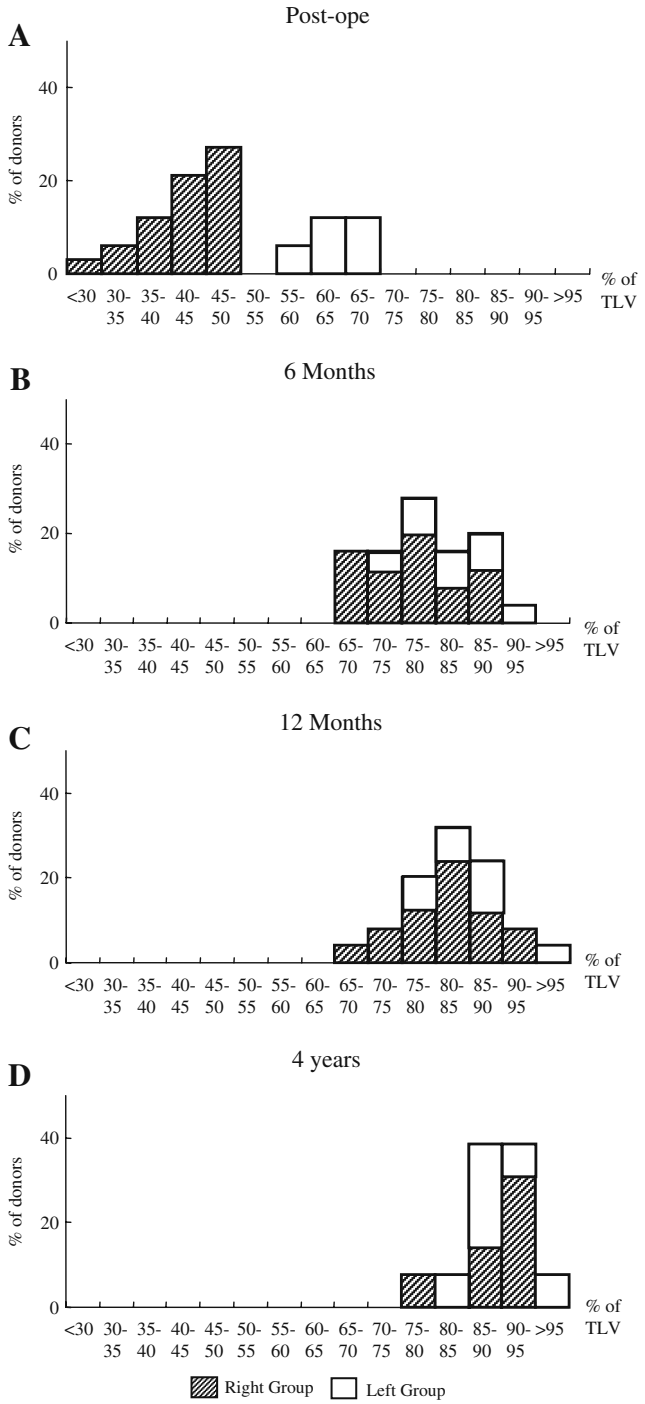

Fig. 2 Histogram of restoration ratio to the preoperative TLV. Restoration ratio to the preoperative TLV at **A** time 0 (preoperative TLV−graft weight), **B** 6 months, **C** 12 months, and **D** 4 years after the donor hepatectomy are shown. The results were stratified according to the right (*black*) and left (*white*) groups. *TLV* total liver volume

rate of regeneration during the early phase after hepatectomy in the present series, in which the percent regeneration was 52%, 57%, and 65% at 1 week, 2 weeks, and 1 month, respectively, after right hepatectomy, was either slightly slower or similar to that in the three aforementioned studies during the corresponding period.

The present study was unique in that we could compare the regeneration response to different extents of liver resection, i.e., right hemiliver (two thirds resection) and left hemiliver (one third resection). Similar comparison has been conducted before in animal models of liver resection, but not in humans. It is worthy of note that the ratio of the restored liver volume relative to the amount of the liver resected at each time-point was similar, irrespective of the extent of liver resection. This finding is consistent with that reported for animal models and signifies that cell proliferative activity, and consequently tissue growth, is proportional to the amount of hepatic mass resected.

The pattern of liver regeneration in the late phase, after 3 months postoperatively, has been investigated rather vigorously until 6–12 months. The percent regeneration to the preoperative TLV has been reported to be 70–80% at 3 months, 72–85% at 6 months, and 83–85% at 12 months,[12–16] and the figures in the present study coincide roughly with the aforementioned results. The majority of previous reports agreed that the donor liver was not restored to the preoperative volume, except for one study that reported 97% recovery at 12 months.[17] In the present series, the percent regeneration to the preoperative TLV was 82% at 12 months and 89% at 4 years postoperatively, and the difference between these time-points was statistically significant (Fig. 1a). This result can be interpreted as being attributable to (1) the donor liver being still under the process of regeneration, albeit at a very slow rate, at 4 years postoperatively or (2) the regeneration process in the human liver terminating between 1 and 4 years postoperatively even if the liver volume is not restored to the preoperative volume.

The mechanism of termination of the process of liver regeneration is poorly understood, although some cytokines such as TNFβ are believed to be involved.[3] In parallel, the goal for liver regeneration in terms of the volume remains unclear. In the present series, large interindividual differences were observed among the subjects in the residual liver volume immediately after liver resection because of the differences in the type of hepatectomy performed and variations in the liver segmental volume ratio,[33,34] while the distribution range became narrower with time (Table 2; Fig. 2). This finding strongly suggests that the liver regeneration process converges to the predestined point, irrespective of the amount of mass resected or the background characteristics. This consideration is further supported by our previous observation in LDLT recipients that both small-for-size and large-for-size grafts increased or decreased in size towards a constant liver-to-body mass ratio after the transplantation.[10]

Table 3 Factors influencing early-phase and late-phase liver regeneration

	Parameter estimate	Standard error	β	P value (Prob>F)
Early-phase regeneration (0–3 months)				
Operative procedure (right compared to left)	0.119	0.042	0.447	0.010
Sex	−0.073	0.037	−0.296	0.059
Peak ALT(IU/L)	0.000	0.000	−0.274	0.099
Late-phase regeneration (3–12 months)				
None of the variables were entered to the model				

Factors influencing the regeneration process are other issues of interest. Animal studies have shown that senescence, steatosis, and ischemic injury had a negative effect on regeneration, while previous studies in LDLT donors did not confirm these findings, except the study by Yokoi et al., which showed a decreased rate of regeneration in older donors,[17] and that by Pomfret et al., which demonstrated a negative effect of female gender.[13] Our results were partially in agreement with those in previous reports: sex and the postoperative peak ALT value were associated with the magnitude of liver regeneration during the early phase; however, no variables that were identified to be related to liver regeneration were identified in the late phase. Although postoperative ALT value is thought to be an indicator of postoperative liver damage most likely caused by the ischemic injury, and indeed this parameter was linked to warm ischemic time in our series (P=0.02), further investigation using a larger cohort will be needed to clarify the relationship between the postoperative ALT value and liver regeneration.

The question of whether functional recovery after a donor hepatectomy occurs in parallel with the volumetric regeneration is another issue of interest. Former studies addressing this issue using quantitative liver function tests have reported contradictory results: our group showed that liver functional recovery as assessed using the intrinsic plasma clearance of antipyrine preceded volumetric regeneration at 12 days postoperatively,[23] while Nadalin et al. reported that functional recovery as assessed using the serum galactose elimination capacity was delayed, compared with the volumetric recovery, at 10 days postoperatively.[15] In the present study, the biochemical parameters that showed albeit mild alterations at 3 months postoperatively had returned to the preoperative levels at 12 months after the surgery, except for the ALB. All these mild derangements were within the normal range at both timepoints in the study; these results agreed with those reported by Chan et al.[18] and Nadalin et al.[15] Also, the ALB at 12 months postoperatively was not associated with the extent of volumetric regeneration of the liver. Furthermore, Nadalin et al. reported that the serum galactose elimination capacity had returned to the preoperative level at 1 year postoperatively. Hence, the liver function appeared to have recovered to the preoperative level at 1 year after surgery, at least from a clinical point of view.

In conclusion, normal liver regeneration in humans followed a similar pattern, albeit being 20–50 times slower, to that observed in rodent models of liver resection; that is, liver regeneration consisted of an early phase of rapid regeneration and a subsequent late phase characterized by a slower rate of regeneration. Although regeneration continued even after 12 months postoperatively, the process seemed to reach a stage close to its final volumetric goal at 4 years after the hepatectomy, when the liver was restored to approximately 90% of its preoperative TLV.

Table 4 Biochemical parameters before, 3 months after, and 12 months after donor hepatectomy

	Before (n=33)	3 months (n=29)	12 months (n=15)
AST (IU/L)	18.3±4.0 (11–30)	23.2±5.1 (16–36)*	20.5±4.8 (15–34)
ALT (IU/L)	17.0±7.2 (7–41)	24.0±9.8 (12–51)*	18.7±7.5 (10–37)
TB (mg/dl)	0.77±0.32 (0.3–1.8)	0.74±0.22 (0.4–1.3)	0.86±0.36 (0.5–1.8)
ALB (g/dl)	4.48±0.27 (4.0–5.1)	4.14±0.31 (3.5–4.7)*	4.30±0.28 (3.6–4.6)*
PT-INR	1.08±0.10 (0.92–1.42)	1.14±0.15 (0.98–1.47)*	1.11±0.19 (0.98–1.46)

Values are expressed as the mean ± standard deviation with the range in parentheses

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *TB* total bilirubin, *ALB* albumin, *PT-INR* prothrombin time international normalized ratio

*P<0.025

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ORIGINAL ARTICLE

Post-Operative Pharmacologic Thromboprophylaxis after Major Hepatectomy

Does Peripheral Venous Thromboembolism Prevention Outweigh Bleeding Risks?

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Abstract
Background Although standard of care after most abdominal surgeries, post-operative pharmacologic thromboprophylaxis after major hepatectomy is commonly withheld due to bleeding risks. The objective of this retrospective study is to evaluate the benefits and risks of post-operative pharmacologic thromboprophylaxis after major hepatectomy at two high volume academic centers.
Methods Demographics, clinicopathologic data, treatments, and post-operative outcomes from patients who underwent major hepatectomy were reviewed.
Results From 2005 to 2010, 419 patients underwent major hepatectomy; 275 (65.6%) were treated with pharmacologic-thromboprophylaxis beginning a median of 1 day after resection. Post-operative symptomatic venous thromboembolism (VTE) occurred in 15 (3.6%) patients. Patients treated with pharmacologic thromboprophylaxis had lower rates of symptomatic VTE (2.2% vs. 6.3%, $p=0.03$) and post-operative red blood cell (RBC) transfusion (16.7% vs. 26.4%, $p=0.02$) with similar rates of overall RBC transfusion (35.0% vs. 30.6%, $p=0.36$) compared to untreated patients. Specifically, isolated deep venous thrombosis (0% vs. 2.1%, $p=0.04$) and pulmonary embolism (2.2% vs. 4.2%, $p=0.35$) occurred less often in treated patients. Analysis of demographics, clinicopathologic data, and treatment factors revealed that pharmacologic thromboprophylaxis was the only variable associated with post-operative VTE.
Conclusions Post-operative pharmacologic thromboprophylaxis lowers the incidence of symptomatic VTE after major hepatectomy without increasing the rate of RBC transfusion.

Keywords Liver resection · Venous thromboembolism · Thromboprophylaxis

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Introduction

Post-operative pharmacologic thromboprophylaxis is widely accepted for venous thromboembolism (VTE) prevention after most general surgical procedures. Multiple prospective randomized clinical trials and meta-analyses of phase III studies over the past 30 years demonstrate that pharmacologic thromboprophylaxis decreases the incidence of asymptomatic deep vein thrombosis (DVT), symptomatic pulmonary embolism (PE), fatal PE, and overall symptomatic VTE in general surgery patients.[1–6] Rates of hemorrhage, subsequent severe morbidity and blood transfusion, and discontinuation of pharmacologic-thromboprophylaxis due to bleeding among general

surgery patients and patients undergoing resection of malignancy are relatively low (0.4–5.9%) compared to VTE prevention benefits.[1–10] Consequently, the American College of Chest Physicians (ACCP) has recommended pharmacologic thromboprophylaxis with low molecular weight heparin, unfractionated heparin, or fondaparinux for moderate risk (major general surgical procedure for benign disease) and high risk (major general surgical procedure for malignancy) patients. In cases of high bleeding risk, mechanical prophylaxis is recommended with prompt institution of chemical thromboprophylaxis when bleeding risk decreases.[6] These are grade IA recommendations—meaning that the benefits of chemical prophylaxis clearly outweigh the associated harm, burden, and/or costs based on the highest quality of evidence.[6] Other organizations, including the European Society of Clinical Oncology, the American Society of Clinical Oncology, the Italian Society of Medical Oncology, the National Comprehensive Cancer Network, and the European Society of Anaesthesiology have similar recommendations.[3,11,12] The Surgical Care Improvement Project (SCIP), representing a collaboration between the Centers of Medicare and Medicaid Services, Centers for Disease Control and Prevention, the American College of Surgeons, and the Department of Veterans of Affairs, have identified VTE prevention in efforts to decrease post-operative complications nationwide. The National Quality Forum, the Joint Commission, and the Agency for Healthcare Research and Quality have all incorporated specific initiatives regarding the use of VTE prophylaxis into general measures of performance. Moreover, the Centers for Medicare and Medicaid Services recently added PE and DVT to their list of "Hospital-Acquired Conditions Initiative," thus limiting hospital reimbursement for treatment of these potentially preventable complications.[13–18]

While advocated for most general surgical procedures, pharmacologic thromboprophylaxis is not widely accepted after hepatic resection. Factors that may lead to a relatively high incidence of post-operative VTE in patients undergoing hepatic resection include malignant diagnoses, distant metastatic disease, prior chemotherapy treatment, advanced age, prolonged general anesthesia, and post-operative immobility.[3,9,19–26] Yet fears of bleeding along the liver transection surface combined with the belief of "auto-anticoagulation" due to post-operative hepatic insufficiency has led many surgeons to either delay or withhold pharmacologic thromboprophylaxis altogether.[27] Unfortunately, liver resection is underrepresented in phase III trials evaluating the benefits of pharmacologic thromboprophylaxis among general surgery patients.[8] The objective of this study was to evaluate VTE prevention benefits and risks of red blood cell (RBC) transfusion from post-operative pharmacologic thromboprophylaxis after major hepatic resection at two high-volume academic centers.

Methods

After obtaining approval from the Institutional Review Board at both institutions, demographics, clinicopathologic data, surgical treatments, and post-operative outcomes from patients who underwent liver resection at the Liver Cancer Center at the University of Pittsburgh Medical Center (UPMC) and at Duke University Medical Center (DUMC) were reviewed. Using Brisbane 2000 terminology,[28] only patients who underwent resection of four or more liver segments were included in this study. Most hepatic lesions were detected pre-operatively with computed tomography, magnetic resonance imaging, and/or positron emission tomography. Intraoperative ultrasonography was used to detect and localize all lesions with respect to major vessels. The extent of hepatic resection was at the discretion of the operating surgeon with the aim of achieving negative surgical margins and a liver remnant of sufficient volume to maintain liver function with intact vascular inflow, vascular outflow, and biliary drainage. Patients with a history of prior VTE, coagulation disorders, pharmacologic anticoagulant use, and/or diseases known to increase thrombotic risk (e.g., systemic lupus erythrematosus, atrial fibrillation, known anti-coagulation factor deficiencies) were not included in this study. Administration, timing, and type of pharmacologic thromboprophylaxis were at the surgeon's discretion. Mechanical thromboprophylaxis (knee-high graduated compression stockings) and early ambulation was ordered for every patient. Ninety-day post-operative morbidity and mortality were recorded. Post-operative complications were graded according to the Clavien–Dindo classification[29] with the following exceptions: (1) grade I complications were largely not recorded except for wound infection and ascites requiring diuresis and (2) the need for blood transfusion was not regarded as a complication. Complications grade III and above were considered severe. No routine surveillance imaging of VTE events were employed—all VTE events noted in this study were symptomatic. Deep venous thrombosis (DVT) and PE were diagnosed after lower extremity ultrasound and chest computed tomography in all cases except one where fatal PE was confirmed on autopsy. Upper extremity DVT after central venous line placement was not included as a VTE event in this study.

Statistical analyses were performed with PASW version.18 (Chicago, IL) software. Data distribution was tested for normality by examining the mean and standard error of the kurtosis and skewness. Continuous variables are reported with medians and 25–75th percentile interquartile ranges (IQR). Comparisons were performed with the Mann–Whitney *U* or Kruskal–Wallis tests for non-normally distributed continuous variables. Chi-square and Fisher's exact (in cases of low frequencies) tests were used for categorical variable comparisons.

Results

From 2005 to 2010, 419 patients without a history of coagulation disorder or pharmacologic anti-coagulant use underwent major hepatic resection and comprised the study cohort. 363 (86.6%) patients underwent resection for malignancy—the most common malignant indications were colorectal cancer metastases and hepatocellular carcinoma (Table 1). Most patients had an American Society of Anesthesiology (ASA) score of II or III. Median pre-operative bilirubin, albumin, hematocrit, platelet, international normalized ratio (INR), and activated partial thromboplastin time (aPTT) values were all within respective normal ranges at laboratories at each institution. 275 patients (65.6%) were treated with pharmacologic thromboprophylaxis at a median of 1 day (IQR 1 day) after partial hepatectomy. A total of 229 (54.7%), 38 (9.1%), and eight (1.9%) patients were administered subcutaneous unfractionated heparin, low molecular weight heparin (enoxaparin), and both unfractionated and low molecular weight heparin (sequentially, not simultaneously) after liver resection, respectively. A greater proportion of patients who underwent resection at UPMC were treated with pharmacologic thromboprophylaxis compared to that at DUMC (91.7% vs. 34.9%, $p<0.001$). More Caucasian and female patients were given pharmacologic thromboprophylaxis compared to untreated counterparts (Table 1). There were no statistically significant differences in patient age, body mass index, diagnoses, or ASA score between treatment groups. While treated patients did have longer pre-operative aPTT, there were no differences in pre-operative bilirubin, albumin, hematocrit, platelet count, or INR between groups (Table 1).

Among the entire study cohort, the most common performed resection was a right hepatectomy (Table 2). Overall, 140 (33.4%) and 14 (3.4%) patients underwent simultaneous major non-hepatic procedures and laparoscopic liver resection, respectively. A total of 140 (33.4%) patients were transfused during their hospital course; 103 (24.6%) were transfused intra-operatively and 84 (20.0%)

Table 1 Demographics, diagnoses, and pre-operative laboratory values for patients who underwent major hepatic resection stratified by post-operative pharmacologic thromboprophylaxis

	All patients (n=419)	Prophylaxis (n=275)	No prophylaxis (n=144)	p
Site of resection: UPMC	227 (54.2%)	208 (75.6%)	19 (13.2%)	<0.001
Age (years)[a]	58 (20)	58 (20)	58 (21)	0.36
Ethnicity				0.02
Caucasian	361 (86.2%)	245 (89.7%)	116 (80.6%)	
African-American	47 (11.2%)	22 (8.1%)	25 (17.4%)	
Other	9 (2.2%)	6 (2.2%)	3 (2.1%)	
Body mass index (kg/m^2)[a]	26.8 (6.5)	27.0 (6.1)	26.4 (7.1)	0.46
Male gender	190 (45.3%)	115 (41.8%)	75 (52.1%)	0.06
Diagnosis				0.11
Colorectal cancer metastases	181 (43.2%)	129 (46.9%)	52 (36.1%)	
Hepatocellular cancer	57 (13.6%)	31 (11.3%)	26 (18.1%)	
Other malignancy	125 (29.8%)	79 (28.7%)	46 (31.9%)	
Benign	56 (13.4%)	36 (13.1%)	20 (13.9%)	
American Society of Anesthesiology Score				0.52
I	8 (2.0%)	5 (1.9%)	3 (2.4%)	
II	118 (29.9%)	74 (27.7%)	44 (34.6%)	
III	250 (63.5%)	176 (65.9%)	74 (58.3%)	
IV	18 (4.6%)	12 (4.5%)	6 (4.7%)	
Pre-operative bilirubin (mg/dl)[a]	0.6 (0.5)	0.6 (0.4)	0.5 (0.5)	0.90
Pre-operative albumin (mg/dl)[a]	4.0 (0.5)	4.0 (0.7)	4.0 (0.7)	0.10
Pre-operative hematocrit (%)[a]	39.4 (6.0)	39.3 (6.2)	39.0 (7.0)	0.91
Pre-operative platelet (10^3/μl)[a]	235 (113)	233 (112)	240 (114)	0.85
Pre-operative international normalized ratio[a]	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.32
Pre-operative aPTT (s)[a]	29.6 (5.2)	30.0 (4.3)	27.8 (6.0)	<0.001

aPTT activated partial thromboplastin time

[a] Continuous variables are reported as median (IQR)

were transfused on or after post-operative day 1. Post-operative mortality, overall morbidity, and severe morbidity occurred in 24 (5.7%), 212 (50.6%), and 86 (20.5%) patients, respectively. Median length of hospital stay after resection was 7 days. Symptomatic post-operative VTE occurred in 15 (3.6%) patients. Most VTE events included PE—only three cases of symptomatic isolated deep venous thrombosis occurred after major liver resection. Patients treated with pharmacologic thromboprophylaxis more often underwent right hepatectomy and less often underwent extended hepatectomy compared to untreated counterparts (Table 2). There were no differences in rates of laparoscopic resection or simultaneous major non-hepatic procedures. Despite greater estimated intra-operative blood loss in untreated patients, there was no difference in the rate of intra-operative RBC transfusion between treatment groups. Similarly, there were no differences in rates of post-operative mortality, overall morbidity, severe morbidity, or overall RBC transfusion between patients treated with and without pharmacologic thromboprophylaxis. Untreated patients were more likely to be transfused on or after post-operative day 1 compared to treated patients. Patients treated with pharmacologic thromboprophylaxis had a lower rates of post-operative VTE (2.2% vs. 6.3%, $p=0.03$) and DVT (0% vs. 2.1%, $p=0.04$) compared to untreated counterparts. Among all demographic, clinicopathologic, and treatment variables, the only factor with a significant difference between patients who did and did not experience symptomatic post-operative VTE was pharmacologic thromboprophylaxis treatment (Table 3). Patients who suffered post-operative VTE had higher pre-operative bilirubin (median 0.7 vs. 0.6 mg/dl, $p=0.06$) and platelet (median 290 vs. $234\times10^3/\mu l$, $p=0.06$) concentrations compared to patients who did not experience post-operative VTE—however, these differences were not statistically significant.

Among patients treated with pharmacologic thromboprophylaxis, the median drop in hematocrit after the start of prophylaxis was 4.0% (IQR 2.0%) and occurred at a median of 3 days (IQR 3 days) after the start of prophylaxis. Of the six patients treated with pharmacologic thromboprophylaxis who experienced post-operative VTE, two were started on pharmacologic thromboprophylaxis treatment greater than 24 h after surgery.

Discussion

In this retrospective study comprising two high-volume academic centers, post-operative pharmacologic thromboprophylaxis decreased the incidence of symptomatic VTE after major hepatectomy without increasing the rate of RBC transfusion. By excluding patients with prior VTE, coagulation disorders, history of pharmacologic anti-coagulant

Table 2 Surgical treatments, intra-operative transfusions, and post-operative outcomes for patients who underwent major hepatic resection stratified by post-operative pharmacologic thromboprophylaxis

	All patients ($n=419$)	Prophylaxis ($n=275$)	No prophylaxis ($n=144$)	p
Resection type[a]				<0.001
Extended left hepatectomy	27 (6.4%)	15 (5.5%)	12 (8.3%)	
Extended right hepatectomy	77 (18.4%)	31 (11.3%)	46 (31.9%)	
Left hepatectomy	48 (11.5%)	39 (14.2%)	9 (6.3%)	
Right hepatectomy	254 (60.6%)	185 (67.3%)	69 (47.9%)	
Other	13 (3.1%)	5 (1.8%)	8 (5.6%)	
Laparoscopic resection	14 (3.3%)	9 (3.3%)	5 (3.5%)	0.91
Simultaneous major non-hepatic procedures	140 (33.4%)	88 (32.0%)	52 (36.1%)	0.40
Estimated intra-operative blood loss (ml)[b]	400 (450)	400 (400)	500 (513)	<0.001
Intra-operative RBC transfusion	103 (24.6%)	73 (26.6%)	30 (20.8%)	0.19
Post-operative RBC transfusion	84 (20.0%)	46 (16.7%)	38 (26.4%)	0.02
Overall RBC transfusion	140 (33.4%)	96 (35.0%)	44 (30.6%)	0.36
Post-operative mortality	24 (5.7%)	13 (4.7%)	11 (7.6%)	0.22
Post-operative overall morbidity	212 (50.6%)	140 (50.9%)	72 (50.0%)	0.86
Length of hospital stay (days)[b]	7 (5)	7 (4)	7 (6)	0.04
Post-operative venous thromboembolism	15 (3.6%)	6 (2.2%)	9 (6.3%)	0.03
Post-operative pulmonary embolism	12 (2.9%)	6 (2.2%)	6 (4.2%)	0.35
Post-operative isolated deep venous thrombosis	3 (0.7%)	0	3 (2.1%)	0.04

[a] The largest component of each resection is reported

[b] Continuous variables are reported as median (IQR)

Table 3 Demographics, demographics, pre-operative laboratory values, surgical treatments, and intra-operative transfusions stratified by post-operative VTE

	Post-operative VTE (n=15)	No post-operative VTE (n=404)	p
Site of resection			0.12
UPMC (n=227)	5 (2.2)	222 (97.8)	
DUMC (n=192)	10 (5.2)	182 (94.8)	
Age (years)[a]	64 (51–70)	58 (49–69)	0.64
Ethnicity			0.70
Caucasian (n=361)	14 (3.9)	347 (96.1)	
Other (n=58)	1 (1.7)	57 (98.3)	
Body mass index (kg/m^2)[a]	26.7 (23.9–30.3)	28.1 (24.8–31.2)	0.20
Gender			0.30
Male (n=190)	9 (4.7)	181 (95.3)	
Female (n=229)	6 (2.6)	223 (97.4)	
Diagnosis			1.00
Malignant (n=363)	13 (3.6)	350 (96.4)	
Benign (n=56)	2 (3.6)	54 (3.6)	
American Society of Anesthesiology Score			0.57
I–II (n=126)	6 (4.8)	120 (95.2)	
III–IV (n=268)	9 (3.4)	259 (96.6)	
Pre-operative bilirubin (mg/dl)[a]	0.7 (0.4)	0.6 (0.5)	0.06
Pre-operative albumin (mg/dl)[a]	3.9 (0.8)	4.0 (0.7)	0.42
Pre-operative hematocrit (%)[a]	40.0 (10.2)	39.4 (6.0)	0.89
Pre-operative platelet (10^3/μl)[a]	290 (185)	234 (112)	0.06
Pre-operative international normalized ratio[a]	1.1 (0.2)	1.0 (0.1)	0.10
Pre-operative aPTT (s)[a]	28.1 (5.3)	29.6 (5.5)	0.31
Liver resection			0.21
Extended right or right hepatectomy (n=331)	14 (4.2)	317 (95.8)	
Other (n=88)	1 (1.1)	87 (98.9)	
Laparoscopic liver resection			0.24
Yes (n=14)	0	14 (100.0)	
No (n=405)	15 (3.7)	390 (96.3)	
Simultaneous major non-hepatic procedures			0.16
Yes (n=140)	8 (5.7)	132 (94.3)	
No (n=279)	7 (2.5)	270 (96.8)	
Estimated intra-operative blood loss (ml)[a]	550 (450)	400 (500)	0.16
Intra-operative RBC transfusion			0.22
Yes (n=103)	6 (5.8)	97 (94.2)	
No (n=316)	9 (2.8)	307 (97.2)	

aPTT activated partial thromboplastin time

[a] Continuous variables are reported as median (IQR)

treatment, and upper extremity VTE secondary to central line catheterization, this study focused on risks of venous thrombosis and bleeding due to factors related specifically to diagnosis, major liver resection, and post-operative pharmacologic thromboprophylaxis. In addition to institution, greater intraoperative blood loss was likely a key factor in the decision not to treat with post-operative pharmacologic thromboprophylaxis (Table 2). The incidence of clinical post-operative VTE after major hepatectomy in the overall cohort was 3.6%. Patients treated with post-operative pharmacologic thromboprophylaxis had lower rates of clinical VTE after major liver resection compared to untreated patients (6.3% vs. 2.2%, p=0.03). Analysis of demographics, clinicopathologic tumor characteristics, and treatment variables revealed that pharmacologic thromboprophylaxis was the only variable associated with post-operative VTE (Table 3). Rates of post-operative and overall RBC transfusion and post-operative mortality, severe morbidity, or overall morbidity were not higher among patients treated with pharmacologic thromboprophylaxis relative to untreated counterparts (Table 2)—suggesting that pharmacologic thromboprophylaxis does not increase the incidence of bleeding leading to further complications or RBC transfusion after major hepatectomy. The median decline in hematocrit

after the start of pharmacologic thromboprophylaxis was only 4% and was most likely due to fluid shifts from the extravascular to intravascular space and not secondary to bleeding. Results in our study are in agreement with that of De Pietri et al. [31] who noted a greater incidence of PE among four of 14 patients who underwent liver resection and were not treated with low molecular weight heparin on the evening of surgery compared to none of 42 patients who were treated (p=0.03).

Our results support the extension of grade IA ACCP guidelines advocating post-operative pharmacologic thromboprophylaxis in general surgery to patients undergoing major hepatic resection. If the risk of post-operative bleeding is particularly high, pharmacologic thromboprophylaxis should be delayed but subsequently started once bleeding risk subsides. The ACCP recommends making group-specific (as opposed to case-by-case patient specific) decisions regarding pharmacologic thromboprophylaxis because of simplicity, the inability to confidently identify groups which do not require prophylaxis, and to facilitate studies regarding safety and efficacy.[6] In this study we focused on patients for which pharmacologic thromboprophylaxis would be most controversial. Our previous work[30] demonstrated that resection of four or more liver segments is associated with highest post-operative mortality, overall morbidity, severe morbidity, and hepatic-related morbidity among all patients undergoing partial hepatectomy and thus should be the criterion for major hepatectomy. These patients are at risk for post-operative VTE due to prolonged general anesthesia and post-operative immobility, the high incidence of malignant indications for resection, large scale release of factor VIII, vWF, and tissue factor from the hepatic transection surface, decreased synthesis of anticoagulant factors and clearance of activated clotting factors due to post-operative hepatic insufficiency, and the acute inflammatory response.[6,31–33] Yet these patients are also at risk of post-operative bleeding because of the large hepatic transection surface area, dilution of procoagulant factors due to large volume colloid and crystalloid infusions, post-operative thrombocytopenia, and decrease in synthesis of procoagulant clotting factors due to post-operative hepatic insufficiency.[31–33] Interestingly, small studies suggest that a pro-thrombotic state is more common after larger volume compared to small volume liver resections. Among living liver donors, Dondero et al. [34,35] note that post-operative PE most often occurred after right hepatectomy compared to after left hepatectomy and left lateral segmentectomy. De Pietri et al. [31] observe lower R time and K time on thromboelastogram after major liver resection for malignancy compared to after minor hepatectomy despite higher INR and PTT after major liver resection. Thus conventional parameters gauging hypocoagulability may not completely reveal thrombotic potential after major liver resection.

Although less common compared to after other general surgical procedures,[1,3,6] post-operative VTE does occur after major liver resection. The overall incidence of post-operative VTE in our study (3.6%) is comparable to that reported in other series (Table 4). Importantly, details on pharmacologic VTE prophylaxis were not available for most of these other studies. Laboratory data also suggest that liver resection results in a prothrombotic state leading to VTE. Cerutti et al. [47] analyzed thromboelastogram profiles from ten patients who underwent right hepatectomy for adult living liver donation. Low molecular weight

Table 4 Literature review of venous thromboembolic events in large liver resection series

Author	*n*	DVT (%)	PE (%)	Comments
Aloia et al. [36]	2,313	47 (2.0)	38 (1.6)	3.5% and 2.8% incidence of DVT and PE after right and extended hepatectomies
Schroeder et al. [37]	587	7 (1.2)	3 (0.5)	–
Jarnagin et al. [38]	1,803	24 (1.3)	16 (0.9)	–
Ito et al. [39]	1,067	15 (1.4)	15 (1.4)	All resections for colorectal cancer metastases
Morris-Stiff et al. [40]	523	4 (0.8)	7 (1.3)	All resections for colorectal cancer metastases
Stewart et al. [41]	137	–	3 (2.2)	All resections for colorectal cancer metastases
Yates et al. [42]	99	–	–	7 (7.1%) incidence of venous thromboembolic events. All resections for malignancy
Lo [43]	1,508	–	4 (0.3)	All living liver donors
Dondero et al. [34,35]	127	4 (3.1)	9 (7.1)	All living liver donors. Seven of nine PE cases observed after right hepatectomy. Routine post-operative chest CT obtained
Broering et al. [44]	165	2 (1.2)	2 (1.2)	All living liver donors
Marsh et al. [45]	121	2 (1.7)	1 (0.8)	All living liver donor right hepatectomy
Umeshita et al. [46]	1,841	–	5 (0.3)	All living liver donors

heparin was administered on post-operative day 1 in all patients. The coagulation index became hypercoaguable in six of ten patients by post-operative day 10.[47] Northup et al. [48] observed a higher ratio of thrombin–antithrombin III complex to plasmin-alpha 2-plasmin inhibitor complex and a lower ratio of tissue-type plasminogen activator to plasminogen activator inhibitor-1 after liver resection compared to after colorectal resection. These results demonstrate a shift toward thrombotic potential in patients after liver resection. Studying blood samples from 20 patients who underwent liver resection for benign indications and were treated with pharmacologic thromboprophylaxis from the day before hepatic resection through day of discharge, Bezeaud et al. [32] observed a 50% post-operative decrease in plasma levels of coagulation inhibitors protein C, S, and antithrombin with an increase in procoagulants factor VIII and von Willebrand's factor. They also noted a greater than 10-fold increase in thrombin–antithrombin complexes and a 2-fold increase in sP-selectin (both prothrombotic markers). Importantly, most of these derrangements persisted on post-operative day 5 (longer than the corresponding increase in prothrombin time), suggesting a prolonged prothrombotic state after liver resection.[32]

The realization of VTE risk and benefits from pharmacologic thromboprophylaxis after liver resection parallels a similar recognition among cirrhotics relative to patients without chronic liver disease.[33,48–51] Traditional markers of coagulation status (including INR, peripheral platelet count, and aPTT) are not associated with VTE in cirrhotics in multiple large series.[33,48–50] Prothrombin time is only sensitive to systemic procoagulant factors and does not reflect the balance between deficiencies in both procoagulant and anti-coagulant factors.[37] A prolonged prothrombin time does not adequately depict levels of other clotting factors (such as VIII, X, and II) that can be more than adequate to promote clot formation.[52] In contrast, low serum albumin is independently associated with VTE among cirrhotics,[48,49,53] suggesting that decreased hepatic synthesis of anticoagulants, such as proteins C and S and antithrombin III, may be a key contributing factor toward VTE. Non-alcoholic steatohepatitis and other facets of the metabolic syndrome also leads to increased thrombotic potential.[33,52] Factors VIII, IX, XI, and XII activities are all elevated in patients with non-alcoholic liver disease and this elevation is related to hepatic fat index and independent of age, gender, or BMI.[54]

Several limitations to this study should be considered. Patterns of post-operative pharmacologic thromboprophylaxis differed starkly by institution. Although incidence of post-operative VTE did not differ by institution (Table 3), varying practices in surgical techniques and post-operative care between centers not accounted for in our analysis may have contributed to differences in post-operative VTE rates between treated and untreated patients. Tumor proximity to the inferior vena cava and surgical duration are factors that may influence the incidence of post-operative VTE not accounted for in this study. Early elevated post-operative INR and aPTT values (particularly on post-operative day 1) likely played a key role in determining which patients were treated with pharmacologic thromboprophylaxis. Because these data were not available for most patients, these variables were not accounted for in this study. Despite the large size of the overall cohort, the small number of VTE events prevented multivariable analysis to explore any factors that may have been independently associated with post-operative VTE and comparisons to distinguish whether low molecular weight or unfractionated heparin better prevents VTE after liver resection. Similarly, this lack of power may have underestimated the association of other factors with post-operative VTE (Table 3). Because only symptomatic VTE were noted, our study may underestimate the benefits of pharmacologic thromboprophylaxis in reducing the total incidence of VTE after major liver resection. Retrospective data collection and possible management of VTE events at other institutions that were not reported to the academic center at which liver resection was performed are additional weaknesses of our study.

Post-operative pharmacologic thromboprophylaxis lowers the incidence of VTE after major hepatic resection without increasing the rate of blood transfusion. Established guidelines for VTE prevention in general surgery should be extended to patients undergoing major hepatectomy.

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ORIGINAL ARTICLE

Pulmonary Resection for Isolated Pancreatic Adenocarcinoma Metastasis: an Analysis of Outcomes and Survival

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Abstract

Objectives This study was conducted to determine if pulmonary metastasectomy (PM) for isolated pancreatic cancer metastases is safe and effective.

Methods This was a retrospective case–control study of patients undergoing PM at our institution from 2000 to 2009 for isolated lung metastasis after resection for pancreatic cancer. Clinical and pathologic data were compared with a matched reference group. Resected neoplasms were immunolabeled for the Dpc4 protein. Kaplan–Meier analysis compared overall survival and survival after relapse.

Results Of 31 patients with isolated lung metastasis, 9 underwent 10 pulmonary resections. At initial pancreas resection, all patients were stage I or II. Other baseline characteristics were similar between the two groups. Median time from pancreatectomy to PM was 34 months (interquartile range 21–49). During the study, 29/31(90.6%) patients died. There were no in-hospital mortalities or complications after PM. Median cumulative survival was significantly improved in the PM group (51 vs. 23 months, $p=0.04$). There was a trend toward greater 2-year survival after relapse in the PM group (40% vs. 27%, $p=0.2$).

Conclusions In patients with isolated lung metastasis from pancreatic adenocarcinoma, this is the first study to show that pulmonary resection can be performed safely with low morbidity and mortality. The improved survival in the PM group may result in part from selection bias but may also represent a benefit of the procedure.

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Keywords Metastatic adenocarcinoma · Pancreatic adenocarcinoma · Metastasectomy

Introduction

Pancreatic cancer (pancreatic adenocarcinoma) remains a highly lethal disease, with 43,140 newly diagnosed cases and 36,800 deaths in 2010 according to American Cancer Society estimates.[1] Median survival for all patients undergoing pancreatic resection is 12.6 months.[2] Because there are no effective screening strategies for this malignancy, most patients present with unresectable, widely metastatic disease. Moreover, nearly 40% of patients who present with potentially resectable disease are not appropriately referred for surgery.[3] Among patients who do undergo surgical resection, the majority will die from disease recurrence, with a 3-year disease-specific survival of only 27%.[4]

The prognosis does appear to be improving, however, as recent figures cite nearly 30% 5-year survival among patients undergoing pancreas resection—an improvement from the historical figures which ranged from 10% to 18%.[5] Among long-term survivors (>5 years) after pancreas resection for adenocarcinoma, the most common site for disease recurrence is the lung.[5,6] Paradigms for pulmonary metastasectomy (PM) have evolved for other cancers with synchronous or metachronous metastatic disease such as colorectal adenocarcinoma, soft tissue sarcoma, uterine carcinoma, and renal cell carcinoma, with a demonstrated survival benefit.[7–13] However, to our knowledge, there are no published reports of PM for pancreatic adenocarcinoma to date. More recently, the recognition of *DPC4* gene status as a prognostic marker may additionally be of use in identifying those patients with less aggressive disease who may best benefit from such interventions.[14,15] Therefore, we examined our institutional experience to test the hypothesis that PM can be performed safely in appropriately selected patients. We also assessed Dpc4 (Smad4/MadH4) protein status in these patients to determine its relationship to the presence of isolated pulmonary metastases.

Materials and Methods

Patient Data

This was a retrospective case–control study of patients undergoing pulmonary resection for isolated pancreatic cancer metastasis at the Johns Hopkins Hospital. Following institutional review board approval, we identified all patients treated for primary pancreatic cancer at the Sol Goldman Pancreatic Cancer Research Center within the Sidney Kimmel Comprehensive Cancer Center from 1996 to 2009. We queried the institutional pancreatic cancer database to identify appropriate patients.

Inclusion criteria were (1) primary diagnosis of pancreatic cancer, (2) no evidence of distant disease at the time of diagnosis, (3) pancreaticoduodenectomy (classic or pylorus-preserving) for curative intent, and (4) development of isolated pulmonary metastasis without evidence of other sites of disease recurrence. Patients were excluded if they manifested multiple sites of disease recurrence. Each patient's clinical scenario is presented at our multidisciplinary pancreas cancer conference, where clinicians from various departments evaluate the patient. All patients treated since the year 2000 underwent a positron emission tomography to rule out other sites of disease. Patients were then stratified into two groups according to whether they had undergone PM, and clinical characteristics and outcomes were compared. Patients who did not undergo PM served as the control group for comparisons of clinical outcomes. Selection criteria included isolated lung metastasis without disseminated disease, and we attempted to match the two groups with respect to age at initial presentation as well as disease burden at the time of recurrence. All patients in this series who underwent PM had their lung resections performed with a presumptive diagnosis of pancreas metastasis. Given the high degree of suspicion, the primary indication for surgery in all cases was pancreatic metastasectomy, with the intent for a curative resection.

All relevant clinical information was extracted from the electronic and paper medical record. Demographic variables included age, gender, race, substance use (tobacco and ethanol), cardiovascular co-morbidities, history of pancreatitis, as well as laboratory values. Staging information from the initial pancreas resection was recorded using the tumor–node–metastasis (TNM) nomenclature adopted from the American Joint Committee on Cancer (AJCC).[2] Also, primary tumor size, lymph node status, histologic grade, and the presence of vascular and perineural invasion were recorded. The types of adjuvant chemotherapy and/or chemoradiation were recorded. To assess functional status, Eastern Cooperative Oncology Group (ECOG) scores at the time of relapse were obtained, as was the date of relapse to determine relapse-free intervals.

Clinical Outcomes

Follow-up information was determined via clinic notes, and the last clinic note determined follow-up time. For survival analysis, vital status was ascertained for all patients using the Social Security Death Index. Postoperative data included length of stay (LOS), in-hospital infections, perioperative complications, and survival. Overall survival, relapse-free survival, and survival after PM were determined, with patient censoring occurring for those patients lost to follow-up.

Histological Assessment and Immunohistochemistry

Collected samples of the primary carcinoma and/or resected pulmonary nodules were formalin-fixed for paraffin embedding, and routine histologic examination was performed. Immunohistochemistry was completed using standard methods described previously in detail.[16,17] Appropriate dilutions of antibodies to the Dpc4 protein (1:100 dilution anti-DPC4 clone B8, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were incubated overnight using a DAKO automated stainer (DAKO, Carpinteria, CA, USA). Immunohistochemical labeling of Dpc4 was scored as intact (positive, retention of labeling) or lost (negative, loss of Dpc4 labeling). Only sections in which internal controls (e.g., lymphocytes, stromal cells) present on the same slide showing intact Dpc4 nuclear labeling were used. Tissue was not banked for all 22 control group patients, so for Dpc4 protein comparisons we used a historical control, which had comparable characteristics to the clinical control group used.

Statistical Analysis

Patients were stratified into two groups for comparisons of clinical characteristics and outcomes: PM vs. no PM. Differences between these two groups were compared using a two-tailed Student's t test for normally distributed continuous variables. Chi-squared or Fisher's exact tests were used for categorical variables as appropriate. Normally distributed continuous variables are presented with the mean ± standard deviation (SD), whereas non-parametric data are presented with median and interquartile range (IQR). Categorical variables are shown in whole numbers and percentages. Baseline comparisons were performed in order to assess the comparability of the two groups. Cumulative survival and survival after relapse were estimated using the Kaplan–Meier method. The log-rank test was used to compare survival according to the two groups.

For the purposes of Dpc4 analysis only, a historical control group was used for comparisons with the nine PM patients. p values less than 0.05 were considered statistically significant for all tests. Analysis was performed using Stata statistical software, version 9.2 (StataCorp, College Station, TX, USA).

Results

Demographics

During the study period, 31 patients were identified to form the cohort for analysis of clinical characteristics and outcomes. Nine patients underwent 10 pulmonary resections, while 22 patients with isolated pulmonary metastasis did not undergo PM and were the control group. For contextual purposes, during the study period, 1,077 patients underwent pancreaticoduodenectomy for invasive primary pancreatic adenocarcinoma.

Mean age for the study cohort was 68±10 years and was similar between the two groups. There were 14 (45%) men in the study overall. Gender and race distributions were similar between the two groups. Eight (27%) patients had a history of smoking, but smoking rates were similar as well. Though patients who received chemotherapy alone were not specifically excluded, all patients received adjuvant chemoradiation with either 5-FU or gemcitabine-based regimens. At the time of relapse, average ECOG scores were lower for PM patients (0±0 vs. 0.9±0.2, p=0.01). The remaining demographic variables were evenly distributed between the two groups and are presented in Table 1.

Staging Information

All patients in this study had undergone a primary pancreaticoduodenectomy (classic or pylorus-preserving) for adenocarcinoma. At the time of initial pancreatic resection, all patients were AJCC/TNM stage I or II. For the PM group, 33% of patients were stage I compared with 0% for the control group; however, this did not reach statistical significance (p=0.08). There was a trend toward PM patients having higher CA19-9 prior to their pancreatectomy (PM 179±175 mg/ml vs. non-PM 78±74 mg/ml, p=0.06). The control group tended to have more positive lymph nodes at pancreatectomy (PM 2.7±2.5 vs. non-PM 6.5±5.6, p=0.06). No patient in the series had an R2 resection. Non-PM patients tended to more commonly have an R1 resection (n=0 (0%) in the PM group vs. n=7 in the non-PM group (31%), p=0.09). The remaining markers of disease burden were similar between the two groups and are depicted in Table 2. Median time from pancreatectomy to first pulmonary nodule on imaging was 29 (IQR 17–47) months and from pancreatectomy to PM was 34 (IQR 21–49) months. Among PM patients, pathology features of resected pulmonary neoplasms are depicted in Table 3.

Outcomes

Twenty-nine (90%) patients died during the study period. Median follow-up for the entire cohort was 21 (IQR 15–38) months after pancreatic resection. PM patients had longer overall follow-up (Table 4). Among the PM cohort, 9 patients underwent 10 lung resections. The majority of lung resections were performed via a standard thoracotomy approach (Table 3). One patient underwent subsequent pulmonary resection after developing a contralateral isolated pulmonary nodule, confirmed to be metastatic pancreatic

Table 1 Baseline demographics

Variables	PM (N=9)	Control (N=22)	p value
Mean age, years (SD)	69.4 (5.5)	67.6 (11.2)	0.63
Male gender, no. (%)	3 (33.3)	11 (50)	0.29
African-American, no. (%)	0 (0)	3 (13.6)	0.22
Diabetes mellitus, no. (%)	2 (22.2)	5 (23.8)	0.92
Coronary artery disease, no. (%)	0 (0)	3 (15.8)	0.21
Smoking history, no. (%)	2 (22.2)	6 (28.6)	0.72
Alcohol abuse, no. (%)	0 (0)	2 (9.5)	0.31
ECOG at relapse, avg (SD)	0 (0)	0.9 (0.2)	0.01
Hypertension, no. (%)	2 (22.2)	4 (19.1)	0.84

cancer. Among the PM group, average LOS after lung resection was 4.2±3.4 days. The only postoperative complication in a PM patient was a single episode of postoperative atrial fibrillation requiring additional intensive care unit stay.

When the entire cohort was analyzed without stratification, after pancreatic resection median cumulative survival and median relapse-free survival were 42 months (95% CI 20–52) and 17 months (95% CI 10–22), respectively. Median survival after relapse was 18.6 months (95% CI 5.6–29.2) for the PM group and 7.5 months (95% CI 3.4–22) for the control group. Kaplan–Meier depiction of overall survival when stratified by PM vs. non-PM is depicted in Fig. 1. Median cumulative survival was significantly improved in the PM group (51 vs. 23 months, p=0.04). Though not reaching statistical significance, there was a trend toward greater 2-year survival after relapse in the PM group (40% vs. 27%, p=0.2) (Fig. 2).

Among the PM patients, two (22.2%) were still alive at the conclusion of the study. Of the seven PM patients who had died, cause of death was available for five (71.4%). One patient developed widely disseminated pulmonary metastases. Two patients developed extrapulmonary metastases (hepatic involvement in one patient and spine metastases in the other). Another patient developed local recurrence in the pancreatic remnant. The fifth patient died of end stage renal disease unrelated to the pancreatic cancer.

Table 2 Staging information

Variables	PM (N=9)	Control (N=22)	p value
Initial TNM stage II, n (%)	6 (67)	22 (100)	0.08
Primary tumor size, cm	3.0 (0.9)	2.7 (0.9)	0.5
Lymph nodes sampled, no.	18 (7)	20 (8)	0.4
Lymph nodes positive	3 (3)	6 (6)	0.06
Positive margins, n (%)	0 (0)	7 (30)	0.09
Perineural invasion, n (%)	17 (85)	4 (44)	0.1
Vascular invasion, n (%)	11 (58)	3 (37)	0.3
Ca 19-9 pre-pancreas resection, mg/ml	179 (175)	79 (74)	0.06

Dpc4 Immunolabeling

Among PM patients, 9 of 10 excised lung specimens were available for Dpc4 immunolabeling. For four patients, the original matched primary carcinoma was also available. Loss of Dpc4 immunolabeling, indicating genetic inactivation of the *DPC4* gene, was present in three of these nine (33%) pulmonary metastases (Fig. 3). In all four matched primary and pulmonary metastases analyzed, Dpc4 status was concordant.

We next compared the frequency of Dpc4 loss in these nine patients with a historical control group for which Dpc4 status has been previously reported.[15] In this control group of eight patients who underwent surgical resection but later developed widespread metastatic recurrence involving multiple organs, including the lungs, eight of eight (100%) primary carcinomas showed Dpc4 loss. A comparison of the frequency of Dpc4 loss in the PM patients to this control group was highly significant (p=0.006).

Discussion

In this single institution retrospective case–control study, we describe our initial experience with 9 patients who underwent 10 pulmonary resections for isolated pancreatic cancer metastasis. It should be emphasized that this is a unique and highly selected group of patients who developed metachronous isolated pulmonary metastasis following pancreatic resection. Eligible patients who are deemed fit enough to undergo pulmonary resection were referred for thoracic surgery consultation only following an extensive multidisciplinary review accounting for tumor biology. A relatively long interval between initial resection of the pancreatic primary and relapse, isolated and stable disease over time, and favorable response to systemic therapy were considered indicative of "good biology" and were requisites to be considered for PM. This study demonstrates that PM can be performed safely with minimal morbidity in this patient population. As the molecular underpinnings of pancreas cancer are further elucidated, targeted chemotherapy regimens will continue to evolve. Thus, we believe that improving chemotherapy agents in the future will enable the paradigm of PM for isolated lung metastasis to apply to pancreas cancer as well.

We compared survival between the two groups using the Kaplan–Meier method. PM patients had improved overall

Table 3 Pathology features of resected pulmonary neoplasms

Variables	PM (*N*=10)
Average lymph nodes sampled, no.	4 (2)
Positive lymph nodes, *n* (%)	1 (10)
Positive margins, *n* (%)	1 (10)
Lobectomy, *n* (%)	6 (60)
Video-assisted lung resection, *n* (%)	3 (30)
Thoracotomy, *n* (%)	7 (70)

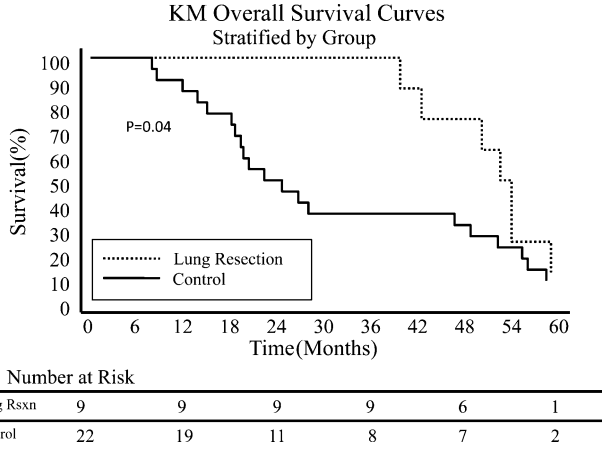

Fig. 1 Kaplan–Meier figure depicting estimates of cumulative survival, stratified by patients who underwent pulmonary metastasectomy vs. those who did not. *P* value determined using Cox–Mantel log-rank analysis

survival with a median survival of 52 months, compared with a median survival of 22 months for non-PM patients. Additionally, there was a trend in favor of PM for post-relapse survival. Patients undergoing PM had a median survival after relapse of 18.6 months, compared with 7.5 months for non-PM patients. This study was underpowered to detect significant survival differences, however. We estimated that a study with 80% power to detect this magnitude of post-relapse survival difference would require approximately 50 patients per group. It is possible that a larger sample size would reveal a significant advantage in favor of PM with respect to survival after relapse.

Overall survival is likely a less reliable indicator of the effectiveness of PM; the longer cumulative survival in PM patients probably reflects a selection bias in that healthier patients were selected for pulmonary resection. In order to minimize this bias, we attempted to identify a contemporaneous matched cohort of patients who had also undergone pancreas resection and developed isolated pulmonary metastasis. There is precedent for this type of study, in highly selected patients undergoing resection for metastatic disease. Much of the paradigm for colorectal cancer with metastasis to the liver stems from retrospective study designs, which are limited by similar selection bias.

The significantly longer follow-up in the PM group likely also contributed to the findings of longer relapse-free survival when evaluated from time of pancreatic resection. We derived the control group for comparing clinical outcomes from a subset of patients from our institutional adjuvant pancreas cancer database. This group had complete follow-up with regard to the parameters in this study. The fields do not exist in the database at large to find a comparable group with respect to follow-up time, and the difference in follow-up time is an added limitation of this study. Propensity score matching could potentially account for differences in follow-up time, but the limited numbers of patients in this study precluded effective propensity score assignment. In the future, prospective evaluation of increased numbers of patients undergoing pulmonary resection for isolated pancreatic metastasis will overcome these limitations.

With respect to treatment of the primary carcinoma, these two groups received equivalent care. There were no statically significant differences in adjuvant therapy between the two groups. Age, gender, and other cardiovascular co-morbidities were also well matched between the two groups. There was no statistically significant difference with respect to initial TNM stage; however, PM patients did have a trend toward a greater proportion of stage I patients at initial pancreas resection. Additionally, PM patients had slightly better ECOG performance status at the time of relapse; this factor may represent a selection bias with respect to PM patients having overall improved functional status at the time of relapse. Due to the limited sample size, we were unable to identify predictors of improved outcomes following PM using a multivariable analysis.

Recent studies have identified *DPC4* gene status as an important marker of prognostic significance.[14,15] In a risk-adjusted model, *DPC4* gene inactivation was associated with

Table 4 Outcomes

Variables	PM (*N*=9)	Control (*N*=22)	*p* value
Follow-up, months	46 (12)	21 (12)	<0.001
Length of stay, days	4.2 (3.4)	–	–
Median relapse-free survival, months	29 (18–47)	14 (8–20)	<0.001
Median survival after relapse, months	18.6 (5.6–29.2)	7.5 (3.4–22.0)	0.4
Median overall survival, months	51 (39–53)	23 (18–52)	0.04

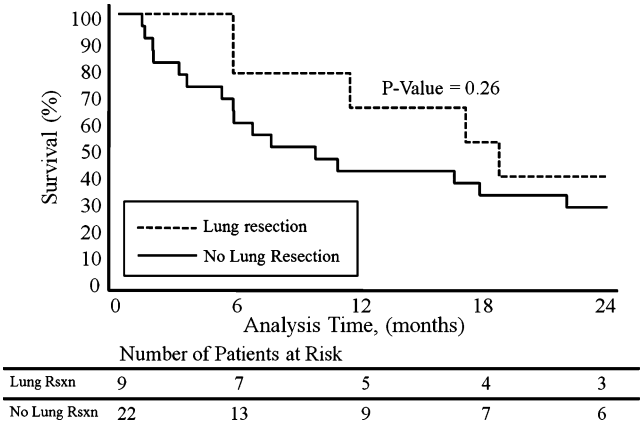

Fig. 2 Kaplan–Meier figure depicting estimates of survival after relapse, stratified by patients who underwent pulmonary metastasectomy vs. those who did not. *p* value determined using Cox–Mantel log-rank analysis

worse overall survival in patients treated with pancreas resection. A rapid autopsy program was instituted at our institution to facilitate the study of genetic markers in patients who died because of advanced pancreatic cancer. Patients were classified into locally destructive vs. widespread disease burden phenotypes. Dpc4 status, as determined by immunolabeling, varied based on disease phenotype as patients with Dpc4 loss were more likely to have a high burden of metastatic disease.

As all patients underwent pulmonary resection at our institution, we were able to review 9 of 10 lung specimens to assess Dpc4 status in the metastatic lesions. In our earlier autopsy series, Dpc4 loss was omnipresent among patients with widespread disease.[15] However, only 33% of the carcinomas resected from the PM patients in this study demonstrated Dpc4 loss. While this finding supports the notion that Dpc4 plays a significant role in dictating the pattern of disease recurrence, there are likely other genetic determinants as well. Tissue for Dpc4 genetic analysis from the clinical control group would ideally have been used. But, tissue was not banked for these patients, so we used a historical control which had comparable characteristics to the clinical control group used. Nevertheless, the use of a historical control group for Dpc4 status is an added limitation of this study.

Conclusion

In summary, we report successful outcomes following pulmonary metastasectomy in patients who had previously undergone pancreaticoduodenectomy for adenocarcinoma of the pancreas. With recent advances in anesthesia and perioperative care, pulmonary resections can be performed safely and with minimal morbidity. In a small, retrospective series, we acknowledge the above-mentioned limitations. A large, multi-institutional effort will be important to validate these findings. Further investigation is required to determine which patients would be best suited for these interventions and the identification of molecular markers in addition to Dpc4 that will be useful in identifying such patients.

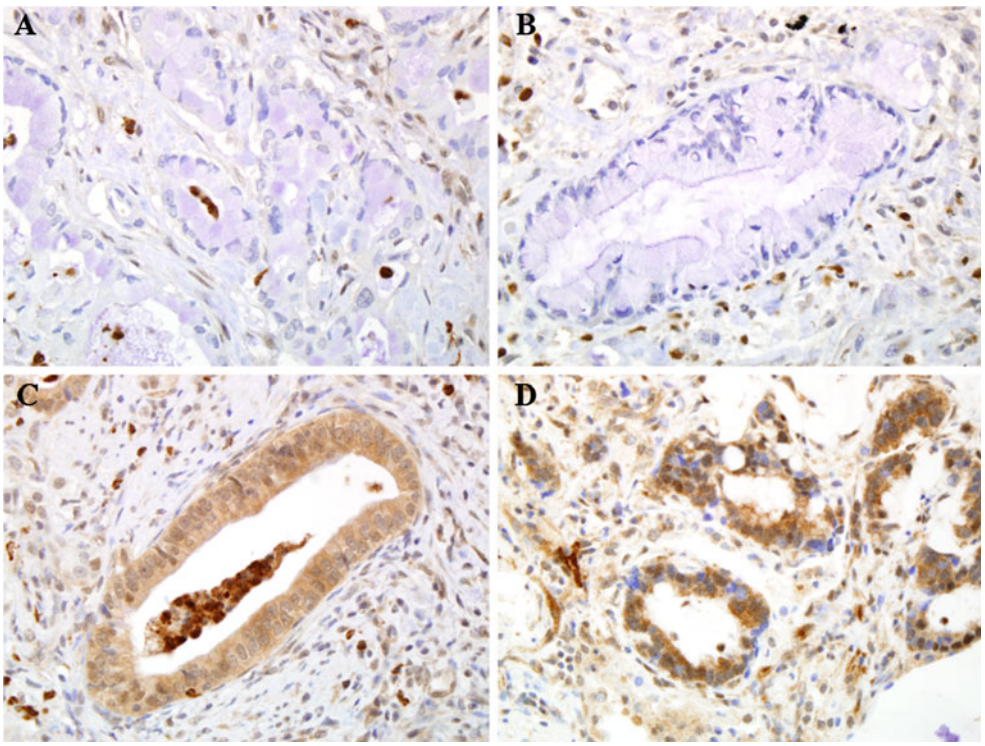

Fig. 3 Histology specimens with assessment of Dpc4 status. Primary pancreas specimen depicting loss of Dpc4 staining (**a**) and lung specimen from the same patient also depicting loss of Dpc4 staining (**b**). Intact Dpc4 status in a primary pancreas specimen (**c**) and lung specimen from the same patient, also demonstrating intact Dpc4 status (**d**)

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Conflicts of Interest The authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

The Changes of Pro-opiomelanocortin Neurons in Type 2 Diabetes Mellitus Rats After Ileal Transposition: The Role of POMC Neurons

Weijie Chen · Zhibo Yan · Shaozhuang Liu · Guangyong Zhang · Dong Sun · Sanyuan Hu

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Abstract
Background Ileal transposition (IT) can effectively resolve obesity and improve type 2 diabetes. IT is associated with increased glucagon-like peptide 1 secretion. The mechanisms mediating the effects of IT on obesity and diabetes remain undefined. Given the role of pro-opiomelanocortin neurons in energy balance, we sought to determine its potential role in these processes.
Methods Twenty non-obese diabetic Goto–Kakizaki rats underwent either IT or sham operation. Various measures including food intake, body weight, fasting plasma glucose, glucagon-like peptide 1 level, activated pro-opiomelanocortin neuron number, and pro-opiomelanocortin mRNA expression were evaluated.
Results The IT group demonstrated significantly improved plasma glucose homeostasis with increased glucagon-like peptide 1 secretion. The IT group ate less and demonstrated reduced body weight gain over time. These effects were also associated with increased central neuronal activity with increased pro-opiomelanocortin and derivative gene expression in the hypothalamus and increased protein expression in the pituitary gland.
Conclusions More pro-opiomelanocortin neurons in the hypothalamus of diabetes rats were activated after ileal transposition. These data suggest a potential important role for pro-opiomelanocortin neurons in the resolution of diabetes after IT.

Keywords POMC · Type 2 diabetes mellitus · Glucagon-like peptide-1

Introduction

Type 2 diabetes mellitus presently afflicts more than 170 million people worldwide[1] and was expected to affect about 300 million persons by 2025.[2] The complications made the disease become a major cause of morbidity and mortality, which strain public healthcare funding.[3] However, there is no radical treatment on type 2 diabetes. Current therapies, including exercise, diet, and medication, can control plasma glucose and reduce the incidence of the complications, but the morbidity and mortality were increasing year by year because of poor compliance of patients.[4,5]

Interestingly, bariatric surgeries were shown to be an effective means of resolving obesity and comorbidities, like type 2 diabetes mellitus (T2DM).[6–8] Because normal plasma glucose after bariatric surgery often occurred long before significant weight loss,[5,9] the control of T2DM seemed to be independent and not secondary to the loss of weight. As a bariatric surgery, ileal transposition (IT) also treated diabetes effectively. It led to normal blood glucose, insulin, and glycosylated hemoglobin level in morbidly obese patients long before weight loss occurred.[5,7,8] The hormonal changes may be indirect and direct mediators of euglycemia[10–13] because more glucagon-like peptide 1 (GLP-1) secretion in IT Goto–Kakizaki (GK) rats was characterized compared with controlled rats,[14] although it had a very short half-life being rapidly degraded by the ubiquitous enzyme dipeptidylpeptidase IV.[15] The "hindgut hypothesis" proposed that the stimulation of the terminal ileum by the early arrival of food could lead to many hormonal

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changes. Nevertheless, the mechanism mediating the effects of IT on obesity and diabetes remained undefined.

The pro-opiomelanocortin (POMC) neurons, a specific population of arcuate nucleus neurons in the hypothalamus, were typical neurons involved in fuel balance.[16] Along with other central neurons, they regulated energy homeostasis and balanced energy intake, expenditure, and storage by receiving and integrating afferent neural and metabolic signals conveying information about the energy status of the body. Studies showed that rising glucose level and GLP-1 could increase POMC neurons firing and affected liver glucose homeostasis and insulin action.[16–20] However, when the glucose level of plasma decreased and GLP-1 increased after IT in T2DM rats, what were the subsequent changes of POMC neurons?

Given the role of pro-opiomelanocortin neurons in energy balance, we sought to investigate the changes of POMC and its potential role after ileal transposition. It might give other clues to reveal the mechanism of T2DM and IT.

Materials and Methods

Animal Model

GK type 2 diabetic rats were the most widely used model of non-obese T2DM to exclude the hormonal effects secondary to the weight loss that IT would obtain.[12,21] Twenty male GK type 2 diabetic rats (8 weeks of age, 150–200 g) were purchased from the National Rodent Laboratory Animal Resources (Shanghai, China) and housed individually in a constant ambient temperature and humidity room on a 12-h light/dark cycle. The procedural protocols were approved and supervised by the ethics committee of Shandong University. After 1 week acclimation, 20 rats were assigned to two groups randomly, each with ten animals: IT and sham IT (S-IT) group.

Surgical Procedures

All operations were performed following an overnight fasting. Inhalation anesthesia used 2% isoflourane in an air/oxygen mixture. Subsequently, IT was performed as previously described.[22] Briefly, an 8-cm ileal segment 5–15 cm proximal to the ileocecal valve was transected, transposed, and anastomosed isoperistaltically with the jejunal 5–10 cm distal to the ligament of Treitz. The opened ends of the ileal segments were anastomosed together using 7-0 silk suture (Fig. 1). Sham surgeries involved the same abdominal incisions, transections, and re-anastomosis of the gastrointestinal tract at multiple sites corresponding to IT, except no ileum transposition. The sham surgeries were prolonged to achieve similar operative times as those observed for IT operations.

The weight and food intake were measured daily for the first two postoperative weeks, twice a week for the following 2 weeks, and then weekly for the following times.

Biochemical Tests

Plasma and Cerebrospinal Fluid Assays At indicated time intervals, blood was collected from tail vein into centrifuge tubes containing 50 mmol/L ethylenediaminetetraacetic acid, 12-TIU/mL aprotinin, and 100 μmol/L dipeptidylpeptidase IV inhibitor, centrifuged at 4,000×*g* for 20 min to extract plasma. Afterwards, the fasting blood glucose level and plasma GLP-1 were measured by enzyme-linked immunosorbent assay using commercially available kits (R&D Systems, Minneapolis, USA).

All rats were sacrificed when they were 45 weeks old. The cerebrospinal fluid (CSF, 50–100 μL per rat) was collected from the cistern magna using a 25-gauge syringe, snap froze, and stored at −80°C until analysis using Elisa kits (R&D Systems, Minneapolis, USA).[23]

Hypothalamic Immunohistochemistry After collecting CSF, the brain was quickly removed, frozen on dry ice, and stored at −80°C. The middle brain was dissected using a cryostat. The micro-dissection of arcuate nucleus, hypothalamus, and pituitary was performed using the procedure described.[24]

Hypothalamic immunohistochemistry was performed as previously described.[25] Briefly, the hypothalamic tissue specimens were fixed in neutral formalin and embedded in paraffin after collection. Subsequently, the 5-μm-thick tissue slides were dewaxed and incubated with 0.01 M natrium citricum for antigen retrieval and with diluted rabbit anti-POMC precursor antibody (Abcam, Cambridge, UK) at 4°C overnight. The following steps were performed using immunostain kit (Fuzhou Maixin Co, Fuzhou, China) according to the manufacturer's instructions. Afterwards, the sections were examined at a magnification of 100 (ten objective and ten ocular lens) under a light microscope to

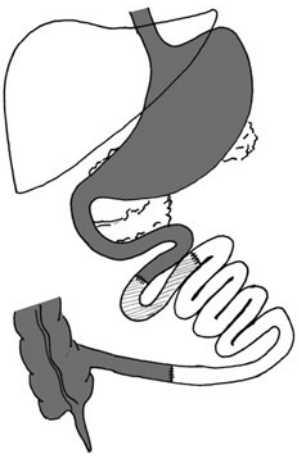

Fig. 1 The procedure of the ileal transposition: 8 cm of the terminal ileum 5–15 cm proximal to the ileocecal valve is transposed isoperistaltically 5–10 cm distal to the ligament of Treita

identify three regions with the highest POMC neurons density. The number of POMC neurons was determined in these areas at a magnification of 200.[26]

Measurements of POMC mRNA Expression in Arcuate Nucleus Total mRNA was extracted from dissected arcuate nucleus using Trizol (Invitrogen, Carlsbad, USA) and treated with RQ1 RNAse-free DNAse (Promega Corp., Madison, USA). One microgram of total mRNAwas reverse transcribed into cDNA using Superscript II reverse transcriptase and random hexaprimers (Invitrogen, Carlsbad, USA). Subsequently, gene expression levels were determined by real-time quantitative RT-PCR (q-PCR) using the iCycler iQ™ (Bio-Rad, Hercules, USA). Analyses were performed on 1 μL cDNA using the iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, USA), in a total PCR reaction volume of 15 μL, containing 50–500 nM of each primer. Relative quantification of the target gene transcripts was done using β-actin gene expression as reference, which was stably expressed in this experiment. Differences in mRNA levels were presented as an *x*-fold induction with respect to S-IT group. The primers for POMC and β-actin, the real-time PCR conditions, and data analysis were performed according to previous report.[27–29]

The Amount of POMC Derivative in Pituitary The amount of POMC was analyzed by western blot assay with an anti-adrenocorticotropic hormone antibody (Abcam, Cambridge, UK).[30] The collected pituitary tissues were weighed and homogenized. The supernatant proteins were subsequently concentrated, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and detected by a polyclonal anti-ATCH antibody. The antibodies recognized the POMC precursor and its processed fragments.[31] Relative concentration of protein was quantified by densitometry using Versa Doc 1000 Imaging System and Quantity One 4.4 software (Bio-Rad, Hercules, USA).

Statistical Analysis

In all cases, experiments were replicated in triplicate and data represented mean±standard deviation. The difference between two groups was analyzed by Student's *t* test. That $P<0.05$ was considered statistically significant in all cases.

Results

Animal Model

Firstly, there was no significant difference between these two groups in terms of weight, food intake, fasting plasma glucose concentration, and GLP-1 level before surgery (Table 1.).

All operations were successful. No death or postoperative complication was observed. Although, there was no difference in operative time and the food intake before the second postoperative week, afterwards the IT rats ate less than the S-IT rats ($P<0.05$; Fig. 2). Both IT and S-IT lead to significant weight loss compared with preoperative weight ($P<0.05$; Fig. 3), but started regaining body weight after the fourth week postoperatively. Moreover, the S-IT rats gained more weight than the IT rats after the eighth week postoperatively ($P<0.05$). The mean weight of the IT rats was 305±15.5 g, the S-IT rats 346±18.7 g at the eighth postoperative week. When sacrificed, the mean weight of the IT rats was 367±32.4 g and of the S-IT rats was 405±35.9 g.

Biomedical Tests

Plasma and Cerebrospinal Fluid Assays Though both groups displayed a decline in fasting glucose level during the first week after operation and a slight rebound before the fourth week, the glucose level in the IT group remained stable lower while the sham operations yield progressive raise (Fig. 4; $P<0.05$). The fasting plasma glucose of the IT rats was between 77 and 99 mg/dL, while the fasting plasma glucose of the S-IT rats fluctuated between 143 and 186 mg/dL.

The intra-assay and inter-assay coefficients of variation were 1.2% and 3.4% for GLP-1 assay, respectively. The assay results showed that the GLP-1 level for the sham group was stable throughout the experiment (Fig. 5), it was between 15 and 36 pmol/L, while the GLP-1 level in the IT group increased promptly after the second week and was higher than that of the S-IT group ($P<0.05$). When the IT rats were sacrificed, the GLP-1 was 65±12.9 pmol/L in plasma and 25±9.3 pmol/L in CSF, while the GLP-1 of the S-IT was 22±8.5 pmol/L in plasma and 12±5.7 pmol/L in CSF.

Table 1 Preoperative data of rats

	IT	S-IT	*P* value
Weight (g)	280.5±12.5	279.1±17.2	NS
Fasting blood glucose (mg/dL)	180.3±13.5	178.9±10.2	NS
Food intake (g)	18.9±3.1	19.2±4.0	NS
GLP-1 value (pmol/L)	13.1±3.7	12.6±2.5	NS

NS no significant difference, *IT* the ileal transposition group, *S-IT* the sham ileal transposition group, *GLP-1* glucagon-like peptide 1

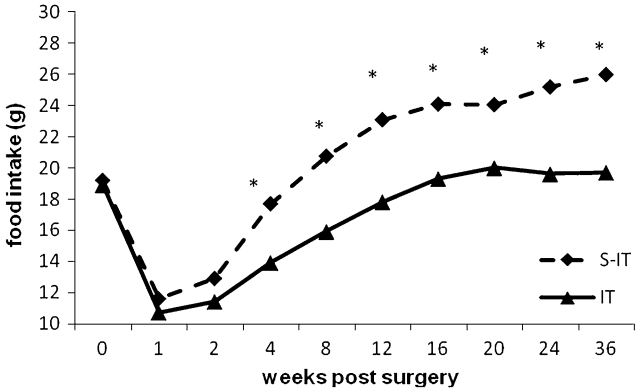

Fig. 2 The food intake of GK rats before and after operation. The food intake of the IT group was smaller than that in the S-IT group since the fourth week. The *asterisk* means that $P<0.05$. *GK rats* Goto–Kakizaki type 2 diabetic rats, *IT* the ileal transposition group, *S-IT* the sham ileal transposition group

Hypothalamic Immunohistochemistry The POMC neurons were stained by immunohistochemistry in two groups (Fig. 6). The highest POMC neurons density was in arcuate nucleus. The mean neuron number at a magnification of 200 was 15.7±5.3 in the IT rats and 5.3±4.1 in the S-IT rats. Therefore, the activated neuron number in the IT group was more than that of the S-IT group ($P<0.05$).

POMC mRNA Expression in Arcuate Nucleus The relative POMC mRNA expression in arcuate nucleus in the IT and the S-IT rats was determined by q-PCR. The POMC gene expression in the IT group was significantly upregulated and 2.16-fold greater than that of the S-IT group (Fig. 7; $P<0.05$).

The Amount of POMC Derivative in Pituitary The relative content level of POMC was 71.4±5.1 in the IT group and 34.6±4.1 in the S-IT group when the rats were sacrificed.

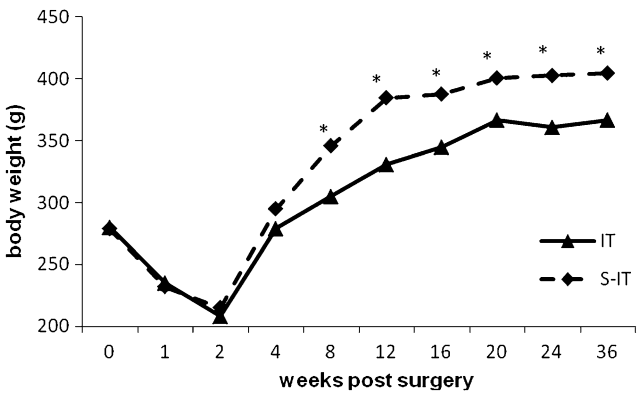

Fig. 3 The weight of GK rats before and after operation. The weight of rat in the S-IT group was higher than that in the IT group since the eighth week. The *asterisk* means that $P<0.05$. *GK rats* Goto–Kakizaki type 2 diabetic rats, *IT* the ileal transposition group, *S-IT* the sham ileal transposition group

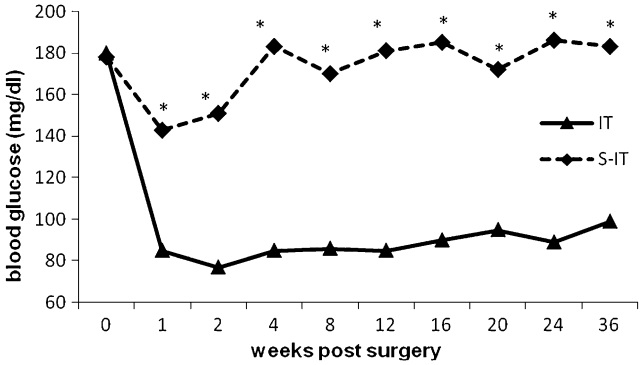

Fig. 4 The fasting plasma glucose of GK rats before and after operation. Though both groups display a decline of fasting glucose level during the first week after operation and a slight rebound afterwards, the glucose levels in the IT remained stable lower while the sham operations yield progressive raise. The *asterisk* means that $P<0.05$. *GK rats* Goto–Kakizaki type 2 diabetic rats. *IT* the ileal transposition group, *S-IT* the sham ileal transposition group

The amount of POMC derivative in the IT group rat was more than that in the S-IT group (Fig. 8; $P<0.05$).

Discussion

Type 2 diabetes was improved significantly by IT procedure in our animal models. The fasting glucose level in the IT group was lower than that in the S-IT group after the second postoperative week, with more GLP-1 excretion after the first week postoperatively. This was in accordance with our previous findings and other reports because IT can significantly improve glucose tolerance, insulin sensitivity, and acute insulin response in GK rats.[7,8,14] Besides, more GLP-1 secretion of the ileal neuroendocrine cell L in IT GK rats

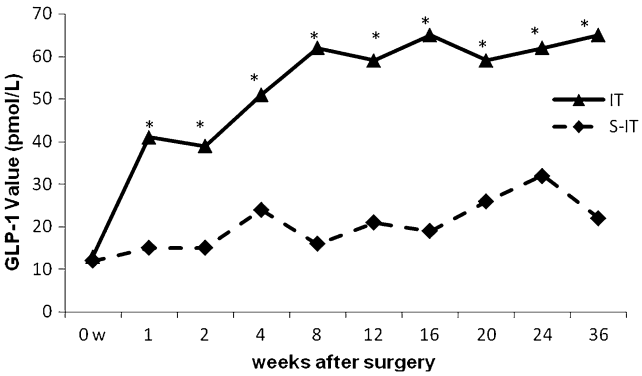

Fig. 5 The GLP-1 level in plasma of GK rats before and after operation. The assay result shows that the GLP-1 level of the S-IT group was stable throughout the experiment. The GLP-1 level for the IT group increased promptly since the second week and was higher than that of the S-IT group. The *asterisk* means that $P<0.05$. *GLP-1* glucagon-like peptide 1, *GK rats* Goto–Kakizaki type 2 diabetic rats, *IT* the ileal transposition group, *S-IT* the sham ileal transposition group

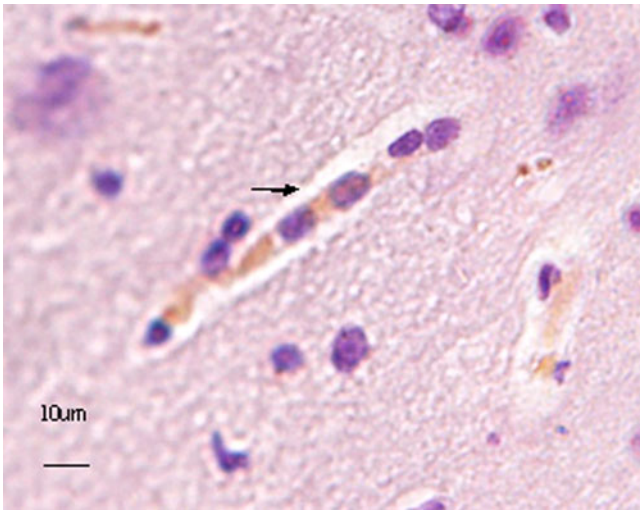

Fig. 6 The POMC neurons stained by immunohistochemistry. The *arrow* indicates the POMC neurons. *POMC* pro-opiomelanocortin neurons

was characterized in response to the early stimulation of carbohydrate and fat in the diet.[14,32] Therefore, higher levels of GLP-1 in plasma and CSF were detected in IT rats than S-IT rats.

Moreover, the IT group rats ate less than S-IT group rats after the fourth postoperative week and gained body weight slower than the S-IT group rats after the eighth postoperative week. Given the role of POMC neurons in regulation of energy homeostasis and balance between energy intake, expenditure, and storage, the change of POMC neurons was investigated. Consequently, the more activated POMC neurons in arcuate nucleus in the IT rats were revealed by immunohistochemistry. Besides, POMC mRNA expression in arcuate nucleus and POMC derivative in pituitary in the IT rats were significantly enhanced.

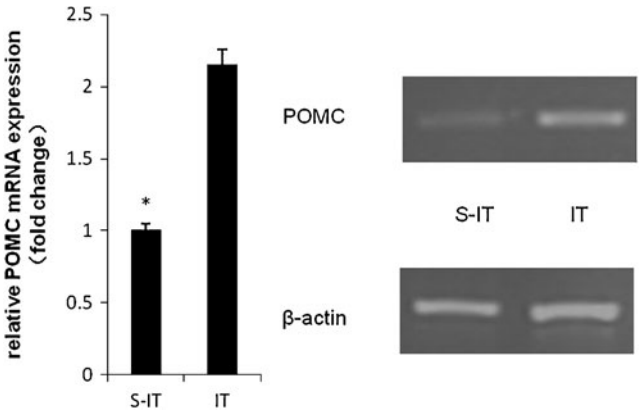

Fig. 7 Significant overexpression of POMC mRNA in the IT rats compared with the S-IT rats. Relative POMC mRNA expression in the IT and the S-IT rats was determined by q-PCR. The mRNA levels were normalized by β-actin mRNA levels. The *asterisk* means that $P<0.05$. *POMC* pro-opiomelanocortin, *IT* the ileal transposition group, *S-IT* the sham ileal transposition group

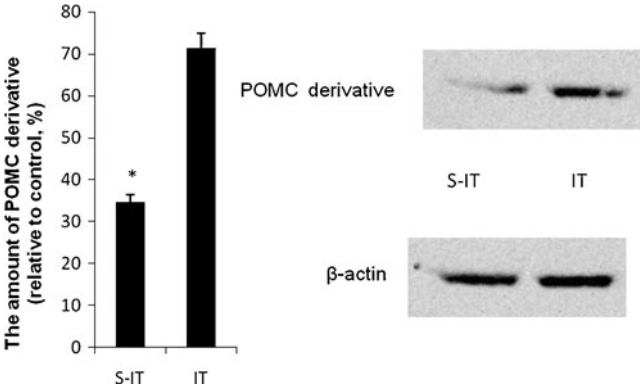

Fig. 8 The quantification of POMC products by Western blot. Densitometry analysis of bands intensities relative to β-actin concentrations showed that the POMC concentration increased in the IT rats. The *asterisk* means that $P<0.05$. *POMC* pro-opiomelanocortin, *IT* the ileal transposition group, *S-IT* the sham ileal transposition group

POMC neurons, like NPY/AgRP neurons, balanced energy intake, expenditure, and storage by receiving and integrating afferent neural and metabolic signals conveying information about the energy status of the body, demonstrating a role for glucose sensing in the overall physiological control of blood glucose, were evaluated.[19,20] These mechanisms were thought to activate glucose transporter 2, glucokinase, and K_{ATP} channels.[16,33–35] Glucose level rising increased POMC neurons firing, and then affected liver glucose homeostasis and insulin action.[16,18–20] However, the glucose level in the IT group was lower than that in the S-IT group. Moreover, it was reported that POMC neurons became defective in obese rats on a high-fat diet, suggesting that loss of glucose sensing by neurons had a role in the development of type 2 diabetes.[20] Therefore, the increased secretion and function of POMC might not result from glucose, but other stimulations.

The POMC neurons might be excited by an increased level of GLP-1. GLP-1 is a gut-secreted hormone which can alter energy balance by sending signals from the gut to the brain and results in brain neuropeptide changes.[36] Its effects included the stimulation of insulin, inhibition of glucagon secretion, inhibition of pancreatic β cells apoptosis, delaying gastric emptying, and so on.[14] Studies showed that GLP-1 could also affect the activities of hypothalamic POMC neurons.[17] GLP-1-sustained secretion was characterized in GK rats that underwent IT in accordance with our results, and the level of GLP-1 in cerebrospinal fluid in the IT group was higher than that in the S-IT group. Therefore, the number of activated POMC neurons, the expression of POMC, and the level of POMC derivative increased in the IT group compared with the S-IT group. The increased secretion, and thus increased level, of GLP-1, not the change of glucose, might play a key role in the activities of POMC neurons.

The activated POMC neurons can reduce food intake and increase energy expenditure. They would release melanocyte-stimulating hormones (α-, β-, and γ-MSH), β-endorphin, and

adrenocorticotrophic hormone when they were activated.[37] Of these hormones, α- and β-MSH can reduce food intake and body weight and increase energy expenditure in animals and humans.[38,39] Thus, the food intake of the IT group was less than S-IT group after the fourth postoperative week; the mean weight of the S-IT rats was more than that of the IT rats after the eighth postoperative week. The GK rats were non-obese type 2 diabetes rats, thus could not show the bariatric effect significantly. However, the slow weight gain indicated the effect of IT and POMC neurons.

In conclusion, though many other aspects need to be further investigated, all these evidences seemed to support the hypothesis that the IT may be a kind of therapy for type 2 diabetes through an enhanced secretion and function of POMC resulting from an enhanced secretion of GLP-1. This gave another potential to change the current concepts of pathophysiology of type 2 diabetes and the treatment of type 2 diabetes.

Conclusion

IT can improve glucose homeostasis in GK diabetes rat. Besides, POMC neurons were activated resulting from an enhanced secretion of GLP-1 after IT indicated that POMC neurons might play an important role in the resolution of type 2 diabetes.

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ORIGINAL ARTICLE

Effect of TachoSil Patch in Prevention of Postoperative Pancreatic Fistula

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Abstract
Background Postoperative pancreatic fistula (POPF) is a severe complication after pancreatic resections. The aim was to assess if application of TachoSil® patch could reduce incidence of postoperative fistulas after laparoscopic distal pancreatic resections.
Methods This is a retrospective study of prospectively collected data after enucleations and distal pancreatic resections. Patients were divided in two groups: with or without application of TachoSil® patch. Demographic and surgical data were analyzed.
Results One hundred twenty-one patients with distal pancreatic resections without additional resections were identified among 230 patients operated by laparoscopic approach at our institution since 1998. They were divided into two groups. In group 1 (n=48), TachoSil® patch was not applied while in group 2 (n=73), the pancreatic stump was covered with TachoSil®. Postoperative fistulas were registered in 8% (4/48) and 12% (9/73) in groups 1 and 2, respectively. The median duration of postoperative hospital stay in group 1 was 5.5 (2–35) days compared with 5 (2–16) days in group 2. No significant difference in surgical outcomes was found.
Conclusions The application of the TachoSil® patch did not affect either occurrence of POPF or duration of postoperative hospital stay. Routine use of TachoSil® patch to prevent pancreatic fistulas does not provide clinically significant benefit.

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Keywords Postoperative complication · Pancreatic fistula · TachoSil · Pancreas resection

Introduction

The laparoscopic approach for distal pancreatic resections has gained increased acceptance for several indications during the past decade and an increasing number of patients is operated by this method both for nonmalignant as well as for malignant diseases. There is increasing documentation that both endocrine tumors, cystic lesions, metastatic lesions, and adenocarcinomas can be safely operated by the laparoscopic approach[1–3] and the implementation of minimally invasive techniques has led to reduced morbidity.[4,5] Despite this, perioperative bleeding and pancreatic leakage still remain a challenge for the surgeons. General morbidity rate and perioperative hemorrhage is generally lower for laparoscopic procedures[6], but there is no

conclusive evidence that minimally invasive surgery leads to reduced POPF rate after distal pancreas resections. Various centers have published different rates of POPF varying from 0% to 32% as defined by the International study group on pancreatic fistulas (ISGPF).[7] In our institution, the overall fistula rate after laparoscopic resections of the pancreas has been 10%.[2]

The continuous search for the new and more effective remedies and techniques to prevent POPF remains is important. In January 2005, we introduced the surgical patch TachoSil® (Nycomed, Pharmaceutical Co. Ltd, Denmark) to cover the resection margin of the pancreas after laparoscopic resections. It was announced as a fast and reliable remedy for haemostasis and sealing of soft tissues. These argued characteristics corresponded to our needs in order to prevent postoperative complications. This study evaluated TachoSil as a prevention remedy for postoperative pancreatic fistula (POPFs) in pancreatic surgery.

Patients and Methods

Patients

A total of 230 patients underwent laparoscopic pancreas resections in our institution from March 1997 to December 2010. After exclusion of local tumor resections (n=36), procedures with additional resections of adjacent organs (n=29), procedures where other types of protection of surgical margin were used (n=14), converted procedures or accomplished as hand assisted (n=5), and explorative and other types of procedures (n=25) a total of 121 patients undergoing distal pancreatic resection (DPR) with or without splenectomy, were left for analysis. All procedures were performed by the same group of surgeons. From January 2005 majority of the procedures have been completed with covering of surgical margin and part of pancreatic remnant by TachoSil patch for to prevent postoperative pancreatic fistulas and potential postoperative bleeding. To that time not all effects of this remedy were clear and good documented, especially about its effectiveness in pancreatic surgery.

Indications to surgical procedures were endocrine tumors, cystic lesions, adenocarcinomas, and others as described in our previous publication.

The patients were retrospectively divided in two groups according to the final management of the surgical margin. In the first group (group 1, consisting of 48 patients (32 women and 16 men) with a median age of 62 (30–81) years and median ASA score of 2 (1–3)), the pancreas was divided by a linear stapler and left without additional covering. In the second group (group 2, consisting of 73 patients (49 women and 24 men) with a median age of 60 (16–82) years and median ASA score of 2 (1–3)), the staple line of the resection margin was covered with a TachoSil® patch. As our method for pancreas division has been described in detail earlier,[2,8] this study focused only on the final part of the procedure.

Data were analyzed retrospectively. Patient's characteristics are presented in Table 1.

Outcome Parameters

According to the ISGPF definition, pancreatic fistula was defined as a drainage fluid beyond the third postoperative day with at least threefold elevation of normal serum amylase. The grading system (grades A, B, and C) of severity of pancreatic fistula was applied (Table 2).[9]

Postoperative complications were registered in accordance with a last revision of the accordion classification, from mild complications (grade 1) to death of the patient—(grade 6) as described in Table 3.[10]

Statistical Analysis

Statistical analysis was conducted using SPSS 16, 0. Data were presented as median (range). For comparison of frequencies, the Chi-square test was performed. For comparison of the continuous variables, Mann–Whitney U test was used.

Results

Out of 230 patients, 121 were included in this study of which 91 were DPR with splenectomy and 30 were DPR performed as spleen-preserving procedures. Details regarding the indications for surgery are summarized in Table 4. All procedures were completed laparoscopically. Data regarding the surgical details are described in Table 5:

In group 1, 38 DPR with splenectomy and ten spleen-preserving resections were performed without using TachoSil. The median operative time for these procedures was 202 (29–350) minutes and the median intraoperative bleeding was 50 (0–1,500) ml. There were three grade 1, two grade 2, four grade 3, and two grade 4 events. Four

Table 1 Patient characteristics

	Group 1	Group 2
Patients	48	73
Gender	32 women	49 women
	16 men	24 men
Median age (years)	62 (30–81)	60 (16–82)
Median ASA score	2 (1–3)	2 (1–3)

Table 2 Main parameters for POPF grading

Grade	A	B	C
Clinical conditions	Well	Often well	Ill appearing/bad
Specific treatment[a]	No	Yes/no	Yes
US/CT (if obtained)	Negative	Negative/positive	Positive
Persistent drainage (after 3 weeks)[b]	No	Usually yes	Yes
Reoperation	No	No	Yes
Death related to POPF	No	No	Possibly yes
Signs of infections	No	Yes	Yes
Sepsis	No	No	Yes
Readmission	No	Yes/no	Yes/no

US ultrasonography, *CT* computed tomographic scan, *POPF* postoperative pancreatic fistula

[a] Partial (peripheral) or total parenteral nutrition, antibiotics, enteral nutrition, somatostatin analog, and/or minimal invasive drainage

[b] With or without a drain in situ

patients developed pancreatic fistula of which all were grade B. The overall morbidity in the group was 30%. The median duration of postoperative hospital stay for group 1 was 5 (2–16) days.

In group 2, we included only those procedures where the pancreatic remnant was covered with TachoSil®. In this group, 53 procedures were DPR with splenectomy and 20 spleen-preserving DPR. The median operative time was 158 (88–608) min, and the median intraoperative bleeding was 50 (5–3,000) ml. The postoperative morbidity included four grade 1 event, three grade 2, one grade 3 event, and three grade 4 events. Postoperative pancreatic leakage was registered in ten patients of which one grade A, six grade B, and three grade C fistulas. The overall morbidity rate in group 2 was 30%, and the median duration of postoperative hospital stay was 5.5 (2–35) days.

Table 3 Revised accordion classification

Grade	Revised accordion classification
Mild	
1	Requires only minor invasive procedures that can be done at the bedside, such as insertion of intravenous lines, urinary catheters, and nasogastric tubes and drainage of wound infections. Physiotherapy and anti-emetics, antipyretics, analgesics, diuretics, electrolytes, and physiotherapy are permitted
Moderate	
2	Requires pharmacologic treatment with drugs other than such allowed for minor complications, e.g., antibiotics. Blood transfusions and total parenteral nutrition are also included
Severe	
3	No general anesthesia: requires management by an endoscopic, interventional procedure or reoperation without general anesthesia
4	General anesthesia or single-organ failure
5	General anesthesia and single-organ failure or multisystem organ failure (>2 organ systems)
Death	
6	Postoperative death

No postoperative mortality was recorded in any of the groups. Detailed description over all postoperative complications is shown in Table 6. We did not find statistical difference in postoperative data between these two groups.

Discussion

Laparoscopic DPR have steadily gained acceptance as a method for surgical removal of both benign and malignant lesions in the tail and body of the pancreas.

The technique not only shows better cosmetic results but is also associated with reduced bleeding and overall morbidity rate compared with traditional open surgery.[11] It is unclear if the method influences the rate of postoperative pancreatic leakage since no randomized studies has been conducted while comparing the techniques. There are, however, studies in which a trend has been reported about nonsignificant reduced rate of POPF after minimally invasive procedures.[6]

Pancreatic leakage is one of the most commonly encountered severe complications following pancreatic resections and leak rates up to 46% has been described.[12] Several different techniques have been attempted in order to prevent fistulas and some authors mean that management of

Table 4 Summary of details regarding the indications for surgery

Indication to surgery	Group 1	Group 2
Cystic lesions	17	34
PNET		
Malign lesions	6	4
Benign lesions	15	14
Exocrine adenocarcinoma	5	9
Metastatic lesions	2	0
Pancreatitis	1	9
Abdominal trauma	0	1
Vascular formation	2	2
Total	48	73

Table 5 Data regarding surgical details

Surgical outcomes	Group 1[a]	Group 2[b]	*p* value
DPR with splenectomy	38	53	
Spleen-preserving resections	10	20	
Operative time (min)	202 (29–350)	158 (88–480)	0.810
Bleeding (ml)	50 (0–1,500)	50 (0–3,000)	0.970
Duration of postoperative hospital stay	5 (2–16)	5.5 (2–35)	0.203

PNET pancreatic neuroendocrine tumor

[a] Pancreatic stump was not covered

[b] Resectional pancreatic stump was covered with TachoSil patch

the resectional margin of pancreatic stump is very important. To develop a standardized technique which can demonstrate a significant decrease in overall morbidity including fistula formation is important.

Better results and safety of the patient is a major concern of any surgical procedures and laparoscopic pancreas resection is no exception. One contribution for the decreased overall morbidity in pancreatic surgery during the last years is the introduction of new staplers, electro-surgical instruments, surgical methods, and other technical and pharmaceutical remedies. TachoSil® is a fixed combination of a patch sponge coated with a dry layer of the human coagulation factors fibrinogen and thrombin. TachoSil® is indicated for supportive treatment in surgery, for improvement of haemostasis, to promote tissue sealing and for suture support in vascular surgery where standard techniques are insufficient.

The haemostatic effect of Tachosil patch in surgical procedures is well documented in the literature[13] in a wide variety of organs.[14]

Since the TachoSil® patch was also reported to be of value in terms of sealing surgical resection surfaces; we postulated it to be of value also in pancreas resections in which fistula formation continued to constitute a problem. Covering of the stapling line on the cut surface of the pancreas therefore became a routine part of the procedure since 2005.

It was described as predicting factors for development of pancreatic fistulas[15], and we tried to look if TachoSil®

Table 6 Detailed description over all postoperative complications

Postoperative complications	Group 1[a]	Group 2[b]	*p* value
Fistula formation	4 (8%)	10 (14%)	0,487
Grade A	0	1	
Grade B	4	6	
Grade C	0	3	
Other morbidity	11 (23%)	11 (15%)	
Severity grade			
Mild			
1	**4**—2 small hematomas around the resection area, urine retention, and wound infection	**3**—urine retention, pleural liquid collection, and wound infection	
Moderate			
2	**3**—postoperative abscess and 2 fibers	**2**—postoperative bleeding and subcapsular splenic hematoma	
Severe			
3	**1**—intraabdominal abscess	**4**—Abscess in operation area, subphrenic hematoma, and 2 wound fractures	
4	**3**—postoperative bleeding	**2**—bleeding and myocardial infarction	
5	0	0	
Death			
6	0	0	
Overall morbidity	15(30%)	21 (30%)	

[a] Pancreatic stump was not covered

[b] Resectional pancreatic stump was covered with TachoSil patch

patch could be beneficial in special cases. In the present series, we found that in the first group (without application of TachoSil) fistulas were registered only in soft glands whereas in the second (where TachoSil was applied on the resectional line) 30% (three out of ten) of all fistulas developed in the hard glands. To make any conclusions based on these results is difficult. We did not experience serious blood loss (≤1,000 ml) in any of these cases.

When we designed this retrospective study we were aware about its limitations and have tried to diminish their possibility. To avoid selection bias only distal resections with or without splenectomy independent of other factors (age, pathology, etc.) were included to the study. Chances that some of the patients fall out of control were equal for both groups. However, due to general low rate of fistulas in our material, one should be aware about possibility of statistical type 2 error.

In this study, however we did not observe significant differences in any of the studied parameters between patients in whom stapling line was covered by TachoSil patch after the resection and those without it. Somewhat surprisingly, grade C fistulas were only observed in patients in group 2, in which TachoSil® was used. The reason for this is unclear. One possible explanation could be that reducing of the natural outflow from the pancreatic remnant can lead to accumulation of ferments and thereby impair the normal process of postoperative healing. To make any conclusion about this, randomized studies are needed.

The haemostatic effect of TachoSil® in various procedures makes the product of great value in complex laparoscopic procedures.

Present data do not support the use of the TachoSil® patch for the prevention of fistulas following distal pancreatic resections.

Conflicts of Interest The authors declare that they have no conflict of interest.

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HOW I DO IT

A Penrose Drain to Help Reflect the Resected "Specimen Side" Jejunum Beneath the Mesenteric Vessels During a Pancreaticoduodenectomy

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Abstract
Introduction Reflecting the resected portion of the proximal jejunum behind the mesenteric vessels during a pancreaticoduodenectomy (Whipple) procedure can be a challenging maneuver.
Methods We describe a simple technique employing a penrose drain that is sutured to the resected "specimen" portion of the jejunum and then pulling it behind the mesenteric vessels.
Results Seven patients underwent this procedure over a 2-month period without difficulties.
Conclusions This simple technique helps simplify one of the key maneuvers in performing a Whipple procedure.

Keywords Whipple · Penrose drain · Pancreaticoduodenectomy

Background

Pancreaticoduodenectomy (Whipple procedure) for either benign or malignant diseases of the head of the pancreas, distal common bile duct, or duodenum is one of the most complex operations in general surgery. The procedure requires a series of complex maneuvers, one of which is resection of a portion of the proximal jejunum, approximately 10 cm distal to the ligament of Treitz, ligation and division of its mesentery, and then reflecting the resected "specimen side" portion beneath the superior mesenteric artery and vein (mesenteric vessels) so that it will lie on the right side of the body. It should be noted that the proximal jejunal segment to be resected should be freed of its mesentery before pulling it to the patient's right side. Reflecting the resected jejunum and duodenum can be difficult to perform, especially if the patient is obese or has a large body habitus. Additionally, we have found that, conceptually, it is not easily grasped by a number of surgical residents.

Surgical Technique

Assuming that the duodenum has already been extensively Kocherized and that the resected portion of the jejunum is prepared to be brought to the right side of the patient's body, a large penrose drain is then delivered posterior to the mesenteric vessels from the right side to the left side of the patient's body; enough length of the drain should be seen on each side of the body. The drain on the patient's left is then attached to the "specimen

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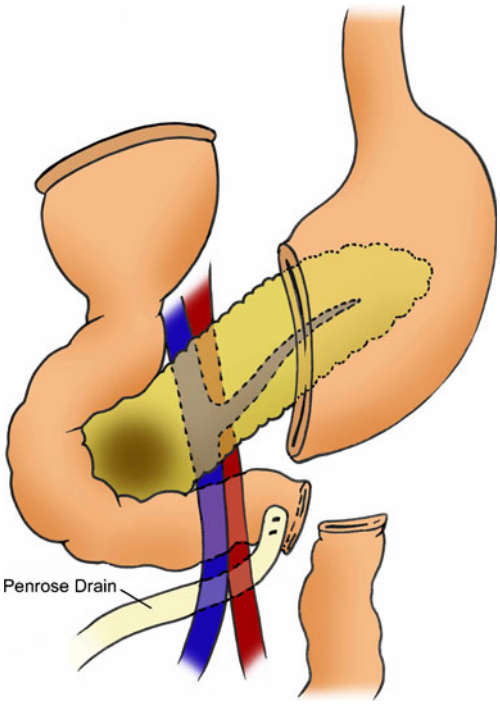

Fig. 1 A penrose is stitched to the "specimen" side of the jejunum to be delivered posteriorly to the mesenteric vessels

side" of the resected jejunum via a U-stitch, using a 2–0 stitch of choice (i.e., silk or prolene; Figs. 1 and 2). A Kelly clamp should be placed on the right side of the patient so as to avoid the drain from being inadvertently dragged through the patient's left side of the body while the drain is being sutured to the jejunum. The clamped end of the drain on the patient's right side is then pulled, thus dragging the attached jejunum from the left side of the body to the right side of the body (Figs. 3 and 4). The completion of the Whipple procedure can then proceed accordingly.

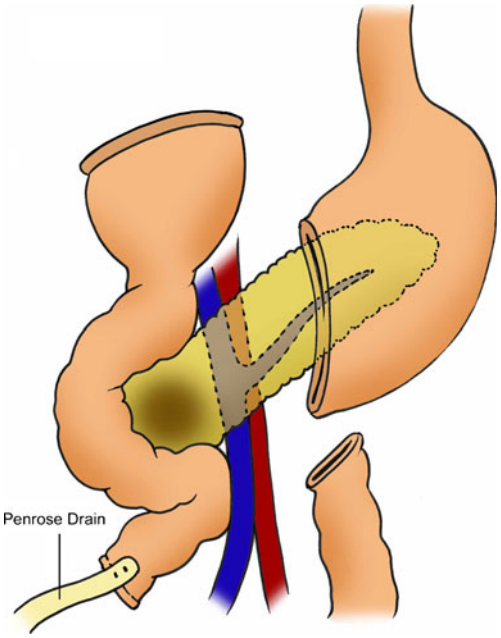

Fig. 3 The "specimen" side of the jejunum is reflected such that it ends up on the patient's right side of the body

Results

Over a 2-month period, we employed this technique on seven patients who underwent the Whipple procedure for a variety of pathologies (Table 1). This technique was described to the surgical residents beforehand and was then performed by them without difficulties.

Discussion

The first successful resection of the duodenum and part of the pancreas was performed by Kausch in 1912.[1,2] This was

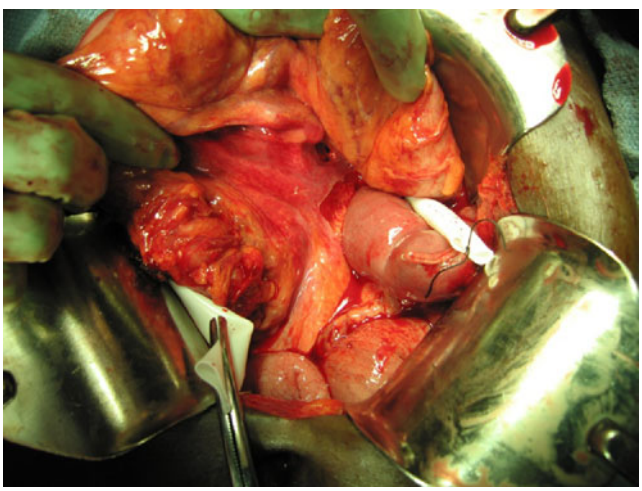

Fig. 2 Intraoperative photographs of the first part of the maneuver

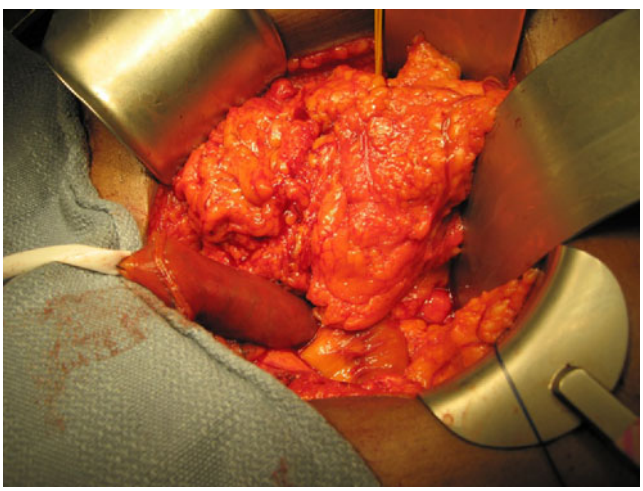

Fig. 4 Intraoperative photographs of the second part of the maneuver

Table 1 Characteristics of patients undergoing a Whipple

Patient	Age	BMI	Gender	Pathology
1	49	47.3	F	Serous cystadenoma
2	25	19.3	M	Solid pseudopapillary
3	43	37.9	F	Neuroendocrine
4	38	46.6	F	Neuroendocrine
5	67	26.4	F	Adenocarcinoma
6	79	33.2	M	Adenocarcinoma
7	61	31.4	M	Adenocarcinoma

BMI body mass index

performed as a two-stage procedure for ampullary cancer.[1,2] In 1935, at the American Surgical Association's Annual Meeting, Alan Oldfather Whipple presented the first report of several successful pancreaticoduodenectomies in the USA.[2,3] Since then, there have been multiple refinements of this technique, although the basic steps of the modified "Whipple procedure" as described by Dr. Whipple in 1942 are relatively unchanged.[2]

In performing the Whipple procedure, we have found that reflecting the resected proximal portion of the jejunum behind the mesenteric vessels could be a challenge, especially in the obese patients or those with a large body habitus. In addition, we found it to be a difficult maneuver for a number of our surgical residents to perform. The penrose technique described herein can be done relatively with ease, as long as there is sufficient room behind the mesenteric vessels to successfully pass the bowel. Our rule of thumb is to take down the tissues that are posterior to the mesenteric vessels such that one can pass one's hand from one side of the vessels to the other with ease.

In summary, the penrose technique can be done with ease and helps simplify a potentially cumbersome maneuver.

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REVIEW ARTICLE

Body Mass Index and Outcomes from Pancreatic Resection: a Review and Meta-analysis

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Abstract
Introduction There are 1.6 billion adults worldwide who are overweight, with body mass indices (BMI) between 25 and 30, while more than 400 million are obese (BMI >30). Obesity predicts the incidence of and poor outcomes from pancreatic cancer. Obesity has also been linked to surgical complications in pancreatectomy, including increased length of hospital stay, surgical infections, blood loss, and decreased survival. However, BMI's impact on many complications following pancreatectomy remains controversial.
Methods We performed a MEDLINE search of all combinations of "BMI" with "pancreatectomy," "pancreatoduodenectomy," or "pancreaticoduodenectomy." From included studies, we created pooled and weighted estimates for quantitative and qualitative outcomes. We used the PRISMA criteria to ensure this project's validity.
Results Our primary cohort included 2,736 patients with BMI <30, 1,682 with BMI >25, and 546 with BMI between 25 and 30. Most outcomes showed no definitive differences across BMIs. Pancreatic fistula (PF) rates ranged from 4.7% to 31.0%, and four studies found multivariate association between BMI and PF (range odds ratio 1.6–4.2). Pooled analyses of PF by BMI showed significant association ($p<0.05$).
Conclusion BMI increases the operative complexity of pancreatectomy. However, with aggressive peri- and post-operative care, increases in BMI-associated morbidity and mortality may be mitigated.

Keywords Pancreatic neoplasms · Obesity · Morbidity · Outcomes

Introduction

Over the last 25 years, the US prevalence of obesity has increased dramatically in all age groups (CDC). More than 400 million adults worldwide are considered obese, with body mass indices (BMI) >30, and nearly 1.6 billion are overweight, with BMI's between 25 and 30.[1] Despite its ubiquity, obesity has been linked to increased risk for developing serious pathologies, including breast, prostate colorectal, endometrial, and other cancers.[2–4] It is simultaneously a common risk factor for chronic cardiovascular disease and diabetes.[5–7] Treatment for obesity-associated illnesses consumes 10% of all US healthcare expenditures,[8] and increased BMI has been linked to poorer outcomes across the board.

More specifically, obesity has been shown to predict the incidence of and poor outcomes from pancreatic cancer,[9–12] as well as to confer a higher risk of developing chronic pancreatitis.[13] In both cases, surgical pancreatic resection is imperative. However, obesity has also been linked to a variety of peri- and post-surgical complications following pancreatectomy, including increased length of hospital stay (LOS),[14–17] surgical site infection,[18–20] blood loss,[21,22] and decreased disease-free and overall survival.[15] Conversely, though increased BMI is frequently cited as a risk factor for deleterious peri-surgical outcomes in patients undergoing pancreatic resection, its application as a universal predictor seems to be unfounded. While several studies have directly

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examined the impact of BMI on outcomes following pancreatic resection,[14–17,21,23,24] their small sample sizes and inconsistent findings have limited the power of their conclusions. Additionally, their results are frequently contradicted within the literature, and their analyses are often limited by a lack of specificity toward BMI-associated outcomes.

However, recent increases in resistant gram-positive and gram-negative bacterial infection and colonization both nationally and within hospitals suggest that identifying predictors of complications before surgery may limit the risk of hospital-acquired morbidity and excess mortality.[25–28] Because 5-year survival following pancreatic resection stands at less than 20%,[29] with post-surgical morbidity as high as 50%,[14] an accurate assessment of BMI-associated complications in pancreatic resection may facilitate targeted pre- and post-operative therapy, greatly improving outcomes and reducing costs in this sick cohort. We undertook this study to identify BMI-associated outcomes in patients undergoing pancreatic resection.

Materials and Methods

This review and meta-analysis was performed using the PRISMA criteria to ensure validity and transparency. We conducted a MEDLINE search using the Boolean operator "AND" to join all combinations of "BMI" or "obesity," with "pancreatectomy," "pancreatoduodenectomy," or "pancreaticoduodenectomy." We did not restrict our search by study design or to core clinical journals, only restricting our initial inquiry to publications in English between January 1990 and June 2010. We included in our review all studies providing information on BMI-associated outcomes in human patients undergoing pancreatic resection for all indications, defining pancreatectomy as an intervention involving any form of pancreatic resection. We assumed that outcomes reported in primary studies were based on individual patients undergoing a single pancreatic resection procedure.

We excluded a priori all case reports/series, reviews not providing new information, studies not assessing outcomes for pancreatic resection, studies failing to provide information about pre-surgical BMI, and studies with equivalent experimental and control group BMIs. A priori sensitivity analyses were not specified, and we did not generate explicit criteria for identifying and excluding primary studies with inherent biases. Our sensitive, rather than specific, inclusion criteria were intended to generate a large study cohort with which to assess a variety of BMI-associated outcomes. Both investigators assessed all articles for failure to meet these criteria. Disagreement was resolved by reaching consensus. All outcomes reported in primary studies are reported with their original qualifying data.

From the studies meeting the inclusion criteria (n=17), both reviewers independently extracted data regarding indication for surgery, type of intervention, patient BMI, all surgery-associated outcomes, and any other pertinent background information. For descriptive BMI-associated outcome analyses, we separated patients into BMI-unit intervals for normal weight (BMI 18–25), overweight (25–30), and obese (>30) patients. Because BMI was reported inconsistently across the primary study cohort, our analyses frequently evaluated BMI <25 versus >25, and BMI >30 versus <30, though we compared all three tiers whenever possible. Several studies reporting BMI as a continuous variable are included in qualitative analyses.

For our meta-analysis, we created pooled rates for all binary outcomes where at least three studies reported those outcomes with corresponding BMI information. We performed Fisher exact tests using R (version 2.12) to identify any differences in outcomes across BMI tiers. We reported the results of these analyses as odds ratios (OR) with 95% confidence intervals and utilized the $p<0.05$ level to specify statistical significance. Similarly, we performed a subgroup analysis of studies using the International Study Group on Pancreatic Fistula (ISGPF) definition of pancreatic anastomosis to illuminate any disparities in the measurement of this outcome among studies not reporting utilization of this standard. All other outcome analyses used definitions of those outcomes used within each individual study, and there was insufficient data to perform sensitivity analyses on these outcomes.

For continuous variables, because we could not extrapolate reported averages backward to their corresponding patients, we created weighted totals by multiplying each reported estimate by its primary-study sample size and dividing that by the pooled sample size. This metric emphasized the importance of sample size in our aggregate estimate for each continuous variable. Since continuous outcomes in primary studies were peri-surgical estimates only, our metric and analyses maintained the descriptive nature of these outcomes. Consequently, we performed minimal statistical analyses of continuous variables. Outcomes that were quantified in only one or two studies are reported separately. Qualitative analyses include the results from univariate and multivariate analyses of BMI-associated surgical outcomes within each primary study.

Results

Study Population

Of 71 studies identified in our broad initial search, we excluded 53 from our review and meta-analysis: those that did not include data from human patients (n=11),[30–40] case

reports/series (n=15),[41–55] review articles not providing new information (n=7),[56–62] those not assessing outcomes for pancreatic resection (n=11),[63–73] those failing to provide information about pre-surgical BMI (n=7),[74–80] and those with equivalent experimental and control group BMI's preventing the extraction of outcomes related to different BMIs (n=2).[81,82]

In all, 17 studies were included in the final set (Table 1).[14–17,21–24,83–91] All primary studies reported retrospective analyses of prospectively maintained databases, suggesting that selection bias may exist in this body of literature. Our 17 study cohort included a total of 4,045 patients, though BMI information was reported heterogeneously. Four studies split BMI into three tiers and yielded 881 patients with a stratified BMI <25, 592 patients with a BMI >30 and 546 patients with a BMI between 25 and 30.[16,21,22,24] Eleven other studies assessed BMI only as >30 versus <30 or >25 versus <25, and these studies provided 1,309 patients with BMI <30 and 328 patients with BMI >25.[14,15,17,23,83–91] One study assessed outcomes for patients with BMI >27 versus <27, and 216 patients therein with BMI >27 were included in our >25 comparator group, though we could not include the remaining 213 patients with BMI <27 from the primary study in any of our analyses.[88] In aggregate, these data yielded a sum total of 2,736 patients with BMI <30, 1,682 with BMI >25, and 546 with BMI definitively between 25 and 30 for various quantitative analyses. Two other studies evaluated BMI as a continuous function and data therein were included only in our qualitative analysis.[84,87] One study provided a stratified BMI measurement, but we could not extract outcomes data corresponding to patients in each BMI tier, and we included analysis offered by this study in our descriptive analysis only.[90] There was insufficient information to generate cohorts of underweight (BMI <18) or class II and III obese patients.

Surgical indications and interventions were diverse across primary studies. This cohort included 2,753 patients with adenocarcinoma, 216 with neuroendocrine neoplasms, 339 with pancreatitis (chronic and immune), 93 with benign neoplasms, and 965 with other operative indications (Table 2). Some patients may have presented with more than one indication for pancreatic resection, but we could not identify outcomes for these patients individually. Reported surgical interventions included classic PD (n=1,001), pyloris-preserving PD (PPPD; n=1,365), unspecified PD (n=1,047), unspecified proximal pancreatectomy (n=100), unspecified distal pancreatectomy (n=756), unspecified partial pancreatectomy (n=17), and total pancreatectomy (n=17). This diversity of indication and intervention was present within many individual studies.[16,20,22,23,64,68,73,75,77,79,80,82,83,85–90] Consequently, we could not stratify outcomes by these conditions, though more research is necessary to illuminate the possibility of

Table 1 Characteristics of 17 studies evaluating BMI-associated morbidity in pancreatectomy

First author, year	BMI categories	# centers	Intervention	Laparoscopic	ISGPF PF	Patients excluded
Benns, 2009[14]	><30	1	M	No	No	LP
Fleming, 2009[15]	><30[a]	1	M	No	No	No BMI (n=7), stage 4 AC (n=4)
Williams, 2009[16]	>30, 30–25, <25	1	PD, PPPD	No	No	Incomplete data (n=20), trauma (n=2)
Noun, 2008[17]	><30	1	PD	No	No	Total PD and CP (n=24)
Tsai, 2010[21]	>30, 30–25, <25	1	PD, PPPD	No	No	Total PD (n=5)
Su, 2010[22]	><25	1	PD, PPPD	No	No	NS
Gaujoux, 2010[23]	><25	1	PD	No	Yes	NS
House, 2008[24]	>30, 30–25, <25	1	PD, PPPD	No	No	NS
Hashimoto, 2010[83]	><30	1	PD, PPPD	No	Yes	Total PD
Schrader, 2010[84]	Continuous	1	M	No	No	NS
Rosso, 2009[85]	><25	1	PD	No	Yes	NS
Akizuki, 2009[86]	><25	1	PPPD	No	Yes	Non-standard procedures (n=16)
Menge, 2009[87]	Continuous	1	M	No	No	NS
Weber, 2009[88]	><27	9	Distal	N=219	Yes	Pancreatic enucleation
Ferrone, 2008[89]	><30	1	Distal	N=13	Yes	NS
Bentrem, 2005[90]	><30	1	PPPD	No	No	NS
Barry, 2003[91]	><25	1	PD	No	No	NS

LP laparoscopic procedures, *NS* not specified, *AC* adenocarcinoma, *CP* chronic pancreatitis, *PD* classic pancreaticoduodenectomy, *PPPD* pyloris-preserving pancreaticoduodenectomy, *M* multiple

[a] Presented as <23, 23–25, 25–29, 30–35, >35, but no valuable outcome measurements except at ><30.

Table 2 Pooled indications for surgical intervention

Indication	# patients
Unspecified adenocarcinoma	1,826
Periampullary adenocarcinoma[a]	914
Duodenal adenocarcinoma	13
Intraductal papillary mucinous neoplasm	270
Neuroendocrine neoplasm	216
Pancreatic metastasis	26
Periampullary tumor	186
Solid pseudopapillary tumor	18
Benign neoplasm	93
Pancreatitis	339
Premalignant	36
Other	335
Not specified	94

[a] Pancreatic, distal bile duct, ampulla, gall bladder

disparate outcomes associated with high BMI in patients undergoing different types of pancreatic resection and with different underlying pathologies.

Outcomes

Eleven studies included BMI as a covariate in multivariate analyses (Table 3),[15,16,21,23,24,83,85,86,88–90] but only PF emerged consistently as a clinically relevant complication of high BMI. Four studies found association between increasing BMI and PF in multivariate analyses, two with BMI >30, one with BMI >27, and one with BMI >25 (range OR 1.6–4.2).[23,83,88,89] Conversely, one study found no association between BMI >25 and PF and another between BMI >30, 25–30, or <25 and PF.[24,85] Of five studies utilizing the ISGPF definition of PF,[23,83,85,86,89] three found a multivariate association between PF and BMI, one found no association, and one did not include a multivariate analysis. Two studies found an association between clinically relevant PF and BMI >27 (OR 3.4) and >30 (OR 6.5).[83,88]

Pooled analyses of PF by pre-surgical BMI, including 485 patients from 11 studies, found significant association between increasing BMI and PF (Table 4).[16,17,21–24,83,85,86,88,89] Overall PF rates ranged from 4.7% to 31.0%. Eighty-five patients in seven studies had BMI <25, while 105 patients in those studies had BMI >25.[16,21–24,85,86,91] This corresponded to PF rates of 8.6% and 12.2% ($p=0.01$), respectively, both of which are slightly lower than the 25% PF rate established by Pratt and colleagues in 233 consecutive PDs.[92] In six studies evaluating BMI >30, 106 patients had BMI >30 and 283 patients had BMI <30, with PF rates of 22.9% and 15.8% ($p<0.001$), respectively.[16,17,21,24,83,89] Three studies utilizing the ISGPF definition of PF had pooled rates of 12.6% and 30.0% for BMI <25 and >25 ($p<0.001$).[23,85,86] Two studies using the ISGPF definition of PF for BMI >30 and <30 had pooled rates of 36.9% and 21.1% ($p<0.001$), respectively.[83,89] However, none provided information about differential outcomes for PF in high-BMI versus low-BMI patients.

Other BMI-associated outcomes were less consistent. One study found a multivariate association between BMI >27 and both any (OR 1.8) and major (OR 3.3) complications.[88] Two studies contradicted this, one finding no association between increasing BMI and any complication (OR 1.0) and the other finding no association between increasing BMI and major complications.[16,24] However, the definition of complication was rarely reported and probably varied across primary studies.

Ultimately, seven studies reported any complication as an outcome of high BMI with rates from 11.6% to 54.3%.[14–17,21,24] Three studies assessing BMI <25 for any complication had pooled rates for BMI <25 and >25 of 40.6% and 39.5%, respectively ($p=0.69$).[16,21,24] Seven studies assessing BMI >30 by any complication had pooled rates for BMI >30 and BMI <30 of 43.7% and 35.5% ($p=0.002$).[14–17,21,24,88] One study reported a significantly decreased multivariate risk of disease-specific mortality with increasing BMI (OR 0.74), but this corresponded to a significant univariate risk increase for any complication with increasing BMI.[21] With complication inconsistently defined, no distinct pattern was visible in these data.

Some less subjective outcomes found agreement across the primary study cohort, but the clinical relevance of any differences seen was questionable. A single study evaluated multivariate association between surgical site infection and BMI.[24] While there was slight association between BMI >30 and increased risk of infection over BMI <25 (OR 1.10, $p=0.03$), there was no association between BMI 30–25 and increased infection rates. Our pooled analysis yielded a similar result (22.9% and 15.8% for BMI >30 and <30 ($p<0.001$), 8.0% and 9.3% for BMI <25 and >25 ($p=0.40$)), with infection rates ranging from 4.6% to 14.0% in six studies reporting that outcome.[14,16,17,21,24,86] Two studies reporting serious infection as an outcome (bacteremia, pneumonia, intra-abdominal infection) found rates of 26.3% versus 51.9% ($p=0.01$) in patients with BMI <25 and >25, and one study found rates of 15.1% versus 15.8% ($p=1.00$) in patients with BMI <30 and >30.[17,22,86] However, Williams and colleagues attribute their equivalent rates of post-operative infections across BMI categories to their meticulous use of appropriate prophylactic antibiotics.[16] A prophylactic regimen not specifically targeted to patients with more widely distributed adipose tissue could reduce serum drug concentrations and, consequently, therapeutic efficacy. Differential prophylactic utilization across primary studies

Table 3 Morbidity predicted by BMI in multivariate analyses

First author, year	BMI stratification	Outcomes with BMI association	OR (range)	*p* value	Outcomes without BMI association	OR (range)	*p* value
Gaujoux, 2010	><25	PF	4.15 (1.49–11.57)	0.01			
Tsai, 2010	>30, 30–25, <25				Risk of disease-specific mortality[c]	0.74 (0.61–0.89)	0.002
Hashimoto, 2010	><30	PF	2.80 (1.06–5.03)	<0.001	DGE	NR	
		Clinically relevant PF	3.44 (1.63–7.23)	0.01			
Fleming, 2009	><35	Positive lymph nodes[a]	2.45 (1.58–93.6)	0.02	Decreased survival	0.49 (NR)	0.08
		Cancer recurrence[b]	1.64 (NR)	0.045			
Rosso, 2009	><25				PF	NR	
Akizuki, 2009	><25	Total dietary intake	7.42 (0.78–70.8)	NS	DGE	NR	
Williams, 2009	><30	Intraoperative blood loss[b]	2.00 (1.12–3.57)	0.02	Severe complications	NR	
		Operative time[b]	2.39 (1.30–4.39)	0.01			
		Length of stay[b]	1.90 (1.03–3.50)	0.04			
Weber, 2009	><27	Any complications	1.84 (1.00–3.27)	<0.05			
		Major complications	3.27 (1.16–9.60)	<0.05			
		PF	1.59 (.81–3.13)	NS			
		Major PF	6.49 (1.79–23.5)	<.01			
House, 2008	>30, 30–25, <25	Post-op wound infection[b]	1.10 (NR)	0.03	PF	1.05 (NR)	0.11
					Any complication	1.04 (NR)	0.07
Ferrone, 2008	><30	PF	NR	0.00			
Bentrem, 2005	><30	ICU admission	2.40 (NR)	0.03			

NS not significant, *NR* not reported

[a] Preoperative therapy associated with lymph node status; significance disappeared when analysis adjusted for tumor size; BMI >35 less likely to have received pre-operative therapy

[b] For BMI 25–30

[c] Remained significant when underweight subjects (BMI <18) analyzed separately

may have contributed to differences seen in infection rates across BMI categories.

Similarly, while Fleming and colleagues report multivariate association between both positive lymph node status (OR 2.45, $p=0.02$) and cancer recurrence (OR 2.00, $p=0.05$) in patients with BMI >30, they note that decreased use of pre-operative therapy in patients with BMI >35 in their study population significantly increased the likelihood of these outcomes ($p=0.02$).[15] Additionally, Su and colleagues note that the bacteria colonizing patients at time of surgery rarely corresponded to bacteria causing post-operative infection, suggesting that prophylactic antibiotics impacted outcomes in unpredictable ways.[22] Though these factors were unaccounted for in nearly all studies assessing BMI's impact on the incidence of both surgical site infection and tumor size, inconsistent or withheld pre-operative therapy may explain increased risk for these outcomes among obese and overweight patients.

Qualitative analyses were similarly inconclusive. Six studies evaluated estimated peri-surgical blood loss as an outcome, with medians from 400 to 1,000 ml (Table 5).[14–16,21,22,24] Weighted overall blood loss estimates (661.2 versus 801.3 ml for BMI <25 and >25 and 615.9 versus 790.1 ml for BMI <30 and >30) corresponded well with each other and reflected consistent increases with increasing BMI seen in all studies reporting this outcome. Similarly, in four studies reporting median operative time (range 363 to 439 min), all found increased operative time with increasing BMI.[14–16,21] Weighted operative times (363.8 versus 382.7 min for BMI <25 and >25 and 351.6 versus 368.7 min for BMI <30 and >30) were similarly disparate, though the small magnitude increases likely reflect increased care by the surgeon and may have little clinical relevance.

No clear relationship emerged between BMI and LOS. Five studies reported this outcome, medians ranged from 8 to 11 days, and means were predictably higher.[14–17,21] All median LOS were similar to those noted by Cameron and colleagues.[29] Weighted LOS for BMI <30 and >30 were 10.0 versus 9.8 days, respectively. However, disparities were within 1 day for all BMI categories in three of four studies, with Williams and colleagues noting a 1.5-day difference between BMI >30 and the other categories.[16] However, Williams and colleagues found 2× increased risk

Table 4 Pooled analyses of BMI-associated morbidity and surgical site infection

First author, year	Pancreatic fistula				Any complication				Surgical site infection			
Body mass index	<25 (%)	>25 (%)	<30 (%)	>30 (%)	<25 (%)	>25 (%)	<30 (%)	>30 (%)	<25 (%)	>25 (%)	<30 (%)	>30 (%)
Gaujoux, 2010	15.8	51.2										
Hashimoto, 2010			17.2	33.0								
Schrader, 2010												
Benns, 2009							50.4	67.7			3.8	7.4
Fleming, 2009							4.9	13.4				
Rosso, 2009	5.1	17.3										
Akizuki, 2009	16.4	13.3							6.0	6.7		
Menge, 2009												
Weber, 2009												
Noun, 2008			15.1	36.8							11.0	15.8
Ferrone, 2008			25.5	40.6								
Bentrem, 2005												
Barry, 2003												
Tsai, 2010	2.8	6.8	3.9	9.4	35.5	40.3	36.7	44.3	6.3	9.6	7.3	11.3
Su, 2010	21.4	16.7										
Williams, 2009	3.9	5.8	4.6	6.1	42.7	55.5	38.4	47.4	5.8	3.7	4.6	4.6
House, 2008	23.4	13.3	15.0	17.1	66.2	30.5	37.1	42.1	22.1	11.8	12.1	21.1
Pooled incidence:	8.6	12.2	15.8	22.9	40.6	39.5	35.5	43.7	8.0	9.3	6.5	11.6

for delayed gastric emptying (DGE) among patients undergoing classic PD (3% versus 7%). Additionally, they note a procedural transition from classic PD to PPPD, a substantial increase in surgical volume during the study period, and a significantly increased use of biliary stents in patients with BMI >30 (p=0.001).[16] Disparate resection procedure utilization and temporal imbalances in BMI distribution could explain the small difference seen in pooled LOS.

With conflicting results, two other studies reported DGE as an outcome (42% versus 53% for BMI <25 and >25, 18% versus 11% for BMI <30 and >30).[17,86] Both studies had small sample sizes (n=92 and n=85) and neither reported univariate significance in this association. Similarly, two studies offered multivariate analyses concluding no association between BMI and DGE, though BMI was not quantified with number of patients experiencing this outcome in one of the two.[83,86] Two studies reported decreased glucose control following pancreatectomy, one noting a significant increase in diabetes among patients with higher BMI (p=0.03) and the other a loss in glucose control (r=0.41, p=0.01).[84,87] However, average BMI for developing diabetes versus not developing diabetes was 24.1 versus 21.9; as the majority of patients in each category are within normal BMI range, the clinical importance of this finding is questionable. Additionally, as neither study included a multivariate analysis of these outcomes, confounding of the data is likely.

Soft pancreatic consistency is widely touted as predictive of PF following pancreatectomy, and five studies addressed this outcome in relation to BMI, though no surgically useful patterns emerged.[17,21,23,24,85] Gaujoux and colleagues found that BMI >25 was predictive of both fatty pancreas and absence of pancreatic fibrosis.[23] These factors were all individually predictive of PF. Age was an independent predictor of fatty infiltration (p=0.03), but BMI was

Table 5 Weighted estimates for continuous variable outcomes

Outcome	<25	>25	<30	>30
Median operative time (min)	364	383	352[e]	369
Median length of stay (days)	–[a]		10	9.8
Mean length of stay (days)			13.1	15.4
Median blood loss (ml)	661	801	616	790
Mean tumor size (cm)	–[b]		3.1	3.2
Lymph nodes harvested (N)	–[c]		17.2	18.1
Any positive lymph nodes (%)	–[d]		66.2	60.9

[a] One study each found no difference in mean or median length of stay for BMI ><25[16,21]

[b] One study found equivalent tumor size in all categories (3), but found statistical significance in ranges[21]

[c] One study found an increase from 17–19 in normal, overweight, and obese patients[21]

[d] One study found identical rates (80%) in all categories. Neoaduvant therapy not administered per institutional policy[21]

[e] One study reported mean as slightly higher than median[14]

significantly associated with soft pancreatic remnants (p=0.04). However, soft consistency most highly correlated with the absence of pancreatic fibrosis (p<0.0001), which Rosso and colleagues confirm (p<0.0001).[85] Three other studies confirm that increasing BMI is associated with increased fat infiltration, two of the pancreas and one in the retroperitoneal space.[17,24,85] However, Rosso and colleagues note that soft pancreatic parenchyma was not associated with increased fatty infiltration (p=0.17). Conversely, Tsai and colleagues report that BMI was not associated with soft pancreatic tissue (p=0.23), though they did not evaluate the study population's level of parenchymal fibrosis.[21]

Comprehensive survival analysis was impossible due to incomplete reporting, though no increased risk with high BMI was evident. As noted above, a single study found multivariate association between decreased risk of disease-specific mortality and increasing BMI (OR 0.74, p<0.01), as well as a significant increase in 5-year survival in patients with BMI >30 and 30–25 (22% and 22% versus 15%, p=0.02).[21] Fleming and colleagues did not find significant association between BMI >35 and decreased survival in a multivariate analysis (p=0.08).[15] Similarly, Benns and colleagues found no significant association between either disease-free or overall survival in patients with BMI ><30 (p=0.50 and p=0.46, respectively).[14] Tsai and colleagues noted that survival was similar among 32 underweight patients and the remaining normal patient cohort (n=398), though this was the only evaluation of outcomes in underweight patients across the study cohort.[21]

Peri-operative mortality was extremely low (range 0.0% to 4.4%) in the four studies reporting it, and none found association between increasing BMI and the incidence of mortality.[17,21,88,91] In multivariate analysis, Bentrem and colleagues found that patients undergoing PD with BMI >30 were more likely to be admitted to the ICU (p=0.003), that BMI >30 was associated with delayed ICU admission from the surgical ward, and that ICU admission was associated with overall decreased survival (p<0.0001), but they did not identify an association between BMI and decreased survival.[90]

Discussion

We have shown that most differential outcomes between high-BMI and low-BMI cohorts undergoing pancreatic resection are far lower in magnitude and far better in prognosis than might be expected. Though several morbidity indices were slightly higher in overweight and obese patients, they rarely reached clinical significance. Higher rates overall of pancreatic fistula in patients with higher BMI are worrisome, but several studies finding no association between BMI and pancreatic fistula rates may offer valuable insight into best-practices scenarios. Consistent use of prophylactic therapeutics, increased peri-surgical care, and better post-surgical management in obese and morbidly obese patients maybe valuable tools for reducing small, but clinically significant disparities in outcomes between patients with different BMIs in this cohort.

Unlike previous studies in this genre, by pooling BMI categories from 17 studies, we were able to establish a large enough cohort to perform a stringent and satisfactory statistical review and meta-analysis for a variety of endpoints previously found to be associated with high BMI. Though outcomes including incidence of PF, surgical site infection, blood loss, and operative time emerged as consequences of increasing BMI in patients undergoing surgical pancreatic resection, outcomes including post-operative LOS, tumor size, harvested lymph nodes, delayed gastric emptying, peri-operative mortality, and decreased overall survival exhibited irregular association or no association with high BMI. These clinically relevant conclusions suggest that BMI alone should not preclude surgical pancreatic intervention.

Importantly, we elucidated the fact that obesity is not a universal predictor of poor outcomes in surgical patients. By rigorously excluding specious outcomes associated with historically significant risk factors like high BMI, therapy may be precisely targeted to individual patients. Because meticulous consideration of all possible risk factors by physicians is a noted contributor to the perfect storm of healthcare over-utilization in the USA,[93] identifying high BMI as a null predictor in patients undergoing pancreatic resection may greatly reduce systemic costs. More importantly, identifying a lack of association between high BMI and many poor surgical outcomes may illuminate imperative post-operative therapeutic considerations, systemically resulting in improved surgical outcomes.

Our study has several limitations grounded within primary study designs and reporting variations. Most significantly, we cannot exclude the possibility of selection bias from individual studies. Each of the 17 studies included in our analysis was a retrospective assessment of prospectively collected data. No randomization was used, and all patients included in our final analysis were selected by individual surgeons to undergo pancreatic resection. However, as we did not exclude studies based on study design, this represents the entire body of literature on this topic. Though targeted pre-operative prophylaxis, peri- and post-operative care may improve outcomes in patients with high BMI, there may be inherent physiological differences, including increased peri-pancreatic fat leading to higher rates of PF that could not be elucidated due to biased inclusion criteria within primary studies. Unfortunately, the direction this bias may take is not predictable from these data.

Additionally, there was no consistency across indicators for surgical intervention within the primary cohort, nor were outcomes reported universally or homogenously. Comorbidities, including diabetes rates, were similarly inconsistently reported, suggesting the possibility of confounding. However, diverse surgical indications and comorbidities are commonplace in this body of literature. To mitigate potential disparities in patient characteristics within individual primary studies, we restricted our pooled analyses to outcomes reported in at least three studies. Additionally, because we could not illuminate the internal architecture of reported continuous variables, we limited statistical testing to variables with discrete expression and conclude significance only with 95% confidence. Unfortunately, incomplete reporting prevented us from identifying any differences in outcomes associated with high BMI in different interventions, but we do not suspect that BMI would substantially increase a patient's risk for complications in one high-risk surgical pancreatic intervention over another.

To mitigate the possibility of serious consequences associated with varied reporting across primary studies because PF is one of the most common and consequential outcomes of pancreatectomy in all patients,[92] we performed a sensitivity analysis using the cohort of studies adopting the ISGPF definition of PF. We found results similar in direction and magnitude to those of our complete study population, suggesting some cohesion among primary study outcome measurements despite potential measurement inconsistencies. Finally, though our study population included more than 4,000 patients and at least 592 with a BMI >30, limited reporting prevented us from creating a truly sound metric for predicting outcomes associated with specific BMI levels. Further research is needed to ensure therapy meets the prophylactic needs of patients, including underweight and class II and III obese surgical patients who we were unable to assess in this review and meta-analysis.

Conclusion

This review identified a clear association between BMI and PF incidence. Given the consistent lack of PF definitions and BMI gradations in these studies, the clinical severity of high-BMI-associated PF could not be ascertained reliably, however. BMI was not found to be associated with LOS, hospital mortality, disease-free survival, or overall survival in a combined analysis of the studies recording these outcomes. Thus, though it appears BMI increases the operative complexity of pancreatic resection, most associated increases in peri- and post-operative morbidity can potentially be mitigated with surgical care and an aggressive patient management schedule. Further research is necessary to explore the impact of BMI on specific pancreatic surgical interventions, as well as the impact of BMI on long-term outcomes following pancreatic resection.

Competing Interests We declare that we have no competing interests.

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CASE REPORT

The Novel Triad of Dorsal Agenesis of the Pancreas with Concurrent Pancreatic Ductal Adenocarcinoma and Nonalcoholic Chronic Calcific Pancreatitis: A Case Series and Review of the Literature

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Abstract
Introduction Dorsal agenesis of the pancreas (DAP) is a rare congenital anomaly, with only 44 cases having been reported in the English literature since 1966.
Materials and Methods A retrospective review of our IRB-approved pancreatic surgery database was performed from November 2005 to November 2010 searching for cases of DAP.
Discussion Disorders in the retinoic acid (*Raldh*) and hedgehog (*Hh*) signaling pathways, which appear to play a role in the development of DAP, have been implicated in other diseases of the pancreas such as pancreatic ductal adenocarcinoma (PDA) and nonalcoholic chronic calcific pancreatitis (NCCP).
Conclusion In this report, we describe three cases of DAP in the setting of PDA, two of which include the third component of NCCP. We provide a discussion of the clinical features of this novel triad and address the molecular pathways that relate to these respective diseases.

Keywords Dorsal agenesis of the pancreas · Pancreatic ductal adenocarcinoma · Nonalcoholic chronic calcific pancreatitis

Introduction

Congenital abnormalities of the pancreas are quite rare. The most common congenital abnormality is pancreas divisum, which has a reported incidence of 1.3–5.8%.[1] Other pancreatic congenital abnormalities are significantly less common, such as annular pancreas, ectopic pancreas, and dorsal agenesis of the pancreas (DAP). Forty-four cases of DAP have been reported in the English literature since 1966. The most common presenting symptom of this condition is abdominal pain, with nearly 68% of patients presenting with vague abdominal discomfort. Additionally, one third of patients are found to have diabetes mellitus at the time of presentation.[2] The diagnosis of DAP is often made by computed tomography (CT) or magnetic resonance imaging (MRI) during the evaluation for abdominal pain, though a significant number of cases have also been identified incidentally during the workup for an unrelated problem. DAP is typically suspected when no pancreatic tissue is seen to the left of the superior mesenteric vessels (Fig. 1). The exact etiology of DAP remains unclear; however, the retinoic acid (*Raldh*) and hedgehog (*Hh*) signaling pathways have been shown to play a role in its pathogenesis. These signaling pathways have also been implicated in the

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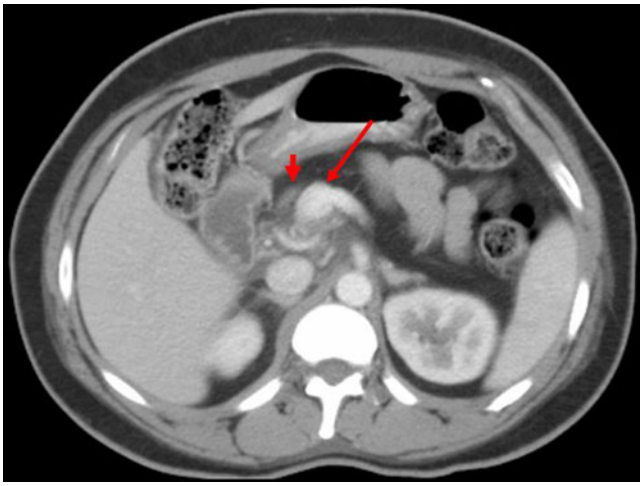

Fig. 1 CT showing agenesis of the dorsal pancreas. Pancreatic tissue does not extend to the left of the superior mesenteric vein. No additional pancreatic tissue to the patient's left of this arrowhead was seen on additional axial CT images. *Arrow head* marks the most distal aspect of pancreatic tissue, *thin arrow* superior mesenteric vein

pathogenesis of pancreatic ductal adenocarcinoma (PDA) and nonalcoholic chronic calcific pancreatitis (NCCP).

While DAP remains a rare disease, NCCP and PDA occur more commonly. NCCP occurs primarily in tropical countries, with a prevalence of 10–15 per 100,000 people. NCCP is characterized by irreversible destruction of the pancreatic parenchyma leading to pancreatic endocrine and exocrine insufficiency.[3] Patients may classically present with a constellation of symptoms including abdominal pain, steatorrhea, and diabetes mellitus. NCCP carries an increased risk for the development of PDA. Chari et al. followed 185 patients for 4.5 years to assess the risk for the development of PDA.[4] During the follow-up period, 25% of patients died from PDA. Although PDA is only the 10th most common cancer in the USA, it remains the fourth leading cause of cancer death. Less than 20% of patients with PDA are candidates for curative surgical resection at the time of diagnosis, and the overall 5-year survival rate is a dismal 4%.

Developing a better understanding of the processes that link these seemingly disparate diseases may offer insight into their early detection and clinical management. In this report, we describe three patients with PDA in the setting of DAP, two of whom had the novel triad of DAP, PDA, and NCCP. Herein, we also place these three patients in the context of the world literature of patients with DAP and PDA.

Materials and Methods

In this study, we performed a retrospective review of the prospectively acquired, IRB-approved pancreatic surgery database in the Department of Surgery of Thomas Jefferson University from 28 November 2005 to 31 November 2010, searching for cases of DAP. We reviewed patient demographics, preoperative studies, operative variables, adjuvant therapy, and survival. We identified three cases of DAP out of 870 patients who underwent surgical resection for pancreatic diseases during the study period. A literature review was conducted searching the English language literature for all cases of DAP with associated pancreatic neoplasms from 1966 to the present. We used the search terms "dorsal agenesis of the pancreas," "pancreatic ductal adenocarcinoma," and "nonalcoholic chronic calcific pancreatitis."

Clinical Material and Results

Case 1

A 37-year-old woman originally from the Indian subcontinent, with a history of insulin dependent diabetes, presented with a history of vague epigastric abdominal pain of 2 months duration. A CT scan (Fig. 1) was performed that showed a 1-cm cystic mass in the uncinate process of the pancreas, associated with peripancreatic fat stranding, consistent with acute pancreatitis. Dorsal agenesis of the pancreas was noted on CT scan as well. Endoscopic retrograde cholangiopancreatography (ERCP) demonstrated a dilated and truncated main pancreatic duct with intraductal calculi (Fig. 2). A pancreatic ductal sphincterotomy was performed with stone extraction and placement of a

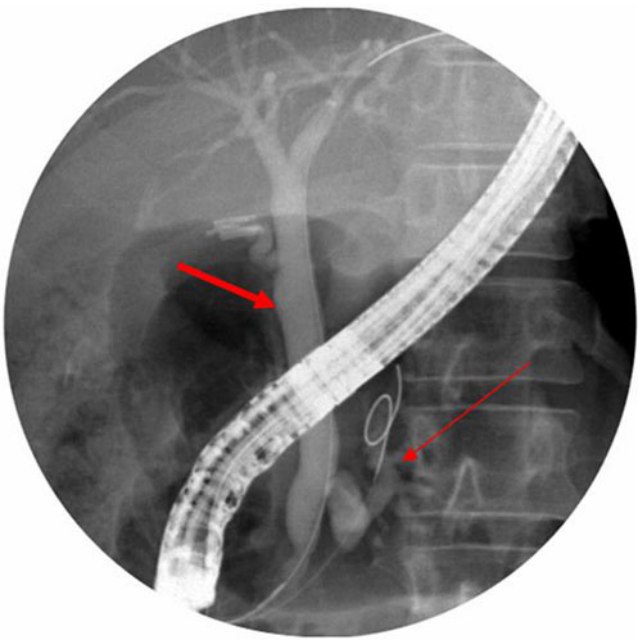

Fig. 2 ERCP demonstrating a dilated and truncated main pancreatic duct with intraductal calculi. Patient's status postcholecystectomy with a somewhat dilated extrahepatic biliary tree. *Thin arrow* dilated and truncated main pancreatic duct with intraductal calculi. *Thick arrow* dilated common bile duct

biliary endoprosthesis. An endoscopic ultrasound (EUS) procedure revealed a 2.2×2.1 cm hypoechoic mass in the uncinate process, with calcifications and an absent pancreatic body and tail. Fine-needle aspiration (FNA) of the mass at the time of EUS revealed atypical cells suspicious for adenocarcinoma. The patient's CA19-9 level was normal at 3 U/mL, but the carcinoembryonic antigen (CEA) level was elevated at 8.2 ng/mL. On operative exploration, a firm mass was identified in the uncinate process of the pancreas, and there was no evidence of metastatic disease. The pancreatic body and tail appeared to be replaced with fat (Fig. 3). A pylorus-preserving resection of the pancreatic head and uncinate process was performed with an end-to-side hepaticojejunostomy and a downstream retrocolic duodenojejunostomy. Pathology revealed a 3-cm moderately differentiated ductal adenocarcinoma involving the uncinate process of the pancreas with negative surgical margins of resection. One of 21 specimen lymph nodes was positive for metastatic disease leading to an American Joint Commission on Cancer (AJCC) pathologic stage of IIb (T3N1M0). The pancreatic neck margin showed benign adipose tissue with no evidence of pancreatic glandular tissue, consistent with an aplastic body and tail. The patient's postoperative course was uneventful, and she was discharged to home on postoperative day 7, on parental insulin therapy and exogenous pancreatic enzyme supplementation. She received adjuvant treatment with gemcitabine-based chemotherapy but died of recurrent malignant disease 17 months postresection.

Case 2

A 59-year-old woman, also originally from the Indian subcontinent and with a history of insulin dependent diabetes mellitus, presented with a 4-month history of abdominal pain and weight loss. The patient had previously undergone a CT scan 2 years earlier which showed a small cystic lesion in the pancreatic head with surrounding calcifications. Repeat CT scan demonstrated a 4×4 cm mass in the head of the pancreas with multiple calcifications causing biliary dilatation and duodenal narrowing, as well as agenesis of the body and tail of the pancreas. Tumor marker analysis revealed a mildly elevated CA19-9 level of 62 U/mL and a normal CEA level of 1.8 ng/mL. The patient underwent operative exploration which revealed a firm mass occupying the entire head and uncinate process of the pancreas, with the pancreatic body and tail replaced by fat. A pylorus-preserving resection of the pancreatic head and uncinate process was performed with a hepaticojejunostomy and duodenojejunostomy. Pathology revealed an 8.5×6 cm moderately differentiated ductal adenocarcinoma of the pancreas. The surgical margins of resection were negative and 1/13 specimen lymph nodes harbored metastatic disease leading to an AJCC pathologic stage of IIb (T3N1M0). No pancreatic tissue was present at the pancreatic neck margin. The patient recovered from surgery uneventfully and was discharged to home on postoperative day 6, on parental insulin therapy and exogenous pancreatic enzyme supplementation. She received adjuvant gemcitabine-based chemotherapy and radiation and remained alive 38 months postresection.

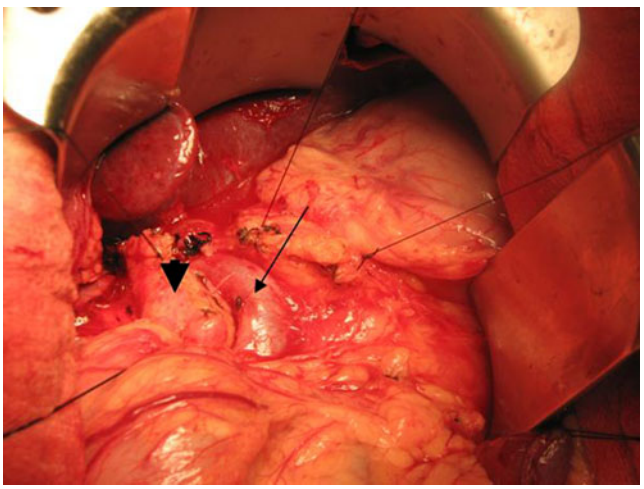

Fig. 3 Intraoperative image of DAP with underlying superior mesenteric vein. There is an absence of pancreatic tissue to the left of the superior mesenteric vein. *Arrow head* marks the most distal aspect of pancreatic tissue. *Thin arrow* superior mesenteric vein

Case 3

A 68-year-old Caucasian man was found to have abnormal liver function tests on routine laboratory analysis performed by his primary care physician. His total and direct bilirubin (2.8/1.8 mg/dL), alkaline phosphatase (461 IU/L), aspartate aminotransferase (227 IU/L), and alanine aminotransferase (418 IU/L) were all elevated. Tumor marker analysis revealed an elevated CA19-9 level of 439 U/mL and a mildly elevated CEA level of 3.1 ng/mL. CT scan revealed a dilated extrahepatic biliary tree with a calcified cystic lesion within the head of the pancreas extending into the uncinate process. The patient underwent an ERCP with sphincterotomy and placement of a biliary endoprosthesis. Upon surgical exploration, the mass in the head of the pancreas was identified, and there was noted to be an absence of pancreatic tissue to the patient's left of the superior mesenteric vessels. As in the two previous cases, a pylorus-preserving resection of the pancreatic head and uncinate process was performed with a hepaticojejunostomy and duodenojejunostomy. Pathology revealed a 2.1-cm moderately differentiated ductal adenocarcinoma of the pancreas with negative surgical margins of resection. None of the 17 specimen lymph nodes harvested harbored metastatic disease, leading to an AJCC pathologic stage of Ib (T2N0M0). The patient was discharged to home on postop-

erative day 10 and underwent adjuvant treatment with gemcitabine-based chemotherapy and radiation. He remains alive 52 months postresection.

Table 1 depicts all of the previously published cases of DAP and associated PDA or NCCP, including our own three cases. Two cases of prior PDA in the setting of DAP have been documented in the English literature, the first having been reported by Matsusue in 1984.[5,6] Since that time, various neoplastic lesions have also been reported including leiomyosarcoma, endocrine tumors, and intraductal papillary mucinous neoplasms in association with DAP. Most cases of concurrent pancreatic neoplasm and DAP in the literature, including our own, underwent a resection of all remaining pancreatic tissue. Balakrishnan reported a case of DAP in the setting of NCCP.[7]

Discussion

This study documents three patients with DAP and PDA (Fig. 4), two of whom had the novel triad of DAP, PDA, and NCCP. Although they never underwent genetic testing, our two patients with NCCP fit the criteria for this diagnosis, in that, they were from an area endemic for NCCP and had chronic abdominal pain, diabetes, and early-onset PDA. The patient who had an ERCP had the presence of large intraductal calculi. All three of our patients underwent successful operative treatment with pylorus-preserving resection of the pancreatic head and uncinate process with construction via a hepaticojejunostomy and duodenojejunostomy. A short description of pancreatic organogenesis and a discussion of the molecular and genetic mechanisms that play a role in the development of these three separate but related entities follow.

The pancreas begins its development in the fourth week of gestation, starting as two separate buds arising from the primordial foregut (Fig. 5). The ventral pancreatic bud develops from the hepatic diverticulum arising from the duodenum, and the dorsal pancreatic bud arises separately from the dorsal aspect of the duodenum.[8] As the duodenum rotates to the right assuming the C shape, the ventral pancreatic bud continues its rotation before fusing with the dorsal pancreatic bud.[9] The dorsal pancreatic bud forms the body and tail of the pancreas and also gives rise to the accessory pancreatic duct which empties into the minor duodenal papilla of Santorini.[8] Any failure in the development of the dorsal bud therefore leads to an absence of a functional pancreatic body, tail, and accessory pancreatic duct. While complete agenesis of the pancreas is a rare congenital disorder that is associated with impaired intrauterine growth and is often fatal,[10] DAP results in only partial impairment of pancreatic function and may often go undetected. The specific etiology of DAP remains unclear,

Table 1 Current series and literature review of DAP associated with PDA or NCCP

Author	Age/Sex	Presentation	Imaging	Operation	Diagnosis	Pathology	Adjuvant Therapy	Survival
Case 1	37/F	Abdominal pain, hyperglycemia	CT, ERCP	Pylorus preserving resection of the pancreatic head and uncinate process	DAP, PDA, NCCP	T3N1M0	Gemcitabine	Dead (17 months)
Case 2	59/F	Weight loss	CT	Pylorus preserving resection of the pancreatic head and uncinate process	DAP, PDA, NCCP	T3N1M0	Radiation, gemcitabine	Alive (38 months)
Case 3	68/M	Elevated LFTs	CT, ERCP	Pylorus preserving resection of the pancreatic head and uncinate process	DAP, PDA	T2N0M0	Radiation, gemcitabine	Alive (52 months)
Ulusan (2006)	72/M	Abdominal pain, jaundice, hyperglycemia	Unknown	Hepaticojejunostomy, cholecystectomy	DAP, PDA	–	–	–
Balakrishnan (2006)	28 F	Abdominal pain, weight loss, hyperglycemia	CT, ERCP	–	DAP, NCCP	–	–	–
Matsusue (1984)	53/F	Abdominal pain, weight loss, hyperglycemia	CT	Total pancreatectomy	DAP, PDA	–	–	–

LFTs liver function tests, *CT* computed tomography, *ERCP* endoscopic retrograde cholangiopancreatography, *DAP* dorsal agenesis of the pancreas, *PDA* pancreatic ductal adenocarcinoma, *NCCP* nonalcoholic chronic calcific pancreatitis

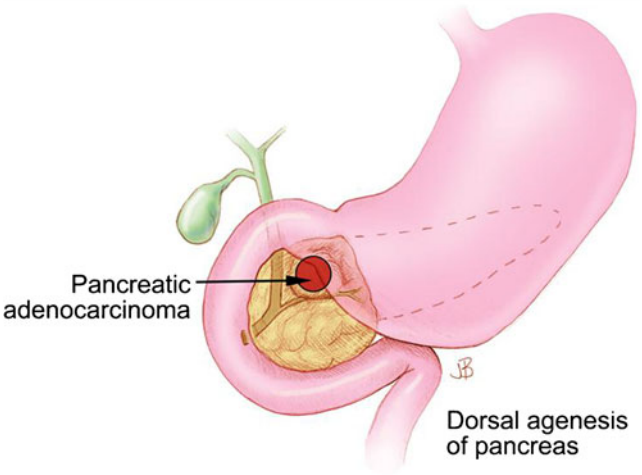

Fig. 4 Illustration of DAP. There is an absence of pancreatic tissue to the left of the neck of the gland. In this case, a cancer of the head of the pancreas is also depicted

but developmental and genetic causes have been implicated in its occurrence.

In mouse models, certain genetic alterations or knockouts are linked to the failure of dorsal pancreatic development. Reduced expression of homeobox gene *Hlxb9* in mice causes failure of dorsal pancreatic development,[11] whereas normal retinoic acid signaling through the *Raldh2* gene leads to the development of the dorsal pancreatic bud. Martin et al. showed that mice with the *Raldh2* knockout fail to develop a dorsal pancreatic bud.[12] Also, early expression of either sonic (*Shh*) or indian (*Ihh*) hedgehog, two members of the hedgehog (*Hh*) cell signaling family, can suppress pancreas development.[13] In addition to DAP, alterations in the *Raldh2* and *Hh* signaling pathways are also implicated in the development of NCCP and PDA, respectively.

Singh et al. demonstrated that increased retinoic acid concentrations inhibited the growth of pancreatic cancer cells in vitro.[14] Conversely, one could hypothesize that perhaps a defect in the retinoic acid signaling pathway may lead to an increased risk of developing pancreatic cancer in patients who have DAP. Despite belonging to different molecular pathways, the *Hh* and *Raldh2* genes represent some of the complementary molecular "on" and "off" mechanisms that are involved in pancreatic development and function. Importantly, when these molecular mechanisms are abnormal, they can lead to aberrant pancreatic phenotypes.

The development of NCCP and other forms of chronic pancreatitis has been associated with a number of genetic alterations. Mutations in SPINK1, a serine protease inhibitor that suppresses intrapancreatic trypsin activity, have been associated with the development of NCCP.[15] Bhatia et al. examined 66 unrelated patients in northern India with a history of NCCP and compared them to 92 healthy control patients from the same region.[15] They found that 50% of the patients with NCCP had a heterozygous NS34S

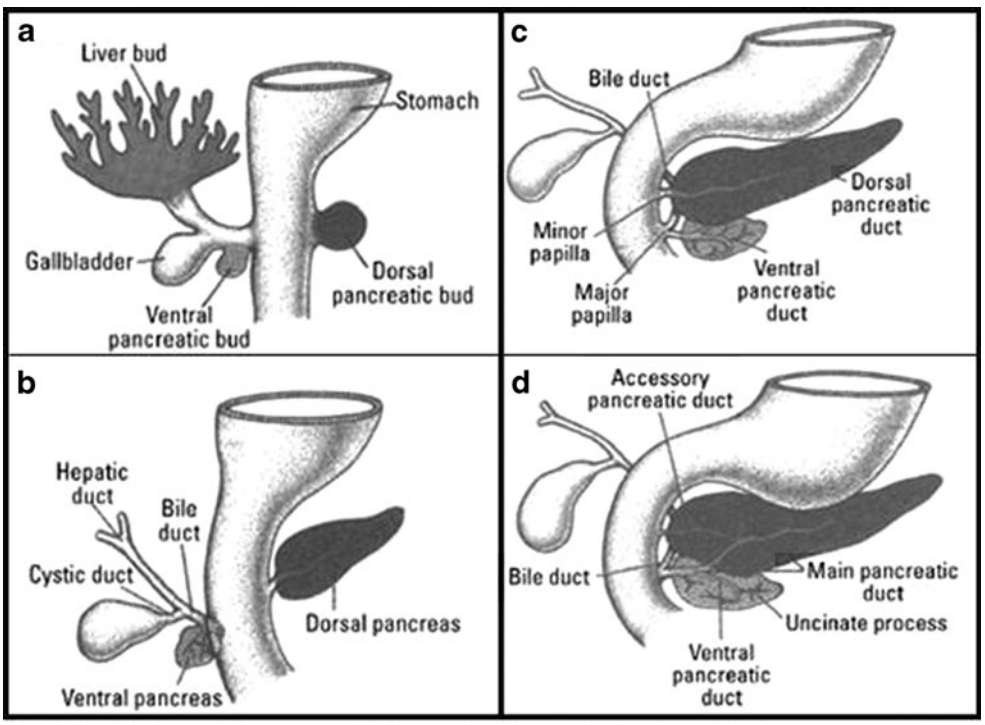

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Fig. 5 Embryology of pancreatic development. **a** 30 days after fertilization. The ventral and dorsal buds develop on opposite sides of the primordial foregut. **b** The ventral pancreatic bud develops from the hepatic diverticulum arising from the duodenum, and the dorsal pancreatic bud arises separately from the dorsal aspect of the duodenum. **c** As the duodenum rotates to the right assuming the C shape, the ventral pancreatic bud continues its rotation before fusing with the dorsal pancreatic bud. **d** The dorsal pancreatic bud forms the body and tail of the pancreas and also gives rise to the accessory pancreatic duct which empties into the minor duodenal papilla of Santorini. Permission to use this figure was obtained from Hugh A. Tilson, Ph.D., Editor in Chief of Environmental Health Sciences on March 01, 2011

SPINK1 mutation, and 14% had a homozygous mutation. Only 2.2% of the healthy control patients had the mutation. *Ihh* and its receptors patched (*Ptc*) and smoothened (*Smo*) are overexpressed in tissues from patients with chronic pancreatitis but weakly expressed in normal ductal cells.[13] Increased *Hh* signaling marks the progression of normal to fibrotic tissue in chronic pancreatitis.[7] These suggest that perhaps the development of these diseases, DAP, PDA, and NCCP, may be rooted in certain common pathways of pancreatic development and function

All three patients in the current study underwent pylorus-preserving resection of the pancreatic head and uncinate process. Parsaik et al. reported the metabolic consequences following total pancreatectomy.[16] One hundred forty-one patients were retrospectively studied, and nearly 89% of patients were on a complex insulin regimen with a mean daily insulin requirement of 35 U. Seventy-nine percent of these patients reported episodic hypoglycemia, which demonstrates the difficulty in balancing postoperative glucose management. Twenty-eight percent of patients reported chronic diarrhea from lack of pancreatic exocrine function postoperatively. It is vital that the patient be educated regarding the metabolic consequences of undergoing a total pancreatectomy prior to surgical intervention.

Difficulty can arise in trying to distinguish congenital agenesis of the pancreas from acquired fatty replacement of the pancreas. Small to large amounts of fat replacement can normally occur in obese and elderly patients.[16] Total fat replacement of the dorsal pancreas has been reported but is quite rare. A proposed mechanism for the replacement of normal pancreatic endocrine and exocrine tissue with adipose tissue is chronic obstruction of the pancreatic duct. Blockage can arise from calculi or tumors. Pancreatic duct obstruction by a stone can cause fibrosis in canine models.[17] Ligation of the pancreatic duct in mice causes complete intralobular replacement.[18] Proposed criteria for confirming true congenital DAP as opposed to fatty replacement include the complete absence of exocrine features, acinar cells, and islets of Langerhans on histologic examination.[19] Suggested radiologic criteria to diagnose dorsal agenesis of the pancreas have included the lack of a dorsal and transverse pancreatic artery on angiography (rarely performed) and the absence of an accessory pancreatic duct on ERP.[19] However, the most common means of diagnosis is by cross-sectional imaging (CT or MRI/MRCP).

Conclusion

In summary, this review documents three patients with DAP and PDA, two of whom had the novel triad of DAP, PDA, and NCCP. All three patients underwent successful operative treatment via pylorus-preserving resection of the pancreatic head and uncinate process. This series raises a number of interesting questions. Is there a potential common molecular pathway in the development of dorsal agenesis of the pancreas and the development of PDA involving the retinoic acid signaling pathway? Did the malignant transformation in two of our patients have a greater association with DAP or NCCP? Patients with known DAP may benefit from early screening given the apparent increased risk of developing pancreatic cancer.

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MULTIMEDIA ARTICLE

Video: Totally Laparoscopic Left Lateral Segmentectomy for Hepatic Malignancies: A Modified Technique

Ajay V. Maker · Wisam Jamal · Brice Gayet

Published online: 1 June 2011

Abstract We present our series of over 30 totally laparoscopic left hepatic lobectomies (hepatic segments II/III) performed only for malignancy. The short- and long-term results support this technique as safe and efficient. This video will illustrate the pertinent issues regarding trocar placement, intrahepatic anatomy, and the technical maneuvers necessary to perform the modified approach using totally laparoscopic techniques.

Keywords Minimally invasive liver surgery · Laparoscopic liver resection · Left lateral segmentectomy · Left hepatic lobectomy · Laparoscopy · Metastasectomy · Segment II · Segment III

Background

We have been modifying our technique of laparoscopic hepatectomy over the last 16 years. This video demonstrates the relevant technical maneuvers necessary to perform our current modified approach to a totally laparoscopic left hepatic lobectomy **(***segments II/III***)**.

Methods

The principal steps of this procedure include laparoscopic ultrasound of the left hepatic vein and portal pedicles to segments II and III, intraparenchymal dissection and division of the segment II and III pedicles, dissection and division of the left hepatic vein, division of the coronary and triangular ligaments, and removal of the specimen. No porta hepatis dissection or pringle maneuver was performed.

Results

A total of 31 laparoscopic left lateral segmentectomies have been performed successfully at our institution since 1998 for the diagnosis of cancer. Fifteen were performed for colorectal liver metastases, as in the patient in the video, nine for hepatocellular carcinoma, three for diagnosis, one for cholangiocarcinoma, and one each for a renal cell and neuroendocrine metastasis. We have been using the current modified technique since 2006 on the last 20 patients. Our short- and long-term results have been similar to those for our open historical control subjects. The median blood loss was 10 mL, and the median operating time was 115 min. No patients required blood transfusions intraoperatively or postoperatively, and no mortalities occurred. There was one major complication of a biliary leak unable to be managed by interventional drain placement that required a return to the operating room. There were three minor complications of a pleural effusion and two postoperative fevers that resolved with conservative management.

Conclusion

This modified minimally invasive technique of hepatic resection is a very safe and efficient approach to a left lateral segmentectomy in selected patients with primary or metastatic disease of the liver.

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GI IMAGE

Utility of Multidetector-Row Computed Tomography and Ultrasonography for Preoperative Planning in a Patient with a History of a Right Gastroepiploic Artery CABG undergoing a Laparoscopic Cholecystectomy

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Abstract
Introduction Laparoscopic cholecystectomy has become the standard procedure for acute cholecystitis.
Methods This procedure, however, is challenging to perform in patients who have had coronary artery bypass grafting (CABG) using the right gastroepiploic artery (RGEA).
Results We completed a laparoscopic cholecystectomy for acute cholecystitis without intraoperative or postoperative cardiac complications in a patient with a history of an RGEA CABG.
Conclusions A critical factor for avoiding disruption to the graft was preoperatively delineating the vascular anatomy of the RGEA graft with a multidetector-row computed tomography (CT) with 3D-CT angiography and ultrasonography.

Keywords Laparoscopic cholecystectomy · Coronary artery bypass grafting (CABG) · Right gastroepiploic artery · Cholecystitis

Introduction

Laparoscopic cholecystectomy has become the first line of therapy for acute cholecystitis given its benefits of decreased pain, shorter hospital stay, and lower health-care cost compared to laparotomy.[1–3] The aging population has led to an increase in the number of surgical procedures performed; however, comorbidities in this population are considerable concern. A significant number of patients have a history of coronary artery bypass grafting (CABG) using the right gastroepiploic artery (RGEA), which presents a significant challenge for any abdominal surgery, particularly laparoscopy.[4] We report the successful completion of a laparoscopic cholecystectomy in a patient with a prior history of an RGEA CABG. Multidetector-row computed tomography (CT) and ultrasonography were used preoperatively to delineate the anatomy of the graft such that the trocars could be safely placed.

Case Report

The patient, a 78-year-old man, was referred to our hospital following the acute onset of upper abdominal pain in the right hypochondrium associated with vomiting and fever. His medical history was notable for three-vessel CABG surgery 12 years previously, involving bypass between the right coronal artery and the RGEA. The laboratory findings were notable for a neutrophil leukocytosis (white blood count of $18.9\times10^3/\mu L$), elevated C-reactive protein of 23.64 mg/dl, and elevated transaminases. A multidetector-row CT scan demonstrated gallbladder wall thickening, cholelithiasis, and choledocholithiasis. 3D-CT angiography demonstrated patency of the RGEA graft located in the upper middle abdomen (Fig. 1). An endoscopic sphincterotomy was then performed and the stones were extracted from the common bile duct using a Dormia basket. An

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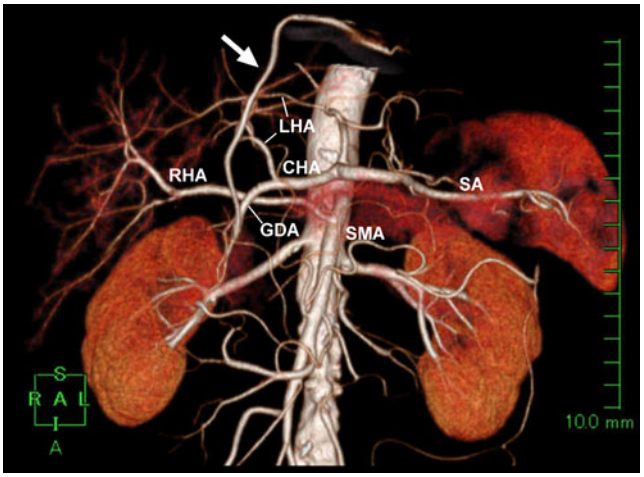

Fig. 1 Abdominal 3D CT angiography showing the RGEA graft to the right coronary artery (*arrow*). The graft is patent and located along the left side of the falciform ligament. *CHA* common hepatic artery, *RHA* right hepatic artery, *LHA* left hepatic artery, *GDA* gastroduodenal artery, *SMA* superior mesenteric artery, *SA* splenic artery

endoscopic nasogallbladder drainage (ENGBD) procedure was performed by cannulating the gallbladder and placing a 7-Fr. tube. Gallbladder aspiration yielded 55 ml of purulent bile. A second attempt to remove the remaining common bile duct stones was performed 3 days after the first ENGBD procedure. The patient underwent a laparoscopic cholecystectomy 2 days after the second ENGBD procedure. A preoperative transabdominal ultrasonography confirmed the location of the RGEA graft along the left side of the falciform ligament and demonstrated good blood flow to the coronary artery (Fig. 2). The first trocar was placed using an open technique through a transumbilical incision. The incision was directed away from the adhesions involving the RGEA graft detected by preoperative imaging studies. The remaining three trocars were then placed as follows: one 5-mm trocar was placed just below the xiphoid process for liver retraction, and two trocars were placed in the upper middle and the right upper quadrant as working ports (Fig. 3). The intraoperative insufflation pressure was maintained below 8 mmHg. No intraoperative ischemic cardiac events occurred. The histological findings confirmed acute suppurative cholecystitis and the bile culture was positive for *Klebsiella oxytoca* and *Morganella morganii* bacteria. The patient went on to have an uncomplicated postoperative course and was discharged home on postoperative day 8 following a complete evaluation of his cardiac function.

Discussion

Laparoscopic cholecystectomy, compared with open cholecystectomy, decreases postoperative pain, allows for a faster return to normal activities, is associated with a lower incidence of postoperative complications, and requires a shorter hospital stay.[1–3] Laparoscopic cholecystectomy is now widely accepted as the treatment of choice for most symptomatic gallbladder diseases,[1] particularly acute cholecystitis.[2,3] The aging of the population has led to an increase in the rates of surgical procedures such as the cholecystectomy, and these patients frequently have a history of cardiac disease. When coronary artery disease is treated with an RGEA bypass, abdominal surgical procedures become highly complicated.[4]

CABG using the RGEA was first described in 1987,[5] and has since gained acceptance as a reliable conduit for

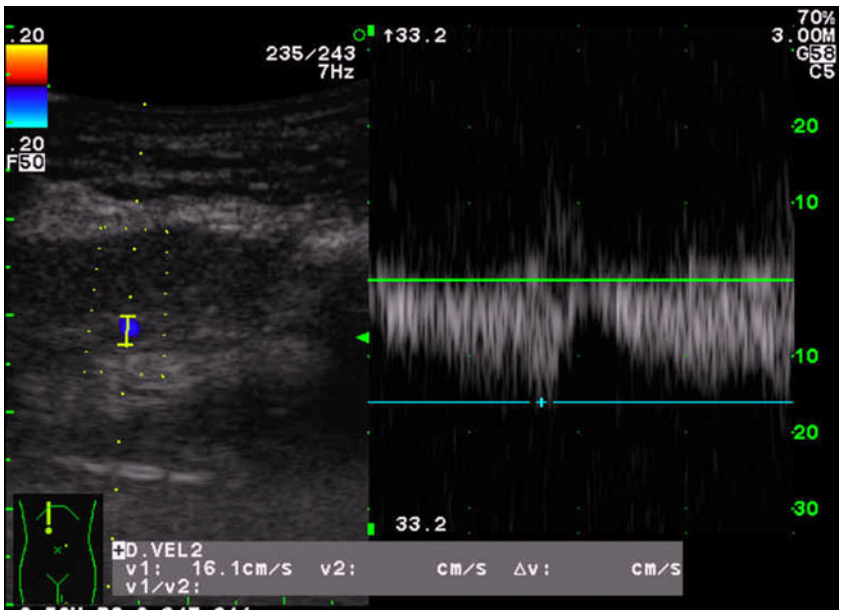

Fig. 2 Transabdominal ultrasonography of the epigastrium in sagittal plane scan showing good blood flow through the RGEA graft to the right coronary artery

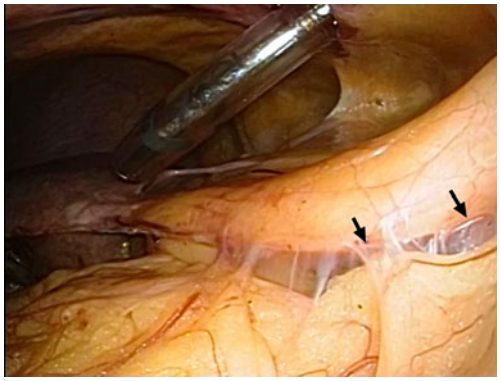

Fig. 3 Intraoperative photograph. A trocar inserted just below the xiphoid process was placed at the right side of the falciform ligament. The RGEA graft was located at the left side of the falciform ligament, and this was not completely visualized due to adhesions (*arrows*)

CABG of the right coronary artery system. A search of electronic databases (MEDLINE, PubMed, and Ovid) using the keywords "laparoscopic cholecystectomy, coronary artery bypass grafting, and cholecystitis" identified a single case report in the English literature.[6] This report describes an elective laparoscopic cholecystectomy in a patient with cholecystolithiasis and a history of RGEA CABG. The authors recommend placing an additional laparoscope and monitoring the RGEA pedicle to avoid unexpected injury of the vessel. Rather than using an additional scope, we employed detailed preoperative imaging studies to plan our operative approach. We utilized a multidetector-row CT with angiography to delineate the three-dimensional configuration of the RGEA graft. An abdominal ultrasonography also provided important information regarding graft location and patency.

With respect to the surgery itself, the first trocar was placed with an open technique. While all methods of trocar insertion carry risk of organ or vessel injury,[7] Hasson's open technique of insertion is safer in the setting of prior abdominal surgery.[8] Identification of the RGEA graft either preoperatively or early in the procedure is critical to prevent any interruption of coronary blood flow and fatal ischemia. In the present case, as the RGEA graft was identified on the left side of the falciform ligament by ultrasonography, all trocars were inserted on the right side, well away from the graft. Finally, to further prevent extension, kinking, or occlusion of the graft, a low intraperitoneal pressure was maintained throughout the procedure.

Conclusion

We report a case of a patient with a history of an RGEA CABG who developed acute cholecystitis and was successfully treated with a laparoscopic cholecystectomy. Critical to the success of this procedure was the preoperative three-dimensional delineation of the graft with multidetector-row CT scan and ultrasonography.

Disclosures Nothing to disclose.

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GI IMAGE

Voluminous Intussusception Involving the Whole Midgut in a Teenager: A Unique Differentiation from Abdominal Cocoon

Lulu Li · Shucheng Zhang

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Abstract
Introduction Intussusception is one of the most frequent complications of Peutz–Jeghers syndrome which has been well described before.
Case Report Herein, a voluminous intussusception with almost the whole midgut involved in a 13-year-old girl is reported, the radiological presentation was so similar with that of abdominal cocoon that she was initially misdiagnosed accordingly. After the laparotomy confirmation, a careful review was taken on the patient's CT images, a few clues for differentiation were found but proved obscure and an overriding premise for identification is just to take the diagnosis of intussusception into consideration.
Conclusion Based mainly on our experience, clinicians should be aware of voluminous intussusception as a unique differentiation of abdominal cocoon and consider it in each case of voluminous abdominal mass.

Keywords Intussusception · Peutz–Jeghers syndrome · Abdominal cocoon · Radiology

Case Report

A 13-year-old girl was admitted in hospital for intermittent abdominal pain for 2 weeks with a sudden exacerbation for 1 day. The abdominal pain was predominantly at the left epigastric region, as well as an intermittent vomiting of gastric content, less stools without blood and abdominal distension were also obtained. Upon physical examination, hyperpigmented macules were seen on her lips. The abdomen was soft; a voluminous and tender mass was palpated on the upper abdomen without rebound tenderness and muscle tension. The girl had no hernias, and no ascites or organomegaly. All laboratory blood analyses, including blood routine, liver and kidney function, were within normal limits. Both ultrasonography and computed tomography (CT) scan of the abdomen revealed a giant mass filling almost the whole upper abdomen, in description, the boundary was a thick, fibrotic, cocoon-like membrane which can be enhanced on the contrast-enhanced CT scanning, and the embedded were the intestinal loops with a little fluid surrounded. Additionally, in some scans, the intestinal dilation was also observed (Fig. 1a, b, c and d). From the radiological findings, the primary diagnosis of abdominal cocoon and incomplete intestinal obstruction was then established; a conservative treatment with antibiotics, fluid infusion, pain-killer drugs and protective agent of gastric mucosa was carried out. Whereas, during the conservative treatment, aggravation of intestinal obstruciton was gradually observed, which were a series of severe signs indicating the progress of peritonitis and potential hazard of intestinal necrosis. As a result, an emergency laparotomy was carried out immediately.

On exploration via right musculus rectus abdominis, a giant cyst was found occupying almost all the abdominal

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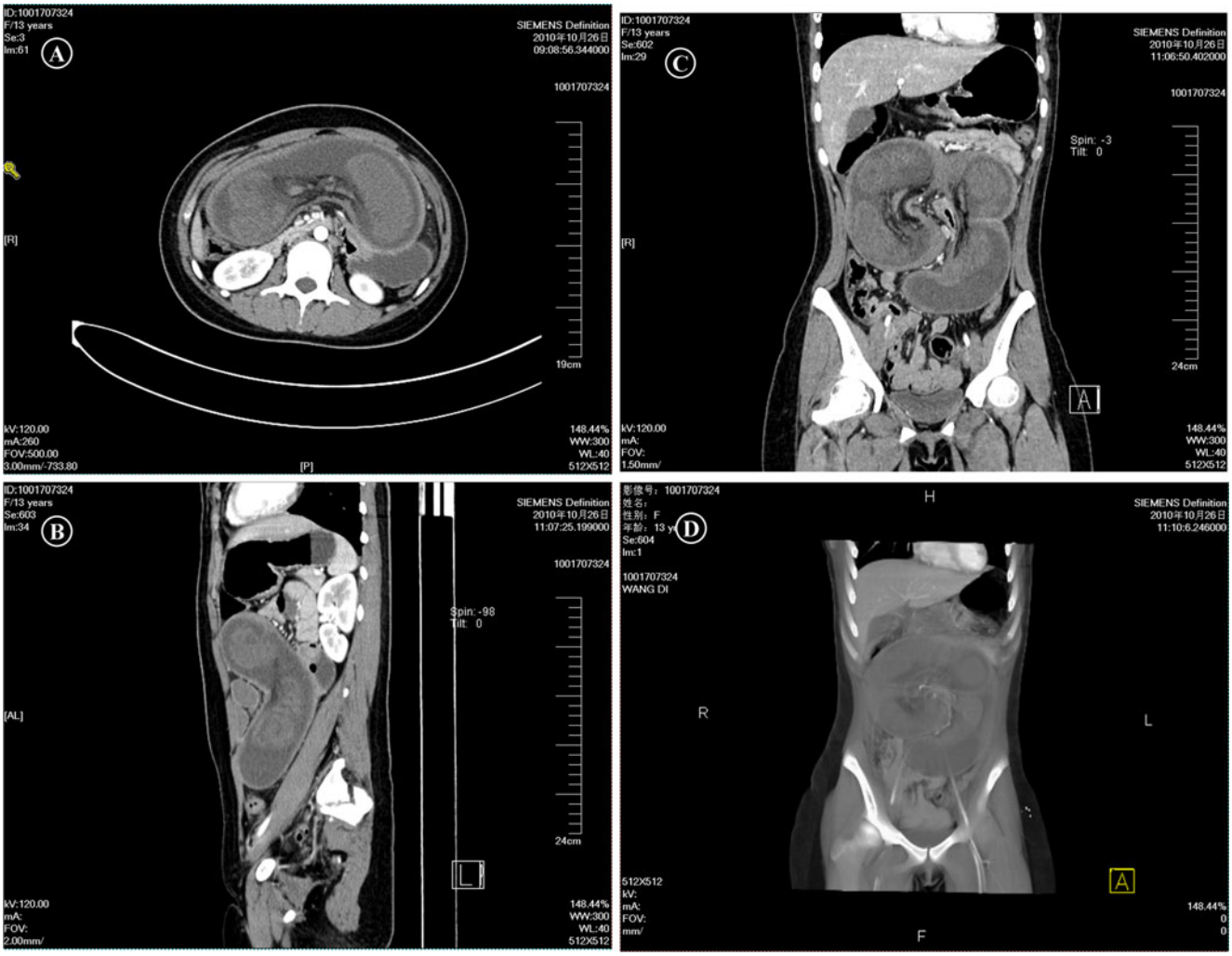

Fig. 1 Contrast-enhanced CT findings in the 13-year-old girl with voluminous intussusception: almost the whole upper abdomen was occupied by a voluminous abdominal cyst, the boundary was a thick, fibrotic, cocoon-like membrane which can be enhanced on the contrast-enhanced CT scanning; and the embedded was the intestinal loops with a little fluids surrounded, in some scans, the intestinal dilation was also observed. **a**, **b**, **c** was the transverse, sagittal and coronal section, respectively, **d** was the 3-D construction of CT images

cavity. After taking the mass out of the abdominal cavity, it turned out to be the extremely dilated intestine instead of soft-tissue sac (Fig. 2a). During further exploration, a voluminous intussusception involving the whole midgut, 1 m in length and 15 cm in diameter, was found 80 cm distal to the Treiz ligament, in addition, vast fluid was found around the intussuseptum (Fig. 2b). Based on the presence of the intestinal vigour, a simple manual reduction was immediately carried out without any resection. Correlating the hyperpigmented macules on her lips with the confirmed intussusception, the probability of Peutz–Jeghers syndrome had to be taken into consideration, initiating a further enteroscopy via a little incision on the intestinal wall for polyps within the intestine. As a result, three polyps at 50, 70 and 100 cm proximal to the ileocecal valve, respectively, were then confirmed (Fig. 2c), the definite diagnosis of voluminous intussusception involving the whole midgut secondary to Peutz–Jeghers syndrome was then established. A simple polypectomy and anastomosis was performed, respectively. The patient had an uneventful postoperative course and was discharged on the eighth postoperative day. After that, the girl received a regular monthly follow-up, at present, she recovered well and no complications were found.

Discussion

Intussusception, as one of the most frequent complications of Peutz–Jeghers syndrome, has been well described before.[1] Nevertheless, such giant and extensive intussusception as reported in this patient, either in size or in range, is still unusual. In spite of this extensive involvement, the abdominal pain, nausea and vomiting were mild, without

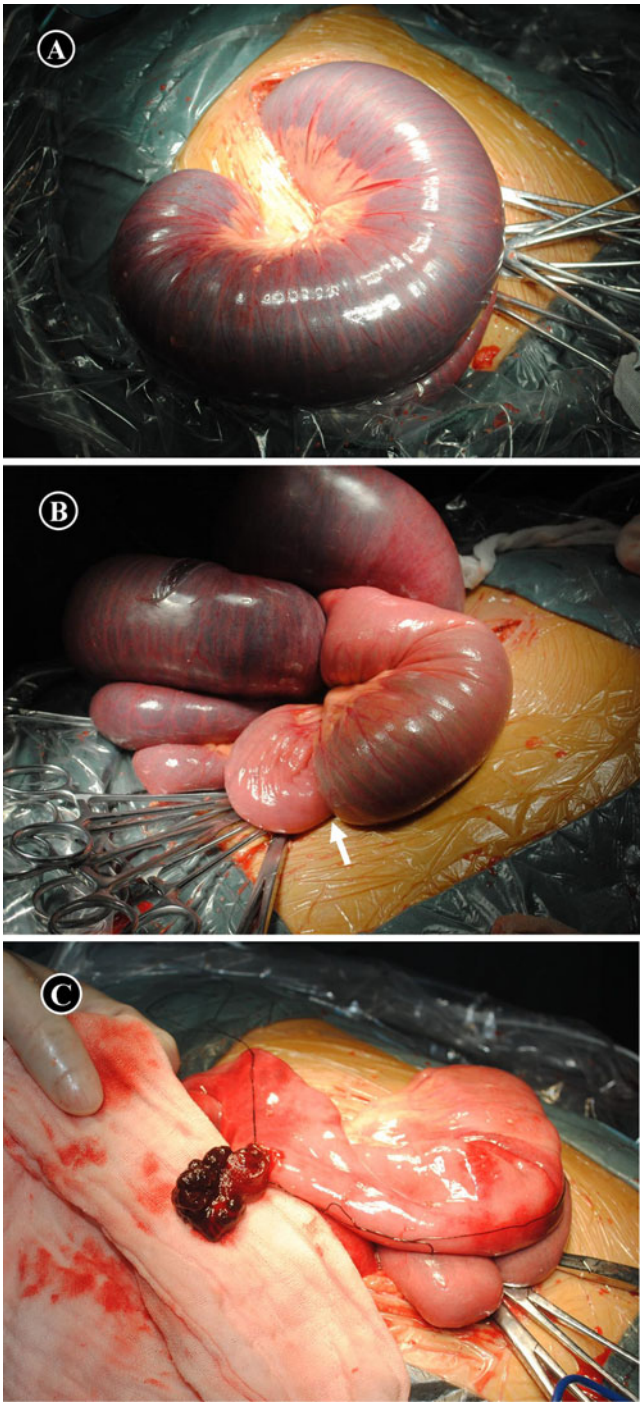

Fig. 2 Intraoperative findings in the 13-year-old girl with voluminous intussusception. **a** A giant cyst was found occupying almost all the abdominal cavity. After taking the mass out of the abdominal cavity, it proved the extremely dilated intestine instead of soft-tissue sac. **b** A voluminous intussusception was confirmed involving the whole midgut, 1 m in length and 15 cm in diameter, and the intussusceptum was surrounded by vast fluid; the *arrow* was just the joint point. **c** Three polyps inside the intestine at 50, 70, and 100 cm proximal to the ileocecal valve were respectively confirmed by enteroscopy via a little incision on the intestinal wall

classic jelly-like bloody stool. A persistent and indolent advancement or a chronic formation of the intussusception without strangulation of the intestine might account for the less-pronounced symptoms.

In our case, the most interesting thing is the CT appearance; it was so similar with that of the abdominal cocoon that it was initially misdiagnosed as abdominal cocoon, accordingly. Not until the intussusception was confirmed in laparotomy, the hyperpigmented macules on her lips was paid attention and the probability of Peutz–Jeghers syndrome was taken into consideration.

"Abdominal cocoon" is a rare cause of intestinal obstruction and primarily affects adolescent girls living in tropical and subtropical regions. Macroscopically, the condition is characterized by a thick, fibrotic, cocoon-like membrane, partially or totally encasing the small bowel. It was firstly described by Foo et al. in 1978,[2] after that, it has been well studied in both the clinical and basic researches. Currently accepted is that the abdominal CT scan can provide more accurate information on the diagnosis of abdominal cocoon and sometimes the degree of obstruction and the types of bowel loops involved.[3] It has been demonstrated that, on the CT scans, a conglomerate of several small-bowel loops (both jejunal and ileal) was seen in the centre of the abdomen, a thick enhancing membrane surrounded the bowel, forming a saclike structure or a cocoon; mild fluid was seen between these encapsulated bowel loops, some of which were closely apposed and probably adhered to each other.[4] Other features include mural thickening of intestinal loops, peritoneal thickening with enhancement, peritoneal or mural calcifications, and reactive adenopathy, described in another literature, were also found to be statistically correlated with the appearance of abdominal cocoon.[5]

The CT appearance in our case was extremely consistent with that of abdominal cocoon, just as shown on CT scan, the intestinal loops were surrounded by a soft-tissue membrane, within which amount of fluids was noted. In addition, the association of loculated fluids, small-bowel faeces sign, small-bowel obstruction which was extremely sensitive and specific in the development of abdominal cocoon was also noted. Therefore it seemed inevitable to make the diagnosis of abdominal cocoon. However, it has not turned to be the reality, if a careful review was taken on this patient's CT images, little clues for differentiation from abdominal cocoon could still be found. Firstly, through an overview of the CT images, it can be found that the outline of abdominal mass in our case was more like a large loop extending along the longitude of the intestines with central vacancy, while abdominal cocoon has a saclike appearance with intestines inside. Secondly, although on CT scan, the soft-tissue membrane seemed identical in intussusception and abdominal cocoon with and without enhancement, the

thickness of telescopic site in the intussuscipien was more significant, and presence of mucous folds within the mildly dilated intussuscipien is also not identifiable on abdominal cocoon. Lastly, for the reason of relatively ischemic condition in intussusception, it is less manifested on contrast-enhanced CT as compared to abdominal cocoon. These three features provide crucial information by which one can distinguish them. In sum, even though the radiological presentations between voluminous intussusception and abdominal cocoon are easy to be confused, a guarded differential diagnosis can still be established, and an overriding premise is just to take the counterpart into consideration.

In addition to the equivocal imaging signs mentioned above, the unremarkable clinical settings in our case, to some extent, also facilitated the misdiagnosis. There is similarity between the presentations of intestinal obstruction caused by intussusception and abdominal cocoon, namely: nonspecific symptoms of abdominal pain, vomiting and a palpable abdominal mass, all of which provided no valuable information for differentiation. Fortunately, in spite of the initial misdiagnosis and delayed treatment encountered, there was no fatal outcome brought in our case consequently. As long as there is an absence of intestinal strangulation, delayed management have not resulted in deleterious clinical outcome. After external reduction and selective polypectomy, the patient had a full recovery.

Conclusion

Limited and tentative conclusions are now possible; based mainly on our experience and that of others, clinicians should be aware of voluminous intussusception as a unique differentiation of abdominal cocoon, although encountered rarely in clinical settings, and consider it in each case of voluminous abdominal mass.

Conflicts of Interest There were no commercial or proprietary interests in any drug, device or equipment mentioned in the submitted article and institutional review board approval was received.

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LETTER TO THE EDITOR

Cholecystokinin Provocation HIDA test

Gareth Morris-Stiff · Gavin Falk · Laurel Kraynak · Steve Rosenblatt

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We thank you for the opportunity of responding to the letter by Dr. Zuckier regarding our paper.[1]

We would actually agree with Dr. Zuckier that there is extensive literature on this topic. Clearly though, we disagree that it is conclusive or can definitively guide clinical practice. There are numerous technical variations in dosing the provoking agents and outcome measures. Our data are not the first to suggest the value of symptom provocation in discriminating patients with and without acalculous biliary disease. Dr. Zuckier cites Smythe and colleagues as evidence against provocation testing; yet, their patients with positive CCK provocation showed that 80% experienced symptom relief with cholecystectomy.[2]

Improvements in the quality of gallbladder ultrasound over the course of the past decade, as well as greater access to a wider range of diagnostic tests to identify the etiology of epigastric and right upper quadrant discomfort, may explain why patients with typical gallbladder pain and a negative ultrasound are more likely to undergo a cholecystokinin dimethyl hepato imino diacetic acid (CCK-HIDA) scan. The overwhelming majority of our patient population were referred by gastroenterologists following a CCK-HIDA scan. In our study population, a CCK-HIDA reproducing symptoms despite a normal EF, was the only positive test out of the myriad performed.

We also agree with Dr. Zuckier that our patients represent a selected group, but this is typical of all clinical studies on this topic. In our judgment, it allows the clinician to also select the most appropriate patient for an operation and does not infer poor methodology. Indeed, we would like to reassure Dr. Zuckier that the individuals reported in this paper do not represent the whole of the population investigated for right upper quadrant pain, but rather a selected population whose histories were convincing for a biliary etiology in the absence of cholelithiasis whose further medical evaluation was otherwise negative. The armamentarium available to evaluate patients with abdominal pain is now much greater than in the day of Smythe et al., and these tests are crucial in the work-up and management of this confusing patient population. We thoroughly investigated our population, and only a small group of patients who were felt to have gallbladder symptoms were submitted to a CCK-HIDA scan.

We did acknowledge in the paper that our data did not allow for a complete statistical analysis. In regard to the extent of a scripted symptom inquiry, we asked our patients after the test whether or not their symptoms were recreated by injection of Sincalide. The subjects were asked in an open-ended manner ("did you notice anything when you had the test?"), as they were not warned in advance that they could potentially expect pain with the study. All patients did report pain and relayed it to be the same as the discomfort that had led them to initially seek medical attention. We did not try to grade the pain; we only reported that the pain was reproduced following injection of CCK. Similarly, during the patients' postoperative follow-up visits, they were asked for the first time if their pain had resolved after cholecystectomy. Indeed all subjects reported symptom resolution in the postoperative clinic. Each patient was also contacted by telephone at the time of the study to see if he or she experienced any symptom recurrence. This interview occurred 3–36 months after cholecystectomy. With the exception of one patient, all patients reported that they still remained pain free.

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In summary, our study was not designed to examine the accuracy of a CCK-HIDA ejection fraction in predicting symptom relief with cholecystectomy. We believe that our results show that a "normal" ejection fraction does not exclude biliary symptoms, which can be discerned by symptomatic response to CCK. Clearly, our findings are provocative and should inspire further prospective work.

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LETTER TO THE EDITOR

Re: Morris-Stiff G, Falk G, Kraynak L, et al.: The Cholecystokinin Provocation HIDA Test: Recreation of Symptoms Is Superior to Ejection Fraction in Predicting Medium-Term Outcomes. J Gastrointest Surg 2010

Lionel S. Zuckier

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While it is important from time to time to visit and even revisit the validity and utility of diagnostic examinations, the recent paper "The cholecystokinin Provocation HIDA Test: Recreation of Symptoms Is Superior to Ejection Fraction in Predicting Medium-Term Outcomes"[1] reports extraordinary findings which are not easily reconciled with published literature and clinical experience.

First of all, 41 of the 42 patients with right upper quadrant pain and normal ultrasound examinations became persistently pain free following cholecystectomy, suggesting a nearly 100% prevalence of biliary dyskinesia in this population. Either the group studied was highly preselected and skewed to disease, or that the methods and criteria of evaluation ("gold standard") were overly lax, which exaggerated the prevalence of disease in this cohort. Secondly, even if we accept that all 41 patients had biliary dyskinesia, it is equally remarkable that they all experienced "typical pain" following a 30-min Sincalide injection, reflecting a 100% sensitivity for this maneuver, which is in contradiction to a large body of prior clinical experience where infusion of Sincalide does not usually elicit pain, even in patients with documented disease.[2]

One has to wonder whether methodological issues are the cause of these anomalous findings. To wit, the paper is very short on details regarding the subjective measurement of pain. It is unclear when the patients were asked about the pain, as we are only told that they were interviewed in the outpatient clinic following the procedure. Was this done immediately following the procedure, or at the 2-week follow-up? Were any written instruments employed? Was there any attempt to grade the severity of the pain? It would seem that the most accurate assessment of symptoms during infusion of Sincalide would be made by directly observing and quizzing the patient during the actual infusion itself, and not by eliciting recollections at a follow-up interview. A similar lack of detail is noted with respect to the 2-week assessment and the subsequent final telephone follow-up.

Finally, even were these extraordinary findings validated, elementary epidemiologic understanding dictates that it is impossible to evaluate a diagnostic study in a population where the prevalence of disease is nearly 100%. In this group, a diagnostic exam which calls every patient positive would be deemed to perform admirably, while the true ability to discriminate normal from abnormal would remain completely untested. Were this test to be ported to a population with a more normally distributed prevalence of disease, it is very possible that the specificity of this highly sensitive examination would be abysmal, leading to a large number of false positive normal patients. Since the study group only included a solitary patient without disease (who in fact experienced pain during Sincalide infusion), there is no way to evaluate specificity.

It is an optimal time to revisit the validity and utility of the gallbladder ejection fraction as a marker of biliary dyskinesia in light of new standardized practice guidelines that have been recently promulgated by expert panels.[3,4] A prospective clinical trial utilizing these newly codified best-

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practice techniques, in combination with meticulous assessment of patient symptoms, including use of real-time survey instruments, should best be able to answer the questions raised by the authors of this study.

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