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2009 SSAT PLENARY PRESENTATION

Preoperative Gastric Acid Secretion and the Risk to Develop Barrett's Esophagus After Esophagectomy for Chagasic Achalasia

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Abstract
Introduction The aim of this study was to determine the contribution of preoperative gastric secretory and hormonal response, to the appearance of Barrett's esophagus in the esophageal stump following subtotal esophagectomy.
Methods Thirty-eight end-stage chagasic achalasia patients submitted to esophagectomy and cervical gastric pull-up were followed prospectively for a mean of 13.6±9.2 years. Gastric acid secretion, pepsinogen, and gastrin were measured preoperatively in 14 patients who have developed Barrett's esophagus (Group I), and the results were compared to 24 patients who did not develop Barrett's esophagus (Group II).
Results In the group (I), the mean basal and stimulated preoperative gastric acid secretion was significantly higher than in the group II (basal: 1.52 vs. 1.01, p=0.04; stimulated: 20.83 vs. 12.60, p=0.01). Basal and stimulated preoperative pepsinogen were also increased at the Group I compared to Group II (Basal=139.3 vs. 101.7, p=0.02; stimulated=186.0 vs. 156.5, p=0.07. There was no difference in preoperative gastrin between the two groups. Gastritis was present during endoscopy in 57.1% of the Group I, while it was detected in 16.6% of the Group II, p=0.014.
Conclusions Barrett's esophagus in the esophageal stump was associated to high preoperative levels of gastric acid secretion, serum pepsinogen, and also gastritis in the transposed stomach.

Keywords Chagasic achalasia · Barrett's esophagus · Cervical esophageal stump · Gastric acid secretion · Serum pepsinogen · Serum gastrin

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Introduction

In the era of globalization, Chagas' disease has also expanded its borders to the northern hemisphere. Economic adversity and political tribulations have increased migration from Chagas' endemic areas, and many of these patients will develop gastrointestinal symptoms. In the USA, more than seven million people from *Trypanosoma cruzi* endemic countries become legal residents between 1981 and 2005.[1–4] Nonetheless, Europe has also witnessed an increased risk, mainly due to the migration to Spain and Portugal. Consequently, US and European clinicians are

likely to see an increasing number of patients with suspected or confirmed chronic Chagas' disease.[5]

T. cruzi, the cause of Chagas' disease, leads to diverse stages of destruction of the intramural plexuses of the gastrointestinal tract. The clinical symptoms are usually limited to the esophagus and colon; however, the entire gastrointestinal tract plexuses are also partially affected.[6]

Chagasic achalasia is a well-defined manifestation of this disease and may be treated by surgical techniques targeting the esophagogastric junction (i.e., Heller myotomy and partial fundoplication) in patients with non-advanced form of chagasic achalasia.[7]

Esophagectomy and gastric pull-up with cervical anastomosis represents the main treatment for the advanced end-stage of this disease, with acceptable morbidity, mortality, and reasonable postoperative outcome for a benign disease.[7,8] However, previous study on postoperative outcome of chagasic patients who have undergone esophagectomy with gastric pull up showed some complications including bleeding severe gastritis, gastric ulcers in the transposed stomach, and also esophagitis, esophageal ulcers, and Barrett's epithelium in the esophageal cervical remnant.[9–14] The contributing factors to Barrett's esophagus development, in this situation, are not completely known.

Thus, the aim of this study was to determine the contribution of preoperative conditions, regarding gastric secretory and hormonal response, to the appearance of Barrett's esophagus in the esophageal stump, in patients who underwent subtotal esophagectomy with gastric pull-up for end-stage chagasic achalasia.

Patients and Methods

Thirty-eight consecutive patients were studied preoperatively and postoperatively, regarding gastric acid secretion (GAS), serum pepsinogen (SP), and serum gastrin (SG). These patients with end-stage chagasic achalasia (severe dysphagia, dilation greater than 7 cm, sigmoid esophagus, and esophageal atony, and complications of previous surgical therapies) were submitted to subtotal esophagectomy and gastric pull-up with pyloroplasty at the Digestive Surgery Division of the Gastroenterology Department of the University of Sao Paulo, School of Medicine. This patient population is part of a published series of 101 esophagectomy patients followed by our group.[13]

Twenty (52.6%) patients were male and 18 (47.4%) were female, with a mean age of 43.6±12.1 ranging from 19 to 65 years old. All were epidemiologically and serologically positive for Chagas' disease. Symptoms of dysphagia, regurgitation, weight loss, and heartburn were present in all patients. Diagnosis of advanced achalasia was confirmed by Barium X-ray, upper endoscopic examination and manometric studies. The patients were followed prospectively for a mean of 13.6±9.2 years (ranging from 2 to 25 years).

This study was approved by the Research and Ethics Committee of the University of São Paulo, School of Medicine, and patient writing consent was obtained from all patients.

Surgical Procedure

A transhiatal subtotal esophagectomy with gastric pull-up to the cervical region was performed. The mean length of the cervical esophageal stump was 5 cm. Anterior extramucosal pyloromyotomy was performed in all patients.

Clinical and Radiological Assessment

Before and after surgical treatment all patients were assessed by clinical, radiological, and endoscopic examinations. Postoperatively, clinical and endoscopic evaluation was performed every 1 to 2 years. The following clinical parameters were studied: dysphagia, regurgitation, heartburn, diarrhea, Dumping Syndrome, fasting gastric residues, and body mass index (BMI). Imaging control, X-Ray study of upper gastro-intestinal tract, and gastric emptying time was studied before and after surgical treatment.

Endoscopic Assessment

Upper gastrointestinal endoscopy and also multiple esophageal and gastric biopsies were performed in all patients every 2 years. The presence of esophagitis (Savary–Miller grades 1–4), metaplastic columnar mucosa and/or Barrett's esophagus was recorded, as well as the length of any esophageal columnar mucosa in the esophageal stump (Fig. 1). The presence of gastritis, or bile in the transposed stomach was also described.

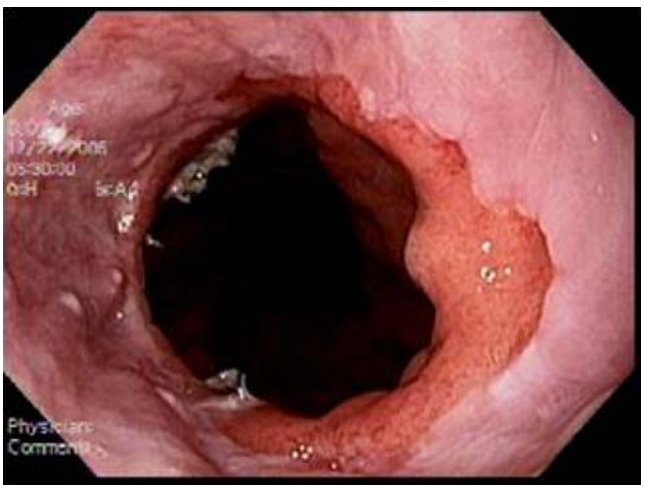

Figure 1 Endoscopic view of metaplastic columnar mucosa in the esophageal stump. Red salmon color mucosa may be seen.

Gastric Acid Secretion in Basal Condition and After Pentagastrin Stimulation

The functional studies were performed between 1 to 4 weeks before the operation to assure data reliability, and at fourth postoperative year.

Due to the achalasia, patients were kept on a liquid diet for 3 days, and the esophagus was cleaned with water using a 30-Fr oral tube on the previous evening, followed by a 12-h fasting period. GAS was evaluated as described elsewhere.[15] The GAS results were expressed in mEq of hydrochloric acid per hour (mEq/h), as basal secretion and peak acid output after pentagastrin stimulation.[15]

Basal and Betazole® Stimulated Serum Pepsinogen Levels

The first blood sample was collected for estimating basal serum pepsinogen. Then, 1.7 mg/kg of body weight of Betazole® was given intramuscularly. Antihistamine products (promethazine and hydrocortisone sodium succinate) were always available in the event of hypersensitivity to the injected drug. Basal blood samples of 8 ml were obtained from a peripheral vein at 60, 90, and 120 min after Betazole® stimulation. Serum pepsinogen was determined according to the method described by Uete et al.[16] and standardized by Saez-Alquezar et al.[17], in the Biochemistry Laboratory of the University Hospital of the University of São Paulo Medical School.

Basal Serum Gastrin

Eight milliliters of venous blood sample was collected to analyze basal serum gastrin in duplicate. Serum gastrin was estimated by radioimmunoassay using Gama Dab/125 Gastrin, Radioimmunoassay Kit, Clinical assays, Division of Travenal Laboratories Inc., Cambridge, Massachusetts, USA.

Pathologic Assessment

Collected biopsies were stained by H&E and the pathological results were reviewed by experienced pathologists. Barrett's epithelium in the esophageal remnant was defined by the presence of esophageal columnar epithelium with specialized intestinal metaplasia (Fig. 2).

Statistical Analysis

Nonparametric data were analyzed using Chi-Square and Fisher's exact test for contingency tables. For parametric data, an unpaired t test was used for comparison of differences between means in the groups. Statistical significance was endorsed by a p value of less than 0.05.

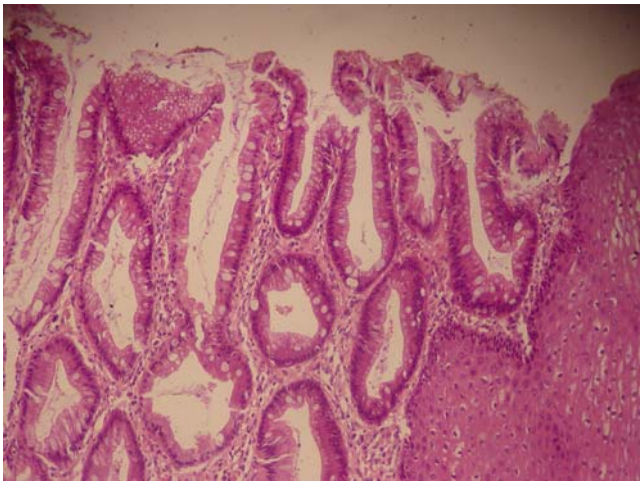

Figure 2 Histological appearance of Barrett's epithelium with the presence of specialized cells (intestinal metaplasia; ×200).

Results

Barrett's esophagus was always preceded by esophagitis and was first observed at 18 months postoperatively, with a mean of Barrett's appearance = 7.73 years (1.6–17 years).

Table 1 shows the relationship between clinical–pathologic parameters and the occurrence of Barrett's esophagus in the esophageal stump. There was a significant association between the development of Barrett's epithelium and the time interval post-esophagectomy. The presence of bile in the gastric conduit, and subjective symptoms of pyrosis and gastritis were also associated with the development of Barrett's. On the other hand, there was no statistically significant association between Barrett's and age, gender, and body mass index (Table 1).

Gastric Acid Secretion

Results of basal and pentagastrin stimulated GAS are presented in Table 1. In the Barrett's group (I), the mean basal and pentagastrin-stimulated gastric acid secretion was significantly higher than in the non-Barrett's group (basal: 1.52 vs. 1.01, $p=0.04$; stimulated: 20.83 vs. 12.60 mEq/h, $p=0.01$). At 4 years postoperative, basal and stimulated GAS were also increased in Group I compared to Group II (basal = 1.38 vs. 1.1, $p=0.28$; stimulated = 15.8 vs. 11.6, $p=0.08$) however, the differences were not statistically significant.

Barrett's development in the esophageal stump after esophagectomy and gastric pull occurred at the mean time of 7 years. Thus, when patients were stratified according to the time of development of Barrett's esophagus, early Barrett's appearance, i.e. less than 7 years postoperatively (eight cases, mean = 4.62±2.25 years), was associated with higher preoperative acidity when compared to those with

Table 1 Comparisons of Patients with and without Barrett's Esophagus in the Esophageal Stump Regarding Clinical and Laboratorial Parameters

	Barrett's (14 patients)	Non-Barrett's (24 patients)	*p* value
Mean age	44 (24–60) SE=2.98	43.12 (19–65) SE=2.57	0.83[a]
Gender	Male = 50%	Male = 45.83%	0.80[b]
BMI	22.97 (19.9–27.78) SE = 0.76	20.92 (19.9–25.3) SE = 0.96	0.77[a]
Symptoms			
Pyrosis	Present = 11/14 (78.6%)	Present = 9/24 (37.5%)	0.02[b]
Regurgitation	Present = 11/14 (78.6%)	Present = 12/24 (50%)	0.10[b]
Endoscopic findings			
Gastritis	Present = 8/14 (57.1%)	Present = 4/24 (16.7%)	0.01[c]
Bile	Present = 11/14 (78.6%)	Present = 11/24 (45.83%)	0.04[b]
Preoperative gastric acid secretion[d]			
Basal	1.52±1.01 mEq/l (0.15–3.9)	1.01±0.53 mEq/l (0.17–1.83)	0.04[a]
M.A.O.	20.83±9.45 mEq/l (7.7–37.4)	12.60±4.57 mEq/l (1.48–18.8)	0.01[a]
Postoperative gastric acid secretion (4 years)[d]			
Basal	1.38±0.94 mEq/l (0.1–3.3)	1.1±0.45 mEq/l (0.4–2.1)	0.28[a]
M.A.O.	15.8±9.20 mEq/l (4.4–32.2)	11.6±5.2 mEq/l (1.76–21.4)	0.08[a]
Preoperative pepsinogen levels			
Basal	139.3±60.9 (65–263 ug/ml)	101.7±37.0 (60–190 ug/ml)	0.02[a]
Stimulated	186.0±63.6 (99–308 ug/ml)	156.5±45.2 (105–248 ug/ml)	0.07[a]
Postoperative pepsinogen levels (1 year)			
Basal	96.6±37.4 (57 to 156 ug/ml)	71.7±21.5 (53–120 ug/ml)	0.008[a]
Stimulated	146.2 + 43.0(92–210 ug/ml)	110.5±32.0 (62 to 192 ug/ml)	0.004[a]
Preoperative basal gastrin			
Gastrin	80.44 (40 to 157 pg/ml)	82.75 (10.1 to 184.74 pg/ml)	0.89[a]

[a] Unpaired t-test
[b] Chi-Square
[c] Fisher's exact test
[d] mEq/l

late occurrence (six cases, mean = 11.76±3.26 years; Table 2). Therefore, higher preoperative GAS levels were also associated with early Barrett's development. (Table 2).

Serum Pepsinogen

Basal and Betazole® stimulated SP are shown in Table 1.

Preoperative basal and stimulated pepsinogen were increased in Group I compared to Group II (basal = 139.35 vs. 101.7, p=0.02; stimulated = 186.05 vs. 156.5, p=0.045).

At 1 year postoperative, basal and stimulated pepsinogen were also increased in Group I compared to Group II (basal = 96.6 vs. 71.7, p=0.008; stimulated = 146.2 vs. 110.5, p=0.004).

Basal Serum Gastrin

There was no difference in basal SG values in the Barrett's patients compared to the non-Barrett' subjects, p=0.89 (Table 1).

Discussion

Esophagectomy and gastric pull-up with cervical anastomosis is currently the procedure of choice in the management of end-stage chagasic achalasia.[7,8] However, this type of reconstruction after esophageal resection causes modifications in anatomy and physiology in the upper gastrointestinal tract.[19–21]

Table 2 Barrett's Esophagus in the Esophageal Remnant and its Relationship to Preoperative Gastric Acid Secretion and Timing of Occurrence

Preoperative gastric acid secretion	Early (8 cases; <7years)	Late (6 cases; >7years)	*p* value
Basal	1.56±1.13 mEq/h (0.2–3.9)	1.26±0.24 mEq/h (0.9–1.4)	0.3[a]
M.A.O.	23.16±8.92 mEq/h (12.3–37.4)	11.91±6.57 mEq/h (4.36–13.3)	0.02[a]

[a] Unpaired *t* test

Long-term follow-up of these patients has shown complications that, if adequately treated, did not influence the clinical outcome, but were associated with downstream clinical findings that raised some concerns: (1) esophagitis in the esophageal cervical stump rose over time and (2) diffuse gastritis and peptic ulcer of the transposed stomach, starting at 5 or more years of follow-up.[9] Based on these findings, our group initiated a prospective study that included quantifying preoperative gastric acid secretion, pepsinogen, and basal gastrin as well as clinical and endoscopic evaluation, biannually.

We have demonstrated that after esophagectomy with vagotomy, the GAS and SP levels decrease from 6 months to 1 year postoperative, and that there is a recovery of the GAS and SP levels to near preoperative values after 4 years of surgical treatment.[9,13]

In 1991 and 1992 we reported for the first time the presence of Barrett's epithelium developing in the esophageal stump of chagasic achalasia patients.[10,11] The occurrence of ectopic columnar metaplasia and Barrett's esophagus in the esophageal stump was not detected in the first 18 months of follow-up but did rise over time; 10.9% between 1 and 5 years; 29.5 between 5 to 10 years and 57.5% at 10 or more years of follow-up.[13]

Resection or disruption of natural antireflux mechanisms, esophagogastric direct anastomosis, pyloroplasty, impairment of gastric motility, recovery of acid secretion from gastric conduit, and impaired motility of esophageal remnant all have the potential to contribute to esophageal stump mucosal damage.[12–14,18]

We sought in this study to evaluate the contribution of preoperative conditions, regarding gastric secretory and hormonal response, to the appearance of Barrett's esophagus in the esophageal stump, in patients who underwent subtotal esophagectomy for end-stage chagasic achalasia.

In patients that underwent esophagectomy mainly for cancer, O' Riordan et al.[19] reported a 50% incidence of columnar metaplasia above the anastomosis in 48 post-esophagectomy patients, with a median follow-up of 26 months (range = 12–67 months). Specialized intestinal metaplasia was detected in 54% of those patients. According to the authors, the prevalence of columnar metaplasia did not relate to the magnitude of acid or bile reflux, to preoperative neo-adjuvant therapies, or to the original tumor histology. However, the duration of the reflux was the most significant parameter, with increasing prevalence over time. The authors concluded that the duration of acid and bile reflux, rather than the volume of reflux, underlies the development of metaplasia.[19]

In the present study, clinical alterations including the presence of gastritis and bile in the gastric conduit noted during endoscopic surveillance, and intense pyrosis were all associated with Barrett's appearance. Unfortunately, we did not have the equipment to measure the presence of bile in locus at that time. It is well known that bile salts injure both the gastric and the esophageal mucosa and their harmful effects are strengthened by the action of gastric secretion.[9,22,23]

In the present investigation, chagasic gastric acid secretion, serum pepsinogen, and basal serum gastrin were analyzed in patients who had developed Barrett's esophagus in the esophageal stump and compared to those who did not develop Barrett's epithelium. In the Barrett's group, the preoperative mean basal and pentagastrin-stimulated GAS was significantly higher than in the non-Barrett's group. Postoperative GAS measured at 4 years was also increased in the Barrett's group, however, statistically marginally significant. These results may be confounded by the presence of duodenogastric reflux due to pyloromyotomy.

Our data demonstrated that GAS and pepsinogen production can be stimulated despite chagasic involvement of the intramural stomach plexuses. This evidence implies that the majority of parietal and chief cells, in chagasic patients, maintain the functional capacity in basal condition and after stimulation.[5] Barrett's group serum pepsinogen values were higher compared to the non-Barrett's group. Other authors have found an association between GAS and serum pepsinogen; however, the scattering of individual values was such that serum pepsinogen could not be used as an index of gastric acid secretion in clinical practice.[24]

In the present research, basal serum gastrin showed a significantly higher basal value in both groups, nonetheless serum gastrin did not influence the appearance of Barrett's metaplasia. A possible explanation for this finding might be the gastric hypoacidity observed in Chagas' disease patients,[5,25,26] acting as a continuous stimuli to the parietal cells. Other mechanisms are postulated to explain the basal hypergastrinemia in Chagas' disease patients: (1) hypersensitivity of the "G" cells due to the autonomic denervation and/or (2) increased production of extra-gastric gastrin under these circumstances.[25–28]

Therefore, the present study enabled us to evaluate clinical, exocrine, and endocrine aspects of the behavior of gastric secretion and their relationship to the Barrett's esophagus in the esophageal remnant. We were able to demonstrate the association between high gastric acid secretion and high serum pepsinogen levels with earlier development of Barrett's esophagus in the esophageal stump. Additionally, the presence of gastritis in the transposed stomach, probably due to exposure to duodenogastric reflux, may also indicate higher risk for developing Barrett in the esophageal stump.

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Dr. Ulysses Ribeiro, Jr, Presenter (University of Sao Paulo, Sao Paulo, Brazil)

Discussant

Dr. Marco Patti (University of Chicago): Congratulations on a very nice presentation and a very interesting study. Clearly your group has a unique experience in the treatment of esophageal achalasia.

I have three questions for you.

As you mention in the manuscript, most of these patients had failed every other modality of treatment, including dilatation or Heller myotomy, before undergoing esophagectomy. How many of these patients had esophagitis or Barrett's esophagus before undergoing esophagectomy?

You showed that there is an increase in the acid gastric secretion and an increased level of pepsinogen. However, based on the endoscopy that is not very reliable, you assume that bile reflux plays a predominant role. Have you decided to assess bile reflux in a more objective way such as by the Bilitec or by pH/impedance monitoring?

Finally, assuming that duodeno gastric esophageal reflux plays a major role in the development of the cervical metaplasia, have consider some form of bile diversion in your reconstruction?

Closing discussant

Dr. Ulysses Ribeiro, Jr: Thank you, Dr. Patti, for your questions.

We have not seen any Barrett's before the surgery in these patients.

The patients did not have important gastroesophageal reflux before surgery because the esophageal junction was functionally closed due to achalasia, with a very high degree of dysphagia. So, dilatation and or myotomy have failled in these patients.

I know that the endoscopic view of the bile is not so reliable, but we didn't have bilitec R to do the measurements. Thus, we have included the bile endoscopic view to evaluate the duodenogastric reflux.

Considering bile diversion, we are thinking about it. But, postoperatively Roux en Y bile diversion would be a difficult and risky operation, with high complication rates.

So, that's a way to go, but I'm not sure if we are going to do it because of the referred complications.

Discussant

Dr. Steve Demeester (USC, Los Angeles): Just a quick question. We know that the severity of reflux after gastric pullup is related to the height of the anastomosis, if it is low in the chest reflux is worse.

In the videos it looked like there was quite a bit of residual cervical esophagus. Do you have an idea of where your anastomosis were placed in most of these individuals?

Discussant

Dr. Ulysses Ribeiro Jr: The mean length of the esophageal stump is around 5 to 6 centimeters.

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2009 SSAT PLENARY PRESENTATION

National Trends in Esophageal Surgery—Are Outcomes as Good as We Believe?

Geoffrey Paul Kohn · Joseph Anton Galanko · Michael Owen Meyers · Richard Harry Feins · Timothy Michael Farrell

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Abstract
Introduction Positive volume–outcome relationships in esophagectomy have prompted support for regionalization of care; however, outcomes have not recently been analyzed. This study examines national trends in provision of esophagectomy and reassesses the volume–outcome relationship in light of changing practice patterns and training paradigms.
Methods The Nationwide Inpatient Sample was queried from 1998 to 2006. Quantification of patients' comorbidities was made using the Charlson Index. Using logistic regression modeling, institutions' annual case volumes were correlated with risk-adjusted outcomes over time, as well as presence or absence of fellowship and residency training programs.
Results A nationwide total of 57,676 esophagectomies were recorded. In-hospital unadjusted mortality fell from 12% to 7%. Adjusting for comorbidities, greater esophagectomy volume was associated with improvements in the incidence of most measured complications, though mortality increased once greater than 100 cases were performed. Hospitals supporting fellowship training or a surgical residency program did not have higher rates of mortality or total complications.
Conclusions The current national mortality rate of 7% following esophagectomy is higher than is reported in most contemporary case series. A greater annual esophagectomy volume improves outcomes, but only up to a point. Current training paradigms are safe.

Keywords Esophagectomy · Training programs · Residency and internship · Factual databases · Trends

Introduction

Through the turn of the millennium, the USA has experienced a steady rise in the incidence of esophageal adenocarcinoma, with annual increases of more than 2% per year between 1998 and 2003.[1] Age-adjusted incidence rates of esophageal cancer now approximate 4.5 cases per 100,000 population,[2] placing it seventh among causes of cancer death.[3]

For over 30 years, surgeons have pondered the association between case volume and patient outcomes for high-risk surgical procedures.[4–8] Esophagectomy, because of its high risk and relatively low volume, has been embraced as a procedure warranting regionalization of care within specialty centers.[9,10]

As a consequence, systems to drive cases to high-volume centers have emerged. For example, the Leapfrog group (Washington, DC), a collaboration of healthcare purchasing organizations that works to initiate improvements in the safety, quality, and affordability of healthcare,[11] has established

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definitions for case volume requirements[12] and tracks outcomes. Investigators have attempted to support or refute case volume thresholds for esophagectomy.[13–15] The definition of what constitutes a high-volume center varies markedly in the literature and is usually arbitrarily defined.[16,17]

On the surface, recent data seem to support improved outcomes in the era of regionalization in esophageal surgery. High-volume centers show superior esophagectomy outcomes,[18] with the best centers reporting mortality rates from 1% to 4%.[16,19–25] However, as systems supporting regionalization gain traction, it remains vital to track national outcomes, since high-volume reporting centers may not represent the rate of actual mortality across the USA. To date, broad efforts to confirm case volume as a surrogate for quality have usually stratified hospital case volume as a categorical variable when comparing statewide or nationwide outcomes via administrative datasets.[26–30]

Paradigm shifts may bring unintended consequences. High-volume centers are also usually the seats of surgical training. Rising numbers of esophageal operations will require these institutions increase both clinical and educational missions. However, since esophageal surgery is often within the domains of specialist surgeons focused on minimally invasive, thoracic, and oncologic practice, the structure of advanced training is heterogeneous and difficult to evaluate. The impact of fellowship programs on patient outcomes after esophagectomy has not been evaluated outside of single-institution experience.[31] The effect of general surgery training programs has rarely been assessed.

Finally, ongoing advances in both surgical and nonsurgical therapeutic modalities and protocols mandate periodic reassessment of our systems intended to regulate delivery of care. Therefore, we report the current state of esophageal surgery in this country with regards to national trends in provision and the impact of case volume and training programs on the safety of esophagectomy.

Methods

The most recently available Nationwide Inpatient Sample (NIS)[32] databases covering the years 1998–2006 were queried. These are the largest all-payer inpatient care databases in the USA, containing data from approximately eight million hospital stays each year. The latest release, the 2006 database, contains all discharge data from 1,045 hospitals located in 38 states, approximating a 20% stratified sample of all nonfederal, short-term, general, and other specialty hospitals in the USA.[32] A dataset was created by merging core and hospital files and filtered to identify esophagectomies using the ICD-9-CM procedure codes 42.4 (esophagectomy), 42.40 (esophagectomy, not otherwise specified), 42.41 (partial esophagectomy), 42.42 (total esophagectomy, excluding esophagogastrectomy), and 43.99 (esophagogastrectomy, also including complete gastroduodenectomy, esophagoduodenostomy with complete gastrectomy esophagojejunostomy with complete gastrectomy, radical gastrectomy, and other total gastrectomy). While these are standard codes for esophagectomy, they also include some gastrectomies without esophagectomy. To correct for this, gastric operations were assumed if associated with a diagnosis code for malignant neoplasm of stomach (151–151.9) or for gastric ulcer (531–531.9) and were excluded. Pediatric patients less than or equal to 17 years of age were excluded. To calculate nationwide case volume totals, the NIS-supplied discharge-level weight was applied. At all other times, the unweighted NIS cohort was utilized for calculating standard errors and performing regression analyses.

Information regarding the presence of a Fellowship Council (FC)-accredited fellowship program in each year of the study period was taken from the Fellowship Council's webpage.[33] The Fellowship Council is an association of minimally invasive, endoscopic, and combined gastrointestinal surgery fellowship directors formed to address the unique needs of fellowship applicants and programs. In 2006, there were 89 listed programs. Information regarding the presence of a thoracic surgery fellowship was taken from the National Resident Matching Program's 2009 website[34] and assumed the presence of such a fellowship throughout all the years of the study. There were 43 such fellowships identified. Information regarding the presence of a Society of Surgical Oncology (SSO) fellowship was taken from this society's website[35] and assumed the presence of such a fellowship throughout all the years of the study. There were 11 such fellowships identified.

A teaching hospital is defined within the NIS as a hospital with residents in any specialty and meeting any of the following criteria: Accreditation Council for Graduate Medical Education (ACGME) residency training approval, membership in the Council of Teaching Hospitals, or a ratio of full-time equivalent interns and residents to beds of 0.25 or higher. Hospitals having a surgical residency were defined as a subgroup. Details of such a surgical residency program were obtained by combining information from the American Medical Association's Fellowship and Residency Electronic Interactive Database Access and the listings of accredited programs on the ACGME webpage.[36,37] There were 192 identified accredited general surgery residencies. The NIS divides hospitals into size tertiles based on bed size, adjusted for region and teaching status.[38]

Comorbidity scores were applied to each inpatient stay record, using the Deyo adaptation of the Charlson comorbidity index.[39] This validated index allocates a score between 0 and 35, with a higher score indicating more comorbidity. The comorbidities examined include myocardial infarction, congestive heart failure, peripheral vascular disease, cerebro-

vascular disease, dementia, pulmonary disease, connective tissue disease, peptic ulcers, chronic liver disease, hemiplegia, renal disease, diabetes, malignancy, leukemia, metastatic cancer, and acquired immune deficiency syndrome.

Perioperative complications were added based on ICD-9-CM codes, in a similar manner to that described by Santry et al.[39] The diagnosis of "any complication" was made if the "died during hospitalization" field=1 or if any of the NIS's 15 diagnosis fields contained one of the following complication or procedure codes: abdominal drainage procedure (5491), acute cerebrovascular accident (43100–43191, 4330–4339, 4340–43491), acute dialysis (3895), acute deep venous thrombosis (4538, 4539), acute myocardial infarction (4100–4109), acute pulmonary embolism (4151, 41511, 41519), acute renal failure (5841–5849), acute respiratory failure (51881), adhesiolysis (5451, 5459), anastomotic leak (9986), bacterial pneumonia (481, 485, 486, 4820–4829), cardiac complications (9971), central nervous system complications (99701–99703), dialysis catheter insertion (3995), foreign body removal (5492), intraoperative hemorrhage (99811), laparotomy (5412), mechanical ventilation (967, 9671, 9672, 9673), postoperative shock (9980), reclosure of abdomen (5461), respiratory tract complications (99973), small bowel obstruction (5600–5609), splenectomy (4143, 415), splenic injury (8650–8651), tracheostomy (311, 3129), transfusion (9904, 9909), urinary complications (9975), wound dehiscence (9983, 99831, 99832), wound infection (9985, 99851, 99859), and wound seroma (99813).

Statistics

SAS 9.2 (SAS Institute, Cary, NC, USA) was used to analyze the data. Logistic regression modeling was performed using generalized estimating equations and assuming a binomial distribution of the data. This allowed control for certain covariables; thus, risk-adjusted outcome measures were calculated. Repeated measure analysis was performed with the experimental unit being hospital identification number clusters. The model was solved for empirical standard error estimates, and p values were based on these estimates. A p value<0.05 was considered significant. Subsequently, the estimates were exponentiated to calculate an odds ratio (OR) and 95% confidence intervals. One of the authors (JAG) holds a Ph.D. in Biostatistics.

Results

Trends in Care

A total of 11,614 esophagectomies were recorded in the NIS database for the study period; this was the cohort utilized for subsequent analysis. NIS weightings indicate this cohort that represents 57,676 total esophagectomies performed in the USA during the 9-year study period of 1998–2006. With a nationwide weighted total of 6,425 esophagectomies being performed in 1998 and 6,032 in 2006, it is evident that the annual number of esophagectomies did not increase over this timeframe, despite the increasing number of new diagnoses of esophageal malignancy[1] (Table 1). At the beginning of the study period, approximately 40% of these operations were performed in teaching hospitals, a proportion which remained constant throughout the study period. The majority of operations were performed in the largest third of hospitals (Table 2). The indications for surgery and the type of operations have remained similar over the same interval (Table 1).

As illustrated in Table 3, high-volume centers for esophagectomy are variously described as performing at least 13 to 20 esophagectomies per year,[12,17,25] and the number of surgical programs meeting these standards has remained stable over time. In 1998, 4.2% of hospitals performing esophagectomies completed 13 or more cases, and 1.2% of hospitals performing esophagectomies completed 20 of more cases. In 2002, these numbers were 7.5% and 2.5% and in 2006 were 12.4% and 5.8%.

Mortality Rates

Concurrent with the stable hospital case volumes, the in-hospital mortality rate for esophagectomies taken as a group has steadily decreased throughout the study period (Fig. 1). The mortality rate of all esophagectomies performed in the USA in 1998 was 12.1%. By 2002, it was 9.0%, and by 2006, it had reached 7.0%. As noted in Table 1, approximately 40% of the operations performed were esophagogastrectomies. Improvements of in-hospital mortality were quite impressive in this subgroup, decreasing from 12.3% at beginning of the study period to 8.9% in 2002 and to 7.8% in 2006. Just fewer than 40% of the operations were partial esophagectomies; mortality rates for this subgroup also fell, from 10.7% in 1998 to 8.6% in 2002 and to 5.9% in 2006. Approximately 16% of operations were total esophagectomies, and here too, mortality rates improved markedly over the study period —15.2% in 1998, 9.8% in 2002, and 6.3% in 2006. The only operation which increased in mortality was "Esophagectomy, not otherwise specified". The numbers performed were small, with 35, 34, and 40 procedures coded in 1998, 2002, and 2006, respectively. Corresponding mortality rates were 11.4%, 8.8%, and 15.0%. These trends in mortality rate occurred synchronously with a steady decrease in every year of the mean Charlson comorbidity scores, from 4.5062 in 1998 to 4.2311 in 2002 and to 3.7997 in 2006.

Table 1 Indications for Operation

	1998	2002	2006
Most frequent diagnoses			
1	Malignancy of cardia or GE junction (41.00%)	Malignancy of cardia or GE junction (40.47%)	Malignancy of cardia or GE junction (37.96%)
2	Malignancy of distal 1/3 esophagus (21.28%)	Malignancy of distal 1/3 esophagus (22.57%)	Malignancy of distal 1/3 esophagus (22.95%)
3	Malignancy of esophagus—multiple or overlapping sites (5.67%)	Malignancy of esophagus—multiple or overlapping sites (5.99%)	Malignancy of esophagus—multiple or overlapping sites (7.06%)
4	Malignancy of middle 1/3 esophagus (5.59%)	Ulcer of esophagus (4.67%)	Malignancy of middle 1/3 esophagus (43.97%)
5	Malignancy of esophagus, NOS (3.29%)	Malignancy of middle 1/3 esophagus (4.20%)	Barrett's esophagus (3.89%)
Operation type, *n* (%)			
Esophagectomy NOS	35 (2.88)	34 (2.65)	40 (3.24)
Partial esophagectomy	457 (37.55)	443 (34.47)	540 (43.80)
Total esophagectomy	165 (13.56)	246 (19.14)	205 (16.63)
Esophagogastrectomy or total gastrectomy	560 (46.01)	562 (43.74)	448 (36.33)
All esophagectomies	1,217	1,285	1,233

Table 2 Characteristics of Hospitals Performing Esophagectomies from 1998–2006

	1998	2002	2006
Teaching hospitals, *n* (%)	144 (43.11)	60 (44.12)	28 (40.00)
Bed size, *n* (%)			
Small	52 (15.57)	25 (18.38)	12 (17.14)
Medium	108 (32.34)	43 (31.62)	24 (34.29)
Large	174 (52.10)	68 (50.00)	34 (48.57)

With all esophagectomies considered together, there was noticeable variation in mortality rates according to expected primary payer status or by self-described racial group (Table 4). The largest three expected payer groups were private including HMO, Medicare, and Medicaid; unadjusted mortality was 5.2%, 12.2%, and 11.3%, respectively. Of the three largest racial groups in which a racial identity was specified, the mortality rates were White 8.9%, Black 12.5%, and Hispanic 7.1%.

Interestingly, as seen in Fig. 1, anastomotic leak rates were quite constant throughout the study period, with little variance about the mean of 1.53 (±0.29).

Effect of Hospital Case Volume

Table 5 examines the independent effect of annual hospital case volume on complication rates, after controlling for the improvements in outcomes seen over the study period and for Charlson comorbidity scores. That is, the risk-adjusted effect of increasing annual case volume is reported. In contrast to previously published studies, artificial case volume groups were not applied and the models were solved for case volume as a continuous variable. An odds ratio <1.0 signifies an inverse correlation between case volume and the complication under review. The odds ratios tend to be very close to 1.0 because the ratios represent the effect of increasing the annual volume by a single case. That is, the effect of each and every case on outcomes is reported. Nearly all analyzed complication categories trended toward an inverse correlation with case volume, with any complication, myocardial infarction, respiratory tract complications, bacterial pneumonia, acute respiratory failure, acute renal failure, postoperative shock, blood transfusion requirement, and splenectomy rates achieving statistically significant improvement. No complication was associated with increasing case volume.

Results which have been tabulated reflect modeling for the linear effect of the variables only in order to simplify presentation. For a more detailed examination of the effects of case volume specifically on mortality rates, modeling was also performed adjusting for year, case volume, and Charlson comorbidity score and additionally the quadratic

Table 3 Hospitals in Each Annual Case Volume Group for Esophagectomies, *n* (%)

Annual case volume, *n* (%)	1998	1999	2000	2001	2002	2003	2004	2005	2006
<13 cases	320 (95.8)	307 (94.2)	282 (94.3)	279 (93.9)	260 (92.5)	264 (92.0)	230 (92.7)	248 (91.8)	212 (87.6)
13–20 cases	10 (3.0)	9 (2.8)	12 (4.0)	11 (3.7)	14 (5.0)	14 (4.9)	9 (3.6)	13 (4.8)	16 (6.6)
>20 cases	4 (1.2)	10(3.1)	5 (1.7)	7 (2.4)	7 (2.5)	9 (3.1)	9(3.6)	9 (3.3)	14 (5.8)
Overall	334	326	299	297	281	287	248	270	242

of case volume. This examines the effect of very high case volume on mortality. When used as predictors in a logistic regression model, both the case volume ($p<0.0001$) and the quadratic ($p<0.0001$) achieved statistical significance, with the predicted trends plotted in Fig. 2. The improvement in mortality rate observed with increasing hospital case volume seems to level out at approximately 30–40 cases per year and then slowly increases after about 80–100 cases per year.

Effect of Training Programs

Table 6 examines the independent effect of fellowship programs on outcomes of esophagectomies. Forty-three hospitals submitting data to the NIS and offering National Residence Matching Program (NRMP)-affiliated thoracic surgery fellowship programs were identified. Examining the independent effect of the presence of a thoracic surgery fellowship, after controlling for yearly variations, annual case volume, and Charlson comorbidity score, it is seen that the rate of any complication was significantly better, as were rates of bacterial pneumonia and incidental splenectomy. Anastomotic leak rates were significantly worse in this group, being nearly double those in hospitals without a thoracic surgery fellowship program (OR 1.81808, 95% confidence interval [1.18347, 2.79297]). Eighty-nine NIS hospitals had Fellowship Council-accredited fellowship programs. Examination of the independent effect of a FC-accredited fellowship on esophagectomy outcomes, after controlling for yearly variations, annual case volume, and Charlson comorbidity score, revealed that anastomotic leak rate was significantly increased (OR 1.71926 [1.09136, 2.70843]). Eleven NIS hospitals offered a Society of Surgical Oncologists-administered fellowship program during the study period. Only a very small number of these institution performed esophagectomies ranging from one to three hospitals per year. In the years 1998, 1999, 2000, and 2004, one of these institutions also offered either a Fellowship Council-accredited fellowship or a thoracic surgery fellowship. There was no significant independent effect of an SSO-administered fellowship on any of the measured variables. There was no detrimental effect of any fellowship program on in-hospital mortality following esophagectomy.

When all of the above fellowship programs were considered together, again controlling for yearly variations, annual case volume, and Charlson comorbidity score, it was noted that the presence of any fellowship program was associated with a decrease in the rate of any complication (OR 0.81655 [0.70613, 0.94425]) and an increase in rates of anastomotic leak (OR 1.64538 [1.12423, 2.40811], myocardial infarction (OR 1.47069 [1.02836, 2.10329]), and requirement for postoperative tracheostomy (OR 1.37774 [1.09114, 1.73961]).

The effects of the presence of an ACGME-accredited general surgical residency program in hospitals submitting data to the NIS are shown in Table 7. There were clear benefits in rates of any complication (OR 0.85656

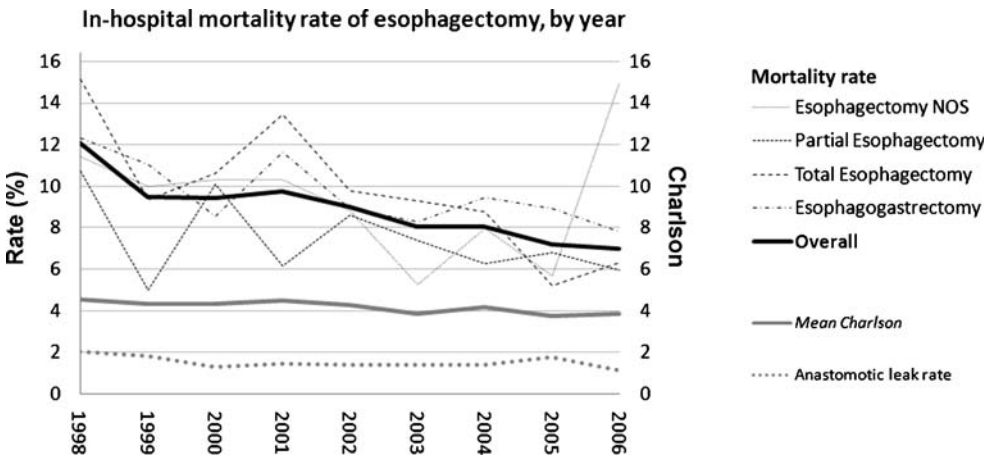

Figure 1 In-hospital mortality rate by year.

Table 4 Unadjusted Mortality Rates by Primary Payer and by Racial Group

	Number of esophagectomies (1998–2006)	Mortality rate (%)
Payer		
Medicare	5,361	12.2
Private (including HMO)	5,039	5.2
Medicaid	655	11.3
Self-pay	213	10.8
No charge	31	3.2
Other	278	4.3
Not specified	21	9.5
Race		
White	7,276	8.9
Black	522	12.5
Hispanic	424	7.1
Asian or Pacific Islander	141	6.4
Native American	17	17.7
Other	136	8.8
Not specified	3,082	8.4

[0.75270, 0.97482]), in-hospital mortality (OR 0.73408 [0.60460, 0.89128]), acute renal failure, acute respiratory failure (OR 0.77169 [0.63680, 0.93518]), and postoperative bacterial pneumonia (OR 0.70775 [0.60034, 0.83437]). The first column shows the independent effect of a surgical residency program, after controlling for yearly variations, annual case volume, and Charlson comorbidity score. The second column shows the effect of a surgical residency program after controlling for any fellowship in addition to the other controlled variables.

Discussion

With the incidence of esophageal adenocarcinoma in the USA increasing, demand for esophagectomies will persist

Table 5 The Incremental Effect of Each Esophagectomy on Annual Outcomes, Controlling for Year, and for Charlson Comorbidity Scores

Outcome variable	OR [95% CI]	*p* value	±
Death	0.98346 [0.97419, 0.99282]	0.0006	↓
Any complication	0.98927 [0.98417, 0.99440]	<0.0001	↓
Anastomotic leak	1.00301 [0.99884, 1.00721]	0.1577	
Acute DVT	0.99462 [0.98666, 1.00264]	0.1879	
Acute PE	1.00064 [0.99237, 1.00898]	0.8796	
Myocardial infarction	0.98895 [0.97981, 0.99817]	0.0189	↓
Other cardiac complications	1.00337 [0.99926, 1.00750]	0.1078	
Bacterial pneumonia	0.99126 [0.98363, 0.99896]	0.0261	↓
Respiratory failure	0.98598 [0.97744, 0.99460]	0.0015	↓
Other respiratory complications	0.99238 [0.98540, 0.99942]	0.0339	↓
Tracheostomy	0.99809 [0.99378, 1.00242]	0.3876	
Post-op shock	0.98720 [0.97659, 0.99792]	0.0194	↓
Splenectomy	0.97594 [0.96103, 0.99107]	0.0019	↓
Acute renal failure	0.98934 [0.98081, 0.99794]	0.0153	↓
Acute CVA	0.99951 [0.99210, 1.00697]	0.8969	
Transfusion	0.98874 [0.98010, 0.99745]	0.0114	↓
Intraoperative hemorrhage	0.99634 [0.99136, 1.00136]	0.1525	
Wound infection	1.00134 [0.99641, 1.00629]	0.5959	
Wound dehiscence	1.00342 [0.99742, 1.00946]	0.2644	

CI confidence interval

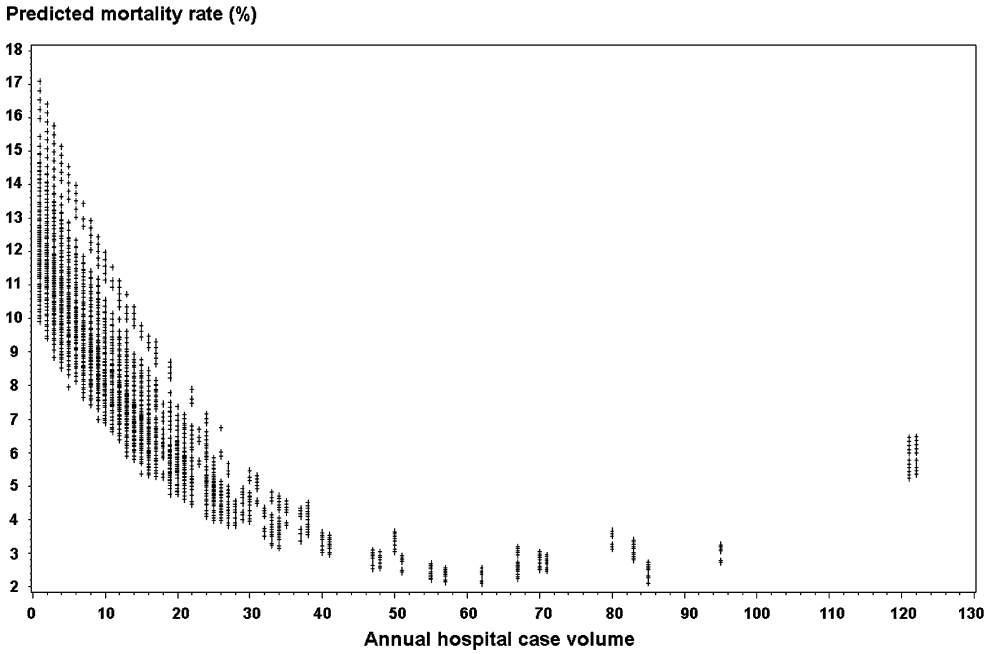

Figure 2 Effect of case volume on mortality rates for esophagectomy, controlling for year, and Charlson comorbidity score.

for the foreseeable future. Several studies have reported an association between increasing hospital esophagectomy volumes and improved outcomes, and these data have been often cited by proponents of centralization of care. However, many of these studies have ignored case mix and comorbidity profiles. There has also been confusion in the studies between mortality rates attributed to institutions and those associated with individual surgeons, especially in hospitals where more than one division performs these operations.[40] A further confounder of volume–outcome

Table 6 The Effect of Fellowship Programs on Outcomes, Controlling for Year, Charlson Comorbidity Scores, and Case Volume

	NRMP thoracic surgery fellowship			Fellowship Council fellowship			SSO fellowship			Any fellowship		
Outcome variable	OR	*p* value	±	OR	*p* value	±	OR	*p* value	±	OR	*p* value	±
Death	0.74540	0.1616		0.64442	0.0520		1.20362	0.5688		0.81876	0.1909	
Any complication	0.76224	0.0025	↓	0.97994	0.7936		0.94781	0.7701		0.81655	0.0063	↓
Anastomotic leak	1.81808	0.0064	↑	1.71926	0.0194	↑	0.82013	0.5418		1.64538	0.0104	↑
Acute DVT	1.40284	0.0680		1.34406	0.1807		0.43378	0.1078		1.24531	0.2344	
Acute PE	0.96735	0.9028		1.09701	0.7590		1.65206	0.1693		0.87970	0.6432	
Myocardial infarction	1.31194	0.1351		1.45736	0.1086		1.57056	0.2886		1.47069	0.0346	↑
Other cardiac complications	0.99563	0.9722		1.04968	0.7362		0.70323	0.1915		1.08368	0.4895	
Bacterial pneumonia	0.75250	0.0297	↓	0.99334	0.9584		1.04377	0.8526		0.84392	0.1386	
Respiratory failure	0.87603	0.3649		0.84606	0.2101		1.64505	0.1904		0.89606	0.4196	
Post-op shock	0.85222	0.6521		0.74101	0.4772		1.88059	0.1494		0.82347	0.5424	
Splenectomy	0.65218	0.0247	↓	1.34382	0.0905		0.85698	0.7017		1.03346	0.8492	
Tracheostomy	1.31743	0.0545		1.31602	0.0934		0.98042	0.9292		1.37774	0.0071	↑
Other respiratory complications	0.89736	0.4647		0.96366	0.7835		1.19115	0.5799		0.91206	0.4308	
Acute renal failure	0.82439	0.1357		0.84315	0.1961		1.39506	0.3252		0.91195	0.4630	
Acute CVA	1.47270	0.1160		1.34735	0.2462		0.72586	0.5282		1.00596	0.9792	
Transfusion	0.80451	0.2266		1.11449	0.4160		1.25370	0.6553		0.97302	0.8312	
Intraoperative hemorrhage	1.15811	0.3325		1.19390	0.3471		0.97744	0.9391		1.25928	0.1314	
Wound infection	1.14559	0.3962		1.09706	0.5600		1.18189	0.5549		1.09991	0.4993	
Wound dehiscence	1.20660	0.3767		1.40928	0.0948		1.43033	0.1176		1.22596	0.2436	

Table 7 The Effect of a Surgical Residency on Outcomes of Esophagectomy

Outcome variable	Controlling for year, Charlson comorbidity scores, and case volume			Controlling for year, Charlson comorbidity scores, case volume, and the presence of any fellowship program		
	OR	*p* value	±	OR	*p* value	±
Death	0.73408	0.0018	↓	0.73871	0.0039	↓
Any complication	0.85659	0.0189	↓	0.90288	0.1662	
Anastomotic leak	1.19260	0.2941		0.87937	0.5191	
Acute DVT	1.40245	0.0191	↑	1.39545	0.0447	↑
Acute PE	1.04715	0.8231		1.14617	0.6013	
Myocardial infarction	1.03786	0.8181		0.83035	0.3602	
Other cardiac complications	1.08972	0.3637		1.07388	0.5243	
Bacterial pneumonia	0.70775	<0.0001	↓	0.69282	0.0002	↓
Respiratory failure	0.77169	0.0082	↓	0.75668	0.0192	↓
Other respiratory complications	0.92581	0.4301		0.94466	0.6071	
Tracheostomy	1.09782	0.3402		0.95042	0.6590	
Postoperative shock	0.86730	0.6150		0.91778	0.8150	
Splenectomy	0.84051	0.1607		0.81263	0.1299	
Acute renal failure	0.73460	0.0017	↓	0.68815	0.0027	↓
Acute CVA	1.23601	0.3335		1.34199	0.2621	
Transfusion	0.84101	0.1314		0.82387	0.1420	
Intraoperative hemorrhage	1.07209	0.5939		0.94721	0.7498	
Wound infection	1.10102	0.3573		1.07930	0.5187	
Wound dehiscence	0.91926	0.5638		0.76096	0.0948	

studies is the categorization of institutions into either low- or high-volume centers based on arbitrary case thresholds.[41] Finally, interpreting such results is difficult when poorly described or suboptimal statistical methodology is utilized.[42]

Despite accruing evidence of the beneficial effects of case volume on cancer surgery outcomes since the end of the twentieth century,[6,26,43] the percentage of esophagectomies being performed in higher-volume hospitals has not increased significantly over the study period. This single fact may explain the discrepancy between the best reported mortality rates and the latest US esophagectomy mortality rate of over 7%. Surgeons and patients discussing informed consent for esophagectomy outside high-volume centers should consider that one in every 14 patients undergoing esophagectomy in this country will die in-hospital.

Although it is encouraging that the mortality rate for esophagectomy has diminished by 60% over recent years, there is no clear association with the movement toward regionalization. Mortality improvement may be partly explained by the decreasing comorbidities of the patient population described above. There have been parallel improvements in perioperative care[44,45] as well as staging and selection.[46,47] Unfortunately, limitations of the NIS database prevent analysis of the effect of tumor stage on outcomes.

Case volume requirements have been determined by various organizations, such as the Leapfrog Group. To meet the standards of this group, at least 13 esophagectomies must be performed by an institution per year. According to the newest Leapfrog criteria, certain nonesophagectomy operations can also be counted toward esophagectomy, such as total gastrectomy and radical gastrectomy.[11] As described above, by excluding operations performed for primary gastric diagnoses such as gastric malignancy or gastric ulcer disease, we have minimized the possibility of inclusion of any cases other than esophageal resection in our study group. Thus, the cohort we reviewed is equally sensitive and more specific for esophagectomy than that used by other groups. The most striking feature of these data is the beneficial effect evident for each and every increment in annual hospital case volume. Nearly every measured complication was seen to significantly improve with increasing annual volume, at least to volumes seen in nonoutlier hospitals. This has now been demonstrated in a very large administrative database, without recourse to artificial case volume groups. It appears that there may be a reversal of these positive volume–outcome associations when hospital volume exceeds 100 cases per year, with some evidence for rising mortality rates. However, the sample size of these very-high-volume hospitals is very

small, which limits interpretation of this interesting and never previously reported finding. We plan further investigation to determine whether this effect is true or perhaps a consequence of case mix or other uncaptured variables.

It is becoming more evident that volume criteria are not the sole determinant of outcome.[48,49] Even with equally experienced surgeons in a high-volume hospital, a variable that differs widely between institutions is the composition of the other members of the surgical team. No previous study has evaluated the effect of fellowship programs or general surgical residencies on outcomes after esophageal resection. If hospital case volume is used as a surrogate for the experience and capabilities of the perioperative team, particular scrutiny should be given to the effect of training programs, which involve multiple and variably rotating trainees in perioperative care of patients and which may sacrifice case volume for educational focus and academic inquiry.

We have identified an overall independent beneficial effect of a fellowship program in hospitals performing esophagectomies. If any fellowship program exists (thoracic, FC or SSO) at a particular hospital, the total numbers of complications decrease, though there is no way to verify from the NIS data whether the esophagectomies were performed by fellowship-affiliated surgeons. This limitation is probably more relevant with the Fellowship Council programs than the SSO or thoracic surgery programs, since the former places emphasis on minimally invasive gastrointestinal surgery and not necessarily surgery for malignancy in the chest. Of much more interest is the apparent increase in the serious adverse events of anastomotic leak, myocardial infarction, and tracheostomy associated with fellowship programs. While we intend to examine this further in future studies, our current hypothesis for the association between anastomotic leaks and fellowships is that, compared with private practice and resident training programs, the fellowship model puts trainees in the position of operating surgeon at crucial stages of an esophagectomy procedure. This complication is not associated with a greater death rate, perhaps as a consequence of better detection and management in these fellowship sponsoring hospitals, but clearly this is an area requiring further investigation.

Outcomes do not clearly stratify along surgical specialties. In this study, hospitals with fellowship programs administered by the Fellowship Council and the SSO had very similar outcomes, whereas a few outcomes, namely rates of incidental splenectomy and rates of bacterial pneumonia, were comparatively better in thoracic surgery fellowship program hospitals. Bias may have been introduced by the small sample size of SSO training hospitals, a result of nonreporting of many of such programs to the NIS. That said, surgeons identifying themselves as thoracic surgeons have been shown to have improved outcomes over those identifying as general surgeons,[50] especially in low-volume centers.

In contradistinction to fellows, residents are usually supervised to a far greater degree during operations. This supervision has been thought to be the major means for ensuring safe outcomes in a teaching environment.[31] In this study, we have shown that an ACGME-accredited general surgical residency program independently improves many of the measured complications, including rates of any complications, in-hospital mortality, acute renal failure, acute respiratory failure, and bacterial pneumonia. It has previously been reported that high-volume centers may minimize the effect of complications by earlier detection and more appropriate management.[25] It is possible that the factor which enables earlier detection of problems is the presence of a strong residency program. The authors hypothesize that a larger house staff permits more frequent physician–patient contact and earlier management of adverse events. Higher deep venous thrombosis rate is reported, and this might be due to either longer operation time with resident training, or because of improved detection by residents in the postoperative period, a consequence of the aforementioned increases in contact with the patient.

Limitations exist in searches of administrative databases related to the accuracy of data entry by institutional coders. The accuracy of coding has previously been reported as suboptimal,[51] though the detection of the presence of a particular diagnosis (as performed in this study) has been validated.[52] It is conceivable that the programs with an active surgical residency might have better entry into the medical record of complications, with disproportionate capture of these measured outcomes in this group.[53] Also, many hospitals are not represented in the NIS cohort, including some of the higher-volume esophagectomy centers in the USA. While attempts have been made to control for this statistically, a larger sample will always provide more accurate representation of the population as a whole. Finally, length of stay was considered by the authors as a variable dependent on the number and severity of complications and so was not used as a control variable in the mathematical modeling. It is, however, conceivable that length of stay is at least partly independent insomuch as the longer a patient remains in hospital the more time is available to capture complications for inclusion in the NIS.

Conclusion

The current 7% esophagectomy mortality rate of hospitals reporting to the Nationwide Inpatient Sample has improved but without evidence for measurable centralization of cases

within high-volume centers. This rate remains higher than that reported in most contemporary series. In this model, the hypothesized positive volume–outcome relationship of esophageal surgery has been validated without the use of arbitrarily assigned case volume categories. This volume-related improvement in mortality is seen to taper with approximately 30–40 annual cases and may reverse in the highest-volume centers. The performance of esophageal resections in training hospitals is safe and with no increase in either mortality or total morbidity, though fellowship training may be associated with a higher anastomotic leak rate.

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Discussion

Dr. Geoffrey Paul Kohn, presenter (University of North Carolina at Chapel Hill, NC, USA).

Discussant

Dr. Jeff Peters (Rochester): This is about the esophagectomy outcome relationships that have been hammered home over the past few years. As you will see a little bit later today, the other side of that equation is the people that do not get resected, and there are a fair number of them. In fact, your observation that the mortality went down—it plateaued, I should say, at about 30 cases per year. Interestingly, this mirrors the number that is true in high resection prevalence institutions as well, and of course, that is much higher than the leapfrog criteria which is 13 and the five per year that have been talked about, and I think a more realistic number.

A couple of questions for you, perhaps one observation and a couple of questions. You interestingly showed that there is no change in the number of esophagectomies per year, and of course you started off we have the thesis that the prevalence of this cancer has increased dramatically over the last 10 or 15 years. Does that imply that we are not operating on the growth in this disease, or the relative proportion of patients that are coming to surgery is less?

You also showed that there was a shift in the number of high-volume hospitals that took care of these patients. So slowly over the decade, I think that you studied, more patients were taken care of in high-volume hospitals. You showed almost a 50% reduction in mortality, 12% to 7%, although you highlighted the 7% as still too high, which is true.

How much of that decrease—that 50% decrease in mortality—was due to that shift? Did you do that analysis?

Lastly, I just quibble with one of your conclusions. You said surgical residents are safe. You showed that resident hospitals were safe. You have no data on who actually did the operation. So, you might want to clarify that a little bit.

Closing Discussant

Dr. Geoffry Paul Kohn (University of North Carolina at Chapel Hill, NC): Thank you, Dr. Peters, for your comments. Addressing the first, the increasing prevalence of esophageal cancer is indeed a real phenomenon, though there is no doubt that the numbers of esophagectomies performed in this country have been relatively stable over the study period.

Data I presented showed the decreasing mean comorbidity score over the period. I think this probably highlights the improved patient selection criteria that we have. Unfortunately, these types of national administrative databases do not have provide any indication as to whether the patients have undergone neoadjuvant therapy. We also do not have very good staging information. However, with the decreasing comorbidity scores, I think we do show that we currently have better or at least more restrictive patient selection, and I think that is the reason that the total case numbers have not increased.

Regarding the cause for the decrease in mortality, we have demonstrated that up to somewhere around 80–100 annual cases, each and every esophagectomy performed in a specific center will improve in-hospital mortality. We did not specifically control for the number of hospitals in each case volume group, but we would expect the observed decrease in mortality to have resulted at least in part from a shift to higher-volume centers.

With regards to the residency point, I completely agree. Again, administrative databases can only determine hospi-

tals in which fellowship programs or residency program exist. No data are available about who actually performed the operation. Your comment is valid and it applies both to the residency institutions and the fellowship institutions.

Our hypothesis, which we have actually started investigating further in a new study, is that the actual vital technical components of the procedure, for example, the construction of the anastomosis, are probably being performed more times by the fellow than by the residents in those institutions. A resident is also probably more strictly supervised by the attending staff. We do not have anything yet to back it up, but I think that is least a possible explanation about data.

Discussant

Dr. Tom Demeester (USC, Los Angeles (Los Angeles, CA)): Dr. Kohn, thank you for the opportunity to review the preprinted manuscript. It is well written and I compliment you for getting the prize for the best manuscript of the meeting.

Your study is based on administrative data with all the shortcomings that are associated with such a database. Yet, you have been able to use the data to help clarify some of the issues regarding esophagectomy for the treatment of esophageal cancer. I have four questions.

Your basic theme has been supportive of other prior investigations that greater hospital volume is related to better outcome. At the recent American Surgical Meeting, a paper by Birkmeyer's group at Michigan suggests that hospitals with large volumes have better outcomes because they are more able to rescue patients from complications. Does your data provide any evidence that high-volume hospitals have better services to allow a better capacity to rescue patients with complications? For instance, surgical intensivists as opposed to medical intensivists, dedicated esophageal anesthesiologists, 24-h availability of interventional radiologists, 24-h operating room availability for surgical therapy of complications, and 24-h surgical endoscopy support to name a few.

My second question focuses on your observation that the survival associated with increasing volume improves to a point, up to about 100 cases. Do you conclude that a hospital will go beyond a safe limit if it exceeds 100 cases per year? In other words, there is an upper limit to the benefit of volume.

My third question regards your statement that overall mortality is going down from roughly 12% to 7%. In the manuscript, you did not show that the reduction was across the board. Was it only due to the effect of the improved mortality in the high-volume hospitals? What happened to mortality in those hospitals that did less than 13, between 13 and 20, and over 20?

The key part of the operation is the esophagogastric anastomosis. Was anastomotic breakdown and sepsis more common in hospitals with resident or fellow? I believe you stated, the leak rate was significantly higher, in fact 50% higher in hospitals with training programs. Further, tracheostomies were more common in teaching hospitals which may be a surrogate for a greater complication rate. If this is true, is it correct to conclude that house officers and teaching programs do not alter safety? Could you comment on this?

My last question deals with where are all these studies going? We continue to talk about high-volume hospitals have better outcomes. Will a point come when organized surgical societies of surgery will recommend criteria for hospitals in order to perform esophagectomies? That completes my questions. I enjoy reading the paper. It was very thought provoking.

Closing Discussant

Dr. Geoffrey Paul Kohn: I think your first and fourth questions are very closely related. The first one was about whether rescue of complications are better at high-volume hospitals and whether the anastomotic leak rates are a concern in fellowship and residency offering hospitals.

Dr. Birkmeyer's group at that meeting did report that, while total complication rates can be similar in high-volume institutions as compared to lower-volume institutions, the outcomes are often superior, probably because of earlier detection and better management. I think that is exactly what our data show. We do show that higher anastomotic leak, and we do show higher risk of certain complications. Some of that might be selection bias because of more attention being paid by the residents in training hospitals, for example, to myocardial infarction. But we do have higher rates of leak, though it does not affect the mortality.

I think there is an improvement in the management of the complications at some of these big institutions. That is the main focus of our next paper that we are in the process of drafting—to look at the outcomes following the index complication.

The second question is, are we doing too many cases? We came into this with the hypothesis that the more cases you did, the better. We discovered that U-shaped curve and we thought that perhaps there was a problem with our analysis; perhaps, the high-volume institutions are choosing more difficult cases. The Charlson Index is a validated comorbidity score, but the specific validation for esophageal cancer has not been attempted. However, since our results have come out, I have had correspondence with surgeons at some of the larger volume institutions. It seems, anecdotally, that this U-shaped curve is a real phenomenon. I am told that when their institutions are ramping up case volume for the first 2 or 3 years, they are noticing a higher morbidity–mortality rate. They think it is probably due to an inability of the facility to accommodate the large increase in volume. It may also

be a staffing or personnel issue. The increased mortality seems to settle down over a few years. This is an interesting phenomenon which has not previously been reported and requires further study.

With regard to your case volume groups question, I think ours is a very powerful model using logistic regression with no artificially allocated case volume groups. We show that, up to a point, each and every single esophagectomy does cause a benefit.

The last question, why are we doing this and what is the likely outcome of this? I think centralization of care is probably going to be forced on us from external regulators to a certain degree. This is already occurring for example in the UK. However, I think we have to be very wary of volume being the only criterion. I think the volume we are using is only a surrogate marker for quality. There are other effects on quality. Additionally, we have to be very aware that by moving cases to high-volume institutions, we are usually moving them to seats of surgical training, and therefore, we have to look at both the effect of and the effect on our educational training system for surgeons.

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2009 SSAT POSTER PRESENTATION

The Metastatic Lymph Node Number and Ratio Are Independent Prognostic Factors in Esophageal Cancer

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Abstract
Objective The current American Joint Committee on Cancer staging system for esophageal cancer is based on lymph node location, irrespective of the number of involved and examined lymph nodes.
Methods We enrolled 488 patients receiving primary curative resection without neoadjuvant therapy for esophageal cancer between 1995 and 2006. The importance of total resected lymph node number (TLN) and metastatic lymph node number (MLN) and ratio (MLR) on patient survival was investigated.
Results The overall 3-year survival rate was 35.4%. The 3-year survival rate was equivalent among patients in N1 (23.3%), M1a (22.0%), and nonregional lymph node metastasis-related M1b (18.5%, $p=0.321$). No survival difference was noted between patients with TLN<15 or ≥15 ($p=0.249$). Both MLN and MLR significantly predicted patient survival. The 3-year survival rate was 52.3%, 29.2%, and 8.0% for patients with MLN=0, 1–3, and ≥4, respectively ($p<0.001$). For patients with MLR=0–0.2 or >0.2, the 3-year survival rate was 28.7% and 9.8%, respectively ($p<0.001$). However, survival rate differences were more evident when TLN was more than 15.
Conclusions We recommend designating both regional and nonregional lymph nodes as N nodes. MLN and MLR, but not TLN, are prognostic factors in esophageal cancer.

Keywords Esophageal cancer · Lymph node metastasis · Prognosis

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Introduction

The current version of the American Joint Committee on Cancer (AJCC) staging system for esophageal cancer has not changed since the fifth edition (1997).[1] It classifies the N stage into N0 (without regional lymph node (LN) metastases) and N1 (with regional LN metastases), irrespective of the number of involved and examined lymph nodes. Since the presence of lymph node metastases in esophageal cancer is a critical determinant in management and prognosis, subclassifying N stage has been often suggested.[2–14] Previous reports have recommended dividing patients into different N subgroups based on the total number of resected lymph nodes and the number and ratio of positive lymph nodes.[2–14] However, most of these studies were either small-scale or from a population-based database.[11–14] Furthermore, most studies included much more esophageal adenocarcinoma than esophageal squamous cell carcinoma (ESCC).[3–6] The purpose of our study was to analyze our experience with a large group of

consecutive patients with esophageal cancer from a single high-volume institution. This cohort had a high percentage of squamous cell carcinoma patients, and all patients received primary surgical resection without neoadjuvant chemoradiation. The importance of total resected lymph node number (TLN), metastatic lymph node number (MLN), and metastatic lymph node ratio (MLR) on patient survival was investigated.

Patients and Methods

Study Design and Patients

A retrospective review was performed on 1,069 consecutive patients with esophageal cancer who were admitted to the Division of Thoracic Surgery, Department of Surgery at Taipei Veterans General Hospital between January 1995 and December 2006. The preoperative workup included physical examination, laboratory tests, esophagogastroduodenoscopy, flexible bronchoscopy, barium esophagography, computed tomography (CT) scans from neck to upper abdomen, ultrasound of the abdomen, and radionuclide bone scans. The exclusion criteria included: (1) patients who were inoperable due to medical unfitness (e.g., poor cardiopulmonary function) or patient refusal; (2) patients unresectable due to extensive locoregional invasion that obliterated the normal tissue planes or presence of distant organ metastasis; (3) patients who received palliative bypass surgery instead of curative radical resection; (4) patients who received neoadjuvant chemoradiation; and (5) patients with 30-day in-hospital mortality (5.1%). This study design was approved by the Institutional Review Board of Taipei Veterans General Hospital.

Surgical Resection

The surgical methods were classified according to the approach methods, reconstructed organs, reconstruction route, and the anastomosis site. Most patients received a tri-incisional approach (McKeown type), which included right-side thoracotomy, midline laparotomy, and left-side cervicotomy. In the thoracic stage, en bloc esophagectomy and radical mediastinal lymph node dissection (including paratracheal nodes, posterior and anterior mediastinal nodes, subcarinal nodes, paraesophageal nodes, and inferior pulmonary ligament nodes) were performed. In the abdominal stage, esophageal substitute mobilization (e.g., gastric tube creation) and dissection of paracardial nodes and enlarged celiac axis nodes (including celiac nodes, left gastric nodes, common hepatic nodes, and splenic nodes) were performed. Then the gastric tube was pulled to the cervical incision for anastomosis. Cervical lymph node sampling was also completed in the cervical stage. The other approaches included transhiatal, thoracoabdominal, and the Ivor Lewis method. Transhiatal esophagectomy was indicated for patients with a small primary tumor without enlarged lymph nodes on CT scan and poor cardiopulmonary function. In the left-side thoracoabdominal approach, the incision extends from below scapula, across the costal margin, and obliquely toward the umbilicus. The left-side pleural cavity and abdominal cavity were exposed simultaneously.

Pathological Examination

After esophagectomy, the periesophageal tissue and lymph nodes were dissected from the esophageal specimen by operator. Each dissected node group was labeled according to AJCC lymph node classifications.[1] Thereafter, the specimens were preserved in 10% neutral buffered formalin overnight and sent for pathological examination. All lymph nodes were cut in 5 μm thickness at several levels along the long axis, embedded in paraffin, and sectioned for H&E staining. The lymph node number was counted under low-power field microscope. Two pathologists examined all slides individually. Description of the tumor (appearance, invasion depth, differentiation) and the lymph nodes (number of involved and examined lymph node in each station) were recorded. The pathological tumor stage was determined according to the tumor–node–metastases (TNM) classification.[1] The total resected lymph node number was the sum of cervical, intrathoracic, and abdominal lymph node numbers. The metastatic lymph node number and ratio of involved to removed nodes were counted. The N status was further subclassified based on the total resected lymph node number (TLN$<$15 or $\geq$15, the value for adequate nodal staging suggested by National Comprehensive Cancer Network guidelines),[15] metastatic lymph node number (MLN=0, 1–3, or $\geq$4, the criteria used in AJCC staging system for colorectal cancer),[1] and metastatic lymph node ratio (MLR$\leq$0.2 or $>$0.2).

Postoperative Follow-Up

Surviving patients were followed up at our outpatient department every 3–6 months for the first 5 years, then annually. Patient information from the Cancer Registry Database in our hospital was also recorded. Overall patient survival, defined as the time from operation to death or last follow-up, was used as a measure of prognosis.

Statistics

A chi-square test was used to compare categorical variables, and ANOVA was used for comparison of continuous variables. The survival curves were plotted by the Kaplan–

Table 1 Patient Characteristics and Univariate Survival Analysis Results

Demographics	Number	3-year survival (%)	Median survival (month)	*p* value
Age, mean (ranges)	63.8 (34–88)	35.4	20±1.5	–
Sex				0.07
Male	461 (94.5%)	34.1	20±1.4	
Female	27 (5.5%)	52.4	41±28.6	
Location				0.39
Upper third	67 (13.8%)	38.0	19±3.5	
Middle third	210 (43.0%)	40.1	24±3.5	
Lower third	211 (43.2%)	29.2	18±2.0	
Histology				0.06
Squamous cell carcinoma	460 (94.3%)	34.9	20±1.6	
Adenocarcinoma	7 (1.4%)	0.0	14±6.5	
Other	21 (4.3%)	41.9	29±5.8	
T				<0.001*
1	68 (13.9%)	62.0	64±11.3	
2	117 (24.0%)	41.9	27±4.7	
3	260 (53.3%)	28.5	17±1.2	
4	43 (8.8%)	16.3	10±1.9	
N				<0.001*
0	235 (48.2%)	51.0	36±8.7	
1	253 (51.8%)	20.7	15±0.9	
M				<0.001*
0	388 (79.5%)	39.6	23±2.3	
1a	42 (8.6%)	22.0	16±2.7	
1b	58 (11.9%)	15.9	10±1.1	
Stage				<0.001*
I	53 (10.9%)	73.2	106±24.9	
II	197 (40.4%)	45.1	30±4.2	
III	138 (28.3%)	18.1	14±0.8	
IV	100 (20.5%)	18.3	14±1.5	
Total resected lymph node number				0.249
<15	183 (37.5%)	30.5	18±2.4	
≥15	305 (62.5%)	37.1	22±2.1	
Metastatic lymph node number				<0.001*
0	218 (44.7%)	52.3	39±9.9	
1–3	166 (34.0%)	29.2	19±2.2	
≥4	104 (21.3%)	8.0	12±1.1	
Metastatic lymph node ratio				<0.001*
0	218 (44.7%)	52.3	39±9.9	
0–0.2	163 (33.4%)	28.7	19±1.8	
>0.2	107 (21.9%)	9.8	11±1.1	
Adjuvant treatment				0.034*
With postoperative chemoradiation	104 (21.3%)	27.8	16±1.2	
Without postoperative chemoradiation	384 (78.7%)	37.4	23±1.9	

Median survival time is presented as an estimate ± SEM (standard error of the mean)

*p value<0.05 was considered significant by log-rank test

Meier method and compared using the log-rank test. Multivariate analysis was performed using the Cox regression model, incorporating all variables found to be significant in univariate analysis. All calculations were performed using SPSS 15.0 software, and a p value of less than.05 was considered significant.

Results

Patient Demographics

Of the 1,069 esophageal cancer patients admitted to our institution, 488 patients were appropriate for this study. The patient characteristics are summarized in Table 1. The mean TLN was 22 nodes. The mean TLN was 23 and 19 in node-positive and node-negative patients, respectively. Fourteen patients were found to have distant visceral metastasis (lung or liver) during the operation and were grouped as M1b. Adjuvant therapy was suggested to all patients with T3 or greater and N1 stage; however, only 104 (21.3%) patients agreed to receive adjuvant chemoradiation. The operative methods are depicted in Table 2. Most patients received a tri-incisional esophagectomy and reconstruction with a gastric tube via the retrosternal route. Two patients received curative resection only, without reconstruction, due to their poor general condition. The approach methods, substitute organs, reconstruction routes, and anastomosis sites had no

Table 2 Operative Methods

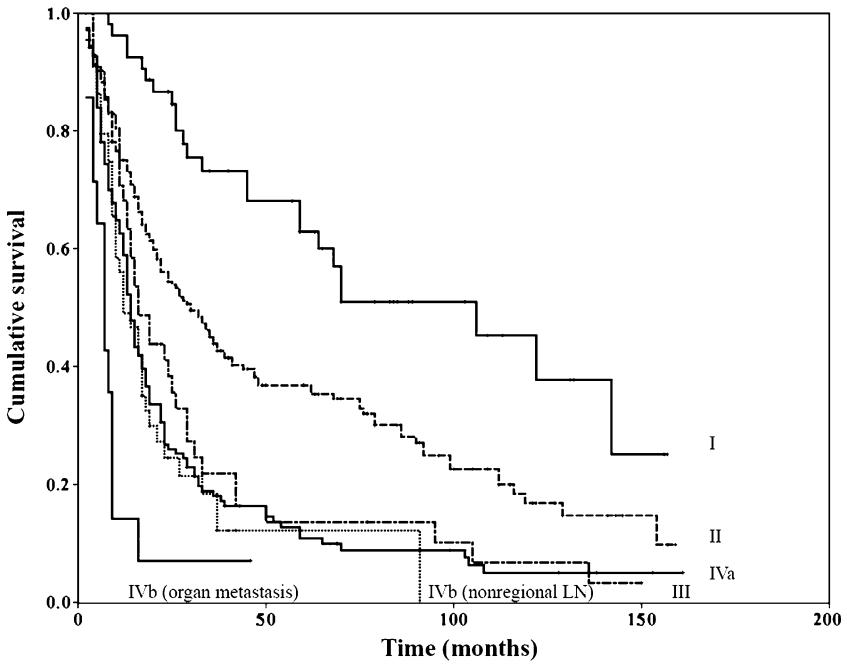
Methods	Number
Surgical approach	
Tri-incisional	407 (83.4%)
Transhiatal	34 (7.0%)
Thoracoabdominal	40 (8.2%)
IVOR Lewis	7 (1.4%)
Substitute Organ	
Stomach	469 (96.1%)
Colon	11 (2.3%)
Jejunum	6 (1.2%)
No reconstruction	2 (0.4%)
Reconstruction route	
Retrosternal	391 (80.1%)
Posterior mediastinal	94 (19.3%)
Subcutaneous	1 (0.2%)
No reconstruction	2 (0.4%)
Anastomosis site	
Neck	471 (96.5%)
Thorax	15 (3.1%)
No reconstruction	2 (0.4%)

impact on patient survival (p=0.761, 0.146, 0.398, and 0.115, respectively).

Lymph Node Metastasis and Survival

Mean follow-up time was 33.7 months. The overall 1-, 3-, and 5-year survival was 70.1%, 35.4%, and 27.1%, respectively. Univariate survival analysis results are shown in Table 1. The "T," "N," "M," and stage grouping all influenced survival significantly. However, the survival curves on a Kaplan–Meier plot were almost identical in stage III (median survival, 14 months; 3-year survival rate, 18.1%), stage IVa (median survival, 16 months; 3-year survival rate, 22.0%), and stage IVb due to nonregional lymph node metastasis (median survival, 12 months; 3-year survival rate, 18.5%; Fig. 1). Since most of the stage III patients were T3N1 and T4N1, it seems that differentiating regional and nonregional lymph node metastasis may not be meaningful. We further compared survival among N1, M1a, and nonregional lymph node metastasis-related M1b stages. The survival difference was insignificant also (N1: median survival, 15 months; 3-year survival rate, 23.3%; p=0.321).

For the importance of the total resected lymph node number on patient outcomes, we found no survival difference between patients with TLN<15 and TLN≥15 (median survival, 18 and 22 months; 3-year survival rate, 30.5% and 37.9%, p=0.249, Fig. 2a). Subgroup analysis revealed that the TLN affected neither node-negative nor node-positive patients (Fig. 2b, c). In contrast, both MLN and MLR significantly predicted patient survival (Figs. 3a and 4a). The 3-year survival was 52.3%, 29.2%, and 8.0% for patients with MLN=0, 1–3, and ≥4, respectively (p< 0.001). The 3-year survival was 52.3%, 28.7%, and 9.8% for patients with MLR=0, 0–0.2, and ≥0.2, respectively (p<0.001). We further divided the patients into TLN<15 and TLN≥15 groups. In the former group, the survival difference was not statistically significant between 1–3 MLN and ≥4 MLN (Fig. 3b), There was also no survival difference between 0–0.2 MLR and >0.2 MLR (Fig. 4b). In the latter group, both MLN (Fig. 3c) and MLR (Fig. 4c)



Patient at risk							
I	53	40	27	16	10	5	2
II	197	99	51	40	19	9	3
III	138	34	17	8	7	4	2
IVa	41	13	5	5	3	2	
IVb (Nonregional LN)	44	8	1	1			
IVb (organ metastasis)	14	1					

Figure 1 Survival curves for patients stratified by TNM stage. The survival curves for stage III, IVa, and nonregional lymph node metastasis-related IVb were almost identical. There was no statistical difference among these three groups. Survival curves were plotted using Kaplan–Meier methods. Statistical differences in survival between groups were analyzed by the log-rank test.

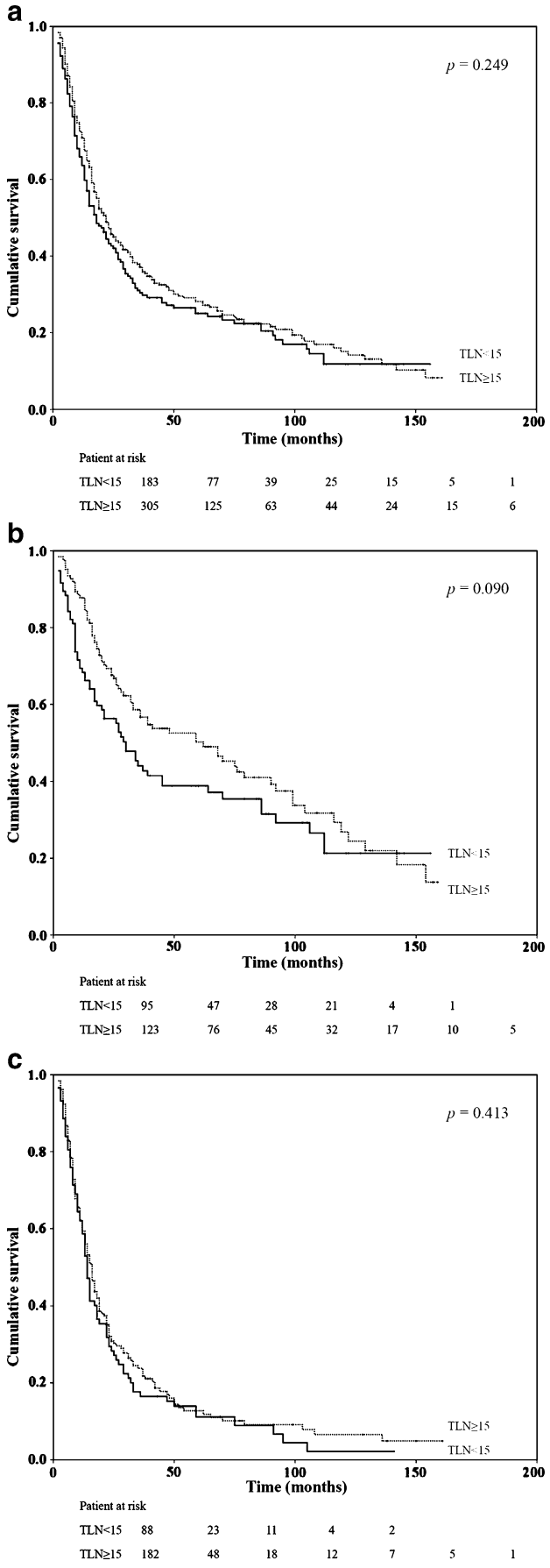


Figure 2 **a** No survival difference between patients with TLN<15 and TLN≥15 was found. Subgroup analysis revealed that the TLN had impact on neither node-negative (**b**, 3-year survival rate, 44.1% for TLN<15 and 58.6% for TLN≥15, p=0.090) nor node-positive patients (**c**, 3-year survival rate, 16.5% for TLN<15 and 23.8% for TLN≥15, p=0.413). Survival curves were plotted using Kaplan–Meier methods. Statistical differences in survival between groups were analyzed by the log-rank test.

remained prognostic factors that differentiated survival in each pairwise stratum.

In the Cox regression multivariate analysis (Table 3), the factors that affected survival in univariate analysis were included except N stage (N0 vs. N1), since it was part of MLN and MLR. Both MLN≥4 and MLR>0.2 were still independent prognostic factors (hazard ratio (HR), 2.38 and 2.23, respectively) for the entire population. After stratification, the multivariate analysis revealed that the hazard ratio was higher in TLN≥15 than TLN<15 group. This implies that adequate lymph node staging highlights the importance of MLN and MLR in survival prediction.

Discussion

The current AJCC staging system for esophageal cancer has not changed since 1997, and there is controversy regarding lymph node designation.[3,4,6,16–19] The staging system version considered celiac lymph nodes as M1a disease for tumors of the lower thoracic esophagus and M1b for tumors of upper/middle thoracic segments. The cervical nodes, in a similar fashion, are designated as M1a for esophageal cancers of the upper thoracic esophagus and M1b for tumors of lower/middle thoracic segment. However, the survival curves on a Kaplan–Meier plot between N1 and M1a were virtually interchangeable in previous reports by Hofstetter et al.[6] and Hagen et al.[17] Furthermore, some authors even showed that there was no survival advantage predicted by subdividing M stage into M1a and M1b, and they suggested abandoning this subclassification.[4,18,19] These findings were confirmed by our results that there was equivalent survival for N1, M1a, and nonregional lymph node metastasis-related M1b. Our findings, taken together with other results in the literature, lead us to suggest designating both regional and nonregional lymph nodes as N nodes and reserving M1 stage for distant organ metastasis.[3,4,6,16–19] This eliminates the need to subclassify the M stage into M1a and M1b.

Another debate is the subclassification of N stage. The current AJCC staging system for esophageal cancer is based on anatomic location only. However, survival is found to be heterogeneous within the N1 classification.[4] To stratify nodal status for better staging according to the total lymph node number, the positive lymph node number and ratio has been recommended.[2–14] However, most reports

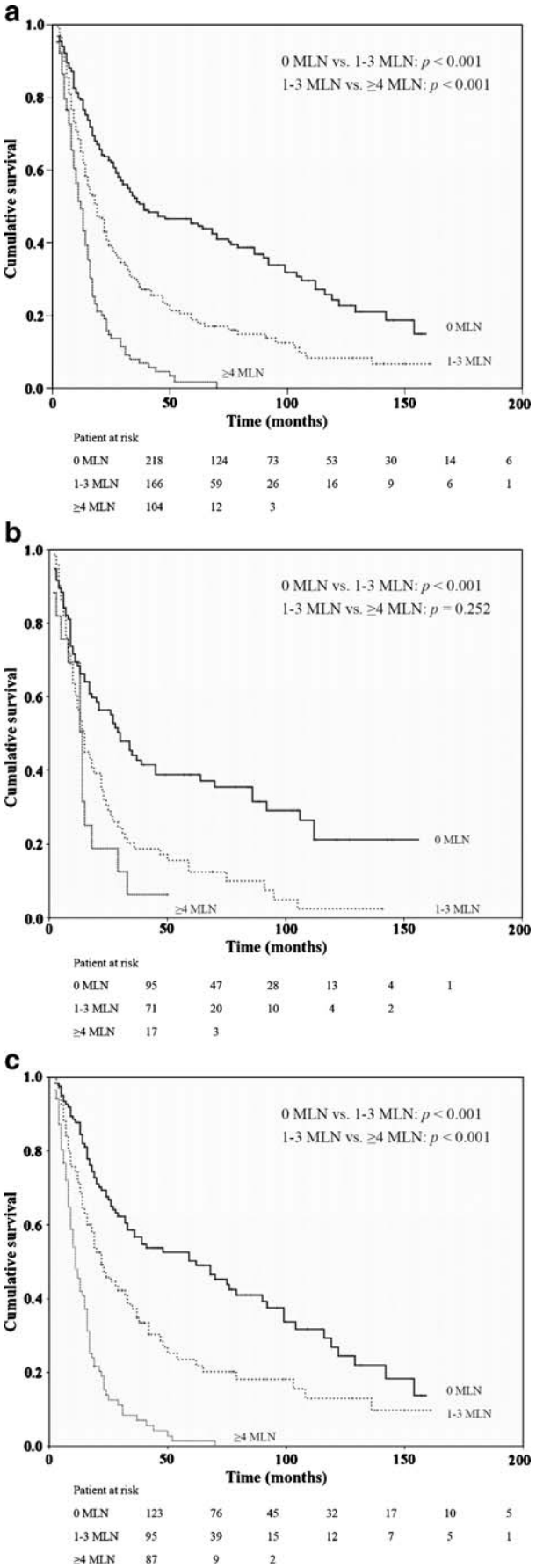


Figure 3 **a** Survival curves of patients subclassified by MLN. **b** After stratifying according to TLN, no survival difference was found between TLN<15/MLN=1–3 and TLN<15/MLN≥4 (3-year survival rate, 18.8% and 6.3%, respectively, p=0.252). **c** The survival difference between MLN=1–3 and MLN≥4 was still significant in the high TLN group. Survival curves were plotted using Kaplan–Meier methods. Statistical differences in survival between groups were analyzed by the log-rank test.

were based on either small-scale or population-based databases. There are shortages and limitations in population-based studies since many confounders, such as preoperative chemoradiation, surgical techniques, variability in pathological evaluation, and hospital volume, have not been well controlled.[9,20] The report of a large group of patients from a single high-volume institution is scarce in the literature. Besides, most studies have much more cases of adenocarcinoma than squamous cell carcinoma of the esophagus.[3–6] Reports based on squamous cell carcinoma-predominant databases are few. In Dhar's study, both the lymph node number (<4 or ≥4) and ratio (≤0.1 or >0.1) were prognostic factors in ESCC in univariate survival analysis but not in multivariate analysis.[7] The current study indicates that both MLN and MLR influence survival. In the univariate analysis, both MLN and MLR were prognostic factors, although the statistical power decreased in patients with low total resected lymph node number. In multivariate analysis, both MLN and MLR were independent survival predictors for the entire population. The survival difference was even more evident in adequate staged (TLN≥15) patients.

The impact of TLN on patient survival is controversial. Peyre and associates proposed TLN as a prognostic factor with a minimum of 23 lymph nodes removed to maximize the survival benefit after esophageal resection.[12] Another investigation by Greenstein and associates, based on the Surveillance, Epidemiology, End Results (SEER) database, also found that a higher number of lymph nodes was associated with a better disease-specific survival in node-negative patients. However, their results were limited to adenocarcinoma histology; the total resected lymph node number had no effect on postoperative survival in ESCC patients.[13] In the current study, based on an ESCC-predominant database, we found no impact of TLN on either node-positive or -negative patient survival. These contrasting results may be attributed to different lymphatic

Figure 4 **a** Survival curves of patients subclassified by MLN. **b** After stratifying according to TLN, no survival difference was found between TLN<15/MLR=0–0.2 and TLN<15/MLR≥0.2 (3-year survival rate, 21.1% and 13.5%, respectively, p=0.109). **c** The survival difference between MLR=0–0.2 and MLR≥0.2 was still significant in the high TLN group. Survival curves were plotted using Kaplan–Meier methods. Statistical differences in survival between groups were analyzed by the log-rank test.

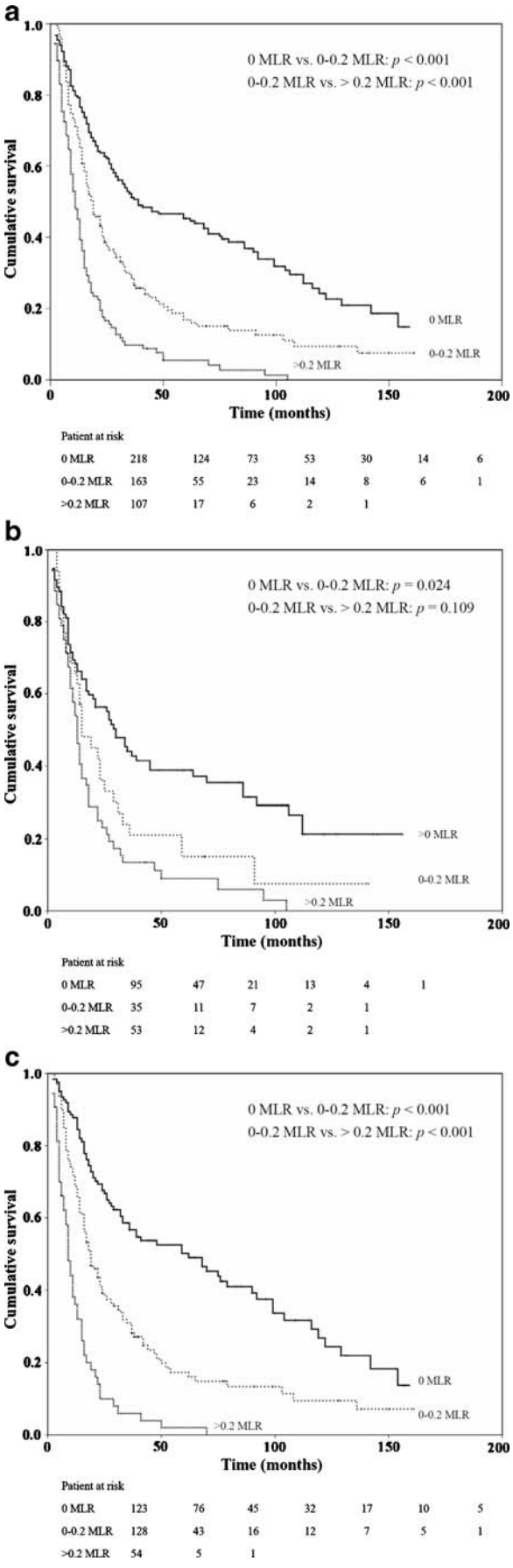


Table 3 Multivariate Survival Analysis Results

Variable	Hazard ratio	95% CI	*p* value
Entire population			
Tumor invasion depth			<0.001
T1/2	1.00	–	
T3/4	1.69	1.351–2.121	
Distant metastasis			<0.001
M0	1.00	–	
M1	2.86	1.612–5.072	
MLN[a]			<0.001
<4	1.00	–	
≥4	2.38	1.849–3.055	
MLR[a]			<0.001
≤0.2	1.00	–	
>0.2	2.23	1.736–2.851	
Chemoradiation			0.975
Without	1.00	–	
With	1.00	0.778–1.295	
TLN<15			
Tumor invasion depth			<0.001
T1/2	1.00	–	
T3/4	1.92	1.334–2.761	
Distant metastasis			<0.001
M0	1.00	–	
M1	5.35	2.099–13.619	
MLN[a]			0.030
<4	1.00	–	
≥4	1.83	1.059–3.161	
MLR[a]			0.006
≤0.2	1.00	–	
>0.2	1.68	1.164–2.430	
Chemoradiation			0.910
Without	1.00	–	
With	0.98	0.650–1.467	
TLN≥15			
Tumor invasion depth			0.009
T1/2	1.00	–	
T3/4	1.47	1.100–1.969	
Distant metastasis			0.034
M0	1.00	–	
M1	2.20	1.062–4.568	
MLN[a]			<0.001
<4	1.00	–	
≥4	3.11	2.293–4.222	
MLR[a]			<0.001
≤0.2	1.00	–	
>0.2	2.97	2.096–4.196	
Chemoradiation			0.855
Without	1.00	–	
With	1.03	0.743–1.432	

Multivariate analysis was performed using the Cox regression model
HR hazard ratio, *CI* confidence interval

[a] Each of these factors was entered into Cox model separately with other factors

patterns in ESCC and esophageal adenocarcinoma. Another possible explanation is that most of our patients received standardized procedures for esophagectomy and lymph node dissection. The extent of lymphadenectomy was similar in most patients in this cohort. Therefore, the number of total resected lymph nodes could not be interpreted as the extensiveness of the lymphadenectomy. The presumption that more lymph nodes harvested means the more radical lymphadenectomy it was is not applicable to our study.

Although there are various proposed cutoff values in the literature, it is very difficult to say which one is the best, or "optimal," value. Among the approaches to generate the cutoff values, one is to compute a statistical significance level for all possible cut-off values and select the one with the smallest p value. Another is to define the cutoff value based on the distribution of marker level among patients. Graphic display of survival curve for different prognostic groups is also a direct way to express the discriminatory power of the model. However, all these methods have bias, and statisticians have recommended abandoning the term "optimal."[21] In our study, the MLN<4 or ≥4 and MLR ≤0.2 or >0.2 were selected since it was most used in the literature. Also, these values showed best discriminatory power on the Kaplan–Meier plots.

In conclusion, there was no survival difference among N1, M1a, and nonregional lymph node metastasis-related M1b patients. We suggest designating both regional and nonregional lymph nodes as N nodes and reserving the M1 stage for distant organ metastasis. There is no need to subclassify M stage into M1a and M1b. We also demonstrated that MLN and MLR, but not TLN, are survival predictors in ESCC. The survival difference between high and low MLN/MLR is more evident in adequate staged (TLN≥15) patients. Our investigation provides further evidence for a revision of the esophageal cancer staging system.

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2009 SSAT POSTER PRESENTATION

Efficacy of a Hepatectomy and a Tumor Thrombectomy for Hepatocellular Carcinoma with Tumor Thrombus Extending to the Main Portal Vein

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Abstract
Introduction Hepatocellular carcinoma (HCC) with major portal tumor thrombus has been considered to be a fatal disease. A thrombectomy remains the only therapeutic option that offer a chance of complete tumor removal avoiding acute portal vein obstruction. However, the efficacy of tumor thrombectomy in addition to hepatectomy has not been well evaluated.
Methods Of 979 patients who consecutively underwent initial HCC resection, 45 (4.6%) HCC patients with tumor invasion of the first branch of the portal vein (vp3) and tumor in the main portal trunk or the opposite-side portal branch (vp4) were retrospectively analyzed to evaluate the efficacy of hepatectomy and tumor thrombectomy.
Results Alpha-fetoprotein, serosal invasion, and intrahepatic metastases were independently significant prognostic factors in all the 45 patients with vp3 or vp4 HCC. The 3- and 5-year survival rates in vp3 and vp4 group were 35.3% and 41.8%, and 21.2% and 20.9%, respectively. There were longer operative times and more intraoperative bleeding in patients with vp4, but no significant difference in mortality, morbidity, and survival between patients with vp3 and vp4.
Conclusion Hepatectomy and thrombectomy for vp4 could not only avoid acute portal occlusion due to tumor thrombus but provide a comparable survival benefit with hepatectomy for vp3.

Keywords Hepatocellular carcinoma · Tumor thrombus · Main portal vein · Hepatectomy · Thrombectomy

Introduction

Hepatocellular carcinoma (HCC) with tumor thrombus extending to the main portal trunk or the opposite-side branch of portal vein has been considered to be an end-stage condition with an extremely poor prognosis, because tumor cells might be already spreading to the entire whole liver.[1] Moreover, tumor thrombus obstructing the portal trunk sometimes rapidly leads to bleeding from esophageal and gastric varices or hepatic failure, directly related to sudden death.[2] The median survival in untreated patients with HCC accompanied by macroscopic portal vein tumor thrombus (PVTT) is 2.7–4.0 months.[1,3,4] Non-surgical therapeutic options, such as transcatheter arterial chemo-embolization (TACE), chemotherapy, and radiation, are not regarded as effective,[5,6] while liver transplantation is not adopted in patients with HCC and major vascular invasion.[7] A surgical resection for this fatal disease has been accepted as a therapeutic intervention that offers a chance of complete tumor removal avoiding portal vein obstruction.[2] However, the appropriate patient selection, perioperative outcomes, or long-term prognostic analysis for HCC with tumor thrombus extending to the main portal trunk have not yet been evaluated.[8–13]

The Liver Cancer Study Group of Japan classifies the tumor invasion of the first branch of the portal vein and

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tumor in the main portal trunk or the opposite-side portal branch into two groups by the degree of vp3 and vp4,[14] because there might be a crucial difference between both groups in terms of therapeutic and prognostic aspects. Tumor thrombus of vp3 can be completely removed by a major hepatectomy with the division of the ipsilateral portal branch near the bifurcation, but a vp4 tumor thrombus must be removed by a thrombectomy or an en bloc combined resection of the main portal trunk, which requires the resection and reconstruction of the main portal vein in addition to a hepatectomy. The surgical intervention for vp4 is not recommended because of higher risk and worse prognosis in comparison to that of vp3, because such surgical procedures seem to be potentially non-curative resections.[13,15]

This study retrospectively investigated the clinicopathological characteristics in 45 patients with vp3 and vp4 HCC, and evaluated the clinical significance of a hepatectomy and thrombectomy for HCC with vp4.

Patients and Methods

Between May 1992 and January 2008, 979 patients with HCC underwent a primary hepatectomy in the National Cancer Center Hospital, Japan. Among these patients, PVTT extending to the first portal branch, main portal trunk, or opposite-side portal branch were confirmed by intraoperative or final pathological examination in the 45 patients (4.6%), and they were enrolled in this retrospective study. The diagnosis and preoperative staging of HCC was based on the findings obtained by such diagnostic modalities as ultrasonography, dynamic computed tomography (CT), and hepatic digital subtraction angiography in combination with CT during arteriography or arterioportography (angiographic CT). Patients with HCC routinely underwent angiographic CT for the preoperative evaluation, in the current study period, because it seemed to be one of the most sensitive diagnostic tools.[16,17] The maximum limit of resectional liver volume was evaluated based on the indocyanine green retention rate at 15 min (ICG15R) and the volume of the remnant liver as estimated by CT was considered when determining the extent of the hepatectomy.

HCC with PVTT extending to the first branch of the portal vein, the major portal vein or the opposite-side portal branch required a right or left hemihepatectomy. The ligation of hepatic artery and portal vein, or the removal of PVTT was performed prior to the mobilization and transection of the liver, in order to eradicate the possibility of tumor scattering from the PVTT to the remnant liver. When PVTT was localized in the first branch of portal vein, the first branch of portal vein could be divided near the bifurcation with a sufficient surgical margin, and the en bloc resection could be performed without exposing the tumor thrombus. The use of either ligation or suturing for closing the stump of the portal vein depended on the condition of the stump of portal vein. When the PVTT extended to the main portal vein or the opposite-side branch of the portal vein, the interruption of the portal venous flow by temporary clamping the trunk and the contra-lateral first branch was performed prior to the thrombectomy. The portal venous wall was incised and the PVTT was peeled off and extracted. A combined resection of the portal vein was required if the tumor thrombus strongly adhered to the portal vein wall. Then, the portal vein was reconstructed by closure of the stump with the running suture or end-to-end anastomosis of the portal vein.

Tumor thrombus invasion of the first branch of the portal vein and tumor thrombus in the main portal trunk or the opposite-side portal branch were classified as vp3 and vp4, respectively, according the classification the Liver Cancer Study Group of Japan.[14] Surgical and postoperative outcomes were examined in patients with vp3 and 4. The following potential prognostic factors associated with survival were investigated as well as an extension of PVTT (vp3/vp4): age, gender, α-fetoprotein (AFP), status of hepatitis virus infection, ICGR15, preoperative TACE, intraoperative blood loss, intraoperative blood transfusion, pathological factors including tumor size, intrahepatic metastases, hepatic vein invasion, bile duct invasion, serosal invasion, histologic grade, distant metastasis, surgical margin (positive = cancer exposed at the resectional cut edge), and residual tumor. Serosal invasion was defined as the invasion to the serosal of the liver, including the exposure from the liver surface, the tumor invasion of the adjacent organs, and tumor rupture. A hepatectomy with residual tumors was defined as: multiple HCCs with apparent residual tumor tissue in the remnant liver and/or extrahepatic distant lesions; and a hepatectomy with tumors in the remnant liver, which were treated with ethanol injection (PEIT) or radiofrequency ablation (RFA). When residual tumors were confirmed 2 or 3 months after surgery based on the imaging results, intrahepatic tumor residues (≤3 nodules) was principally managed using local ablation therapy (PEIT or RFA), if possible, and multiple recurrences were treated using TACE. After discharge, all patients were closely followed at the outpatient clinic every 3 months by measurement of the serum AFP levels, US, and CT. Adjuvant therapy was not administrated during the current study period. Local ablation therapy, TACE, or a repeated hepatectomy was performed in the event of recurrence, according to the recurrence pattern.[18]

Any statistical difference among the groups was analyzed with the chi-square test. Overall survival and recurrence-free survival estimates were calculated by the Kaplan–Meier method. Recurrence-free survival was defined as the time from surgery to any first recurrence or the last follow-up, and

Table 1 Surgical Procedures and Outcomes in Patients with HCC and PVTT vp3 and vp4

Surgical procedures	vp3 (n=26)	vp4 (n=19)	P value
Types of hepatectomy			
Sectionectomy	1 (3.8%)	0 (0%)	0.523
Left hepatectomy	12 (46.2%)	7 (36.8%)	
Right hepatectomy	13 (50.0%)	12 (63.2%)	
Types of portal vein resections and closures			
Ligation of the portal vein	20	0	<0.001
Closure of the stump by suturing	6	0	
The removal of PVTT and the closure of the stump by suturing	0	14	
The removal of PVTT and the end-to-end anastomosis	0	5	
Total operative time (min)	388 (240–777)	448 (323–685)	0.034
Blood loss (ml)	1107(429–4193)	1963(1020–4703)	0.041
Mortality	0 (0%)	0 (0%)	–
Morbidity	6 (23.1%)	4 (21.1%)	1.000

PVTT portal vein tumor thrombus, *vp3* tumor thrombus extending to the first branch of portal vein, *vp4* tumor thrombus extending to the trunk or contra-lateral first branch of portal vein

analyzed for patients without residual tumor. Univariate comparisons of the survival curves were made with the log-rank test. Association were considered to be significant if $P<0.05$. A multivariate regression analysis was performed using the Cox proportional hazards model and variables associated $P<0.10$ were entered into the final model adopted. $P<0.05$ was considered to be significant. All statistical analyses were performed using the SPSS for Windows 11.5 statistical software package (SPSS, Chicago, IL, USA).

Results

All 45 patients underwent a right or left hemihepatectomy, except one patient who underwent right an anterior

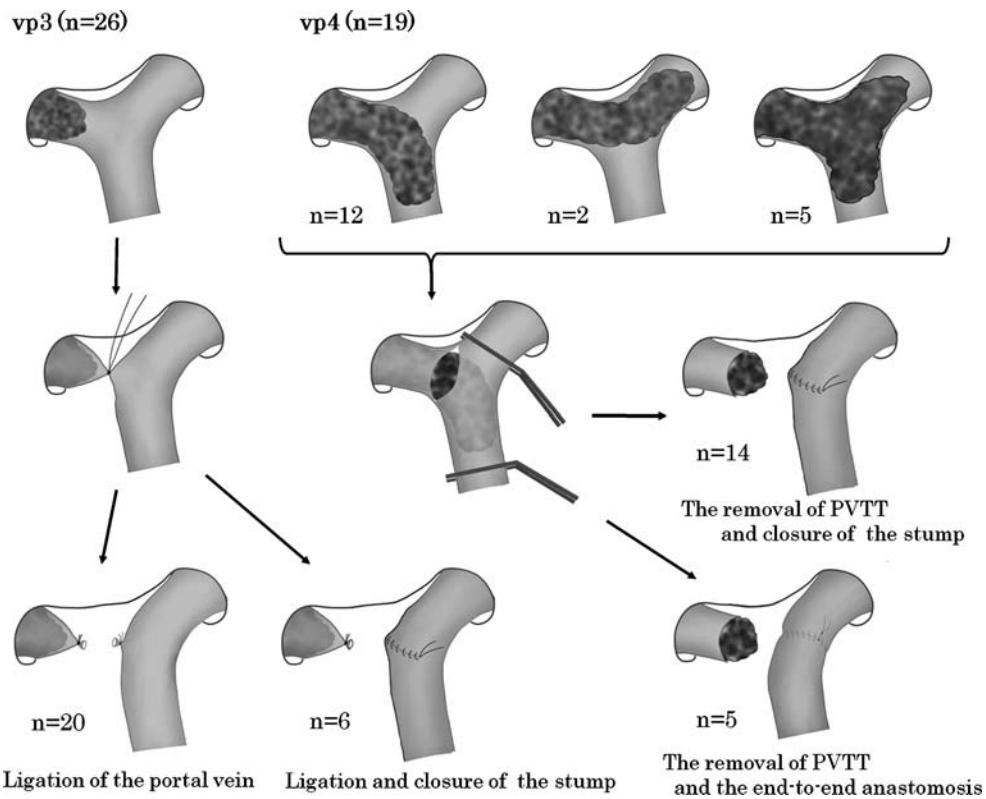

Figure 1 The surgical procedures for PVTT. A schematic illustration shows the surgical procedure for PVTT and reconstruction of the portal vein in patients with vp3 (n=26) and vp4 (n=19). All patients with vp3 underwent the ligation of the portal vein near the bifurcation. The portal vein could be divided by only ligation in 20 of the vp3 patients. Six patients with vp3 underwent suturing and closure of the portal venous stump. PVTT of vp4 patients protruded into bifurcation or main portal trunk (n=12), contra-lateral branch (n=2), and both of them (n=5). Fourteen of the vp4 patients underwent suturing and a closure of the portal venous stump, five of them underwent an end-to-end anastomosis, following a thrombectomy by the removal of PVTT in all vp4 patients.

Table 2 Univariate Analysis of Prognostic Factors in Patients with HCC and PVTT vp3 and vp4

Variables		No. of patients (%)	Median survival (months)	3-year survival (%)	5-year survival (%)	*P* value
Overall		45	20	37.4	22.4	
Age	≥60	22 (49)	21	39.6	0	0.932
	<60	23 (51)	17	35.0	28.0	
Gender	M	41 (91)	21	39.9	23.4	0.087
	F	4 (9)	9	–	–	
ICG R15 (%)	≥10	24 (53)	28	42.2	25.3	0.151
	<10	21 (47)	15	30.9	20.6	
α-Fetoprotein (ng/ml)	≥2,000	22 (49)	56	20.2	0	0.002
	<2,000	23 (51)	37	52.8	42.3	
HBV-Ag	(−)	26 (58)	36	47.0	18.8	0.195
	(+)	19 (42)	13	22.3	22.3	
HCV-Ab	(−)	30 (67)	17	21.9	21.9	0.092
	(+)	15 (33)	56	63.0	25.2	
Child–Pugh	A	38 (84)	20	37.9	21.7	0.938
	B	7 (16)	28	38.1	–	
Preoperative TACE	No	22 (49)	20	36.8	24.6	0.879
	Yes	23 (51)	23	38.4	20.5	
Blood loss (ml)	≥1,500	21 (47)	21	30.5	15.2	0.857
	<1,500	24 (53)	20	42.8	25.7	
Intraoperative transfusion	No	36 (80)	23	42.1	22.6	0.260
	Yes	9 (20)	10	16.7	16.7	
Tumor size (cm)	≥7.0	24 (53)	13	25.9	0	0.002
	<7.0	21 (47)	36	49.2	49.2	
Intrahepatic metastasis	(−)	20 (44)	56	54.3	43.4	0.001
	(+)	25 (56)	10	25.2	8.41	
Portal vein invasion	vp3	26 (58)	18	35.3	21.2	0.821
	vp4	19 (42)	28	41.8	20.9	
Hepatic vein invasion	(−)	39 (87)	21	35.9	25.6	0.648
	(+)	6 (13)	7	50.0	0	
Bile duct invasion	(−)	38 (84)	23	39.3	26.9	0.335
	(+)	7 (16)	20	25.7	0	
Serosal invasion	(−)	36 (80)	23	45.0	27.0	0.018
	(+)	9 (20)	7	0	0	
Histologic grade	mod	13 (29)	–	52.2	52.2	0.061
	por	32 (71)	18	31.4	9.41	
Surgical margin	Negative	35 (78)	28	41.6	28.5	0.047
	Positive	10 (22)	13	22.2	0	
Residual tumors	(−)	39 (87)	23	39.3	23.6	0.030
	(+)	6 (13)	4	20.0	–	

HBV-Ag hepatitis B virus surface antigen, *HCV-Ab* hepatitis C virus antibody, *ICG R15* indocyanine green retention rate at 15 min, *TACE* transcatheter arterial chemoembolization, *mod* moderately differentiated, *por* poorly differentiated, *vp3* tumor thrombus extending to the first branch of portal vein, *vp4* tumor thrombus extending to the trunk or contra-lateral first branch of portal vein

sectionectomy with extraction of the PVTT, because his liver function was insufficient to tolerate a hemihepatectomy (Table 1). The surgical procedures for PVTT are described in Fig. 1. Twenty patients with vp3 underwent only the ligation of the portal veins. Six of them underwent the closure of the stump by a running suture of the portal vein, because it was impossible to ligate the portal vein with an adequate margin. Fourteen patients with vp4 underwent a thrombectomy and the closure of the stump by a running suture of the portal vein. Five of them under-

went end-to-end anastomosis of the portal vein following the thrombectomy. There were no patients, whose thrombus had adhered to the portal vein wall, thus requiring combined resections. The surgical time and intraoperative blood loss in the vp4 group was longer and more than those of the vp3 group (Table 1—P=0.034 and P=0.041, respectively). The morbidity was similar in both groups, and no mortalities occurred in either group.

The median follow-up interval was 17 months (range, 1–100 months) in 45 patients with vp3 and vp4. The 1-, 3-, and 5-year survival rates were 69.6%, 37.4%, and 22.4%, respectively, with median survival time of 20 months, while the 1-, 3-, and 5-year recurrence-free survival rates were 30.4%, 21.2%, and 0%, respectively. The AFP level (>2,000 ng/ml; P=0.002), tumor size (not less than 7.0 cm; P=0.002), presence of intrahepatic metastases (P=0.001), presence of serosal invasion (P=0.018), surgical margin (positive; P=0.047), and presence of residual tumor (P=0.030) were found to be significantly associated with a worse prognosis (Table 2). The multivariate analysis showed that the value of α-fetoprotein (≥2,000/<2,000 ng/ml), intrahepatic metastases (present/absent), and serosal invasion (present/absent) were independently associated with poor survival, with a hazard ratio (95% confidence interval) of 2.64 (1.13–6.15; P=0.025), 4.95 (1.97–12.5; P=0.001), and 4.40 (1.58–12.3; P=0.005), respectively.

There was no significant difference in the overall survival and recurrence-free survival between vp3 and vp4 (P=0.821 and 0.710, respectively). Figure 2 shows the overall survival rate of vp3 and vp4. The 1-, 3-, and 5-year survival rates in vp3/vp4 were 72.0/65.8%, 35.3/41.8%, and 21.2/20.9%, respectively, while the 1-, 3-, and 5-year recurrence-free survival rates in vp3/vp4 were 27.3/35.0%, 16.4/28.0%, and 0/0%, respectively. There was no difference in the clinicopathological background between the two groups, including all the factors in the present study.

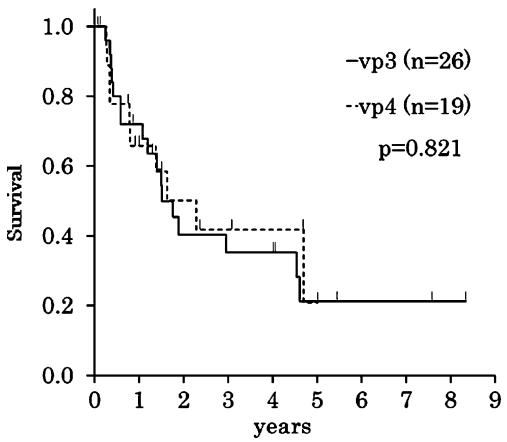

Figure 2 The overall survival curves for vp3 (n=26) and vp4 (n=19). There was no statistical difference between the groups (P=0.821).

During follow-up study, two patients with vp4 died of unknown causes 56 months and 17 months after surgery, respectively. The detail of the recurrence in these patients could not be evaluated and they were excluded from the recurrence analysis. Recurrence developed in 32 (82.0%) of 39 patients who could undergo a macroscopically complete resection (Table 3). The dominant pattern of recurrence was intrahepatic metastases in 27 patients (69.2%). In addition, no differences in the recurrence patterns were observed between patients with vp3 and vp4.

Discussion

Tumor thrombus located in the first branch of the portal vein or in the main portal trunk sometimes causes a rapid obstruction of the portal trunk, thus leading to acute death related to gastrointestinal bleeding or hepatic failure.[2] An en bloc hepatectomy or hepatectomy with a tumor thrombectomy is the most effective procedure to prevent acute portal hypertension and hepatic failure.[2,8–13,19] Hepatic surgeons might generally pursue an en bloc hepatectomy for HCC with tumor thrombus in the first portal branch (vp3), but hesitate to perform a thrombectomy for a thrombus in the major portal vein or the opposite-side branch of the portal vein (vp4), because this surgical intervention could not contribute a long-term survival benefit with higher surgical risk. Kondo et al.[15] reported that all patients with vp4 died within 400 days after surgery. In the current study, the 5-year survival rate in the vp3 and vp4 group were 21.2% and 20.9%, respectively. There was no difference in terms of the short- or long-term outcomes, though the surgical time and intraoperative blood loss were longer and more in the vp4 group. Previous studies reported the 5-year survival rates in patients with vp4 range from 0% to 28.5% (Table 4).

To achieve a favorable postoperative survival, an anatomical resection or hepatectomy with a wide surgical margin are advocated.[20,21] An en bloc resection of the portal vein seems to be a theoretically superior procedure without exposure of the tumor thrombus, but is complicated with a high incidence of morbidity and mortality.[2,22] On the other hand, a thrombectomy seems to be a potentially non-curative resection, because tumor cells might be exposed in the surgical field in spite of extensively careful management. Inoue et al.[10] reported no survival difference in the patients with macroscopic portal tumor thrombus undergoing a thrombectomy and en bloc resection. There are possible explanations for the lack of a therapeutic difference between vp3 and vp4: (1) treatment efficacy might depend not on the surgical procedures but on the tumors' biological aggressiveness; (2) cancer cells in HCC with a macroscopic tumor thrombus such as vp3 or vp4 could spread to the entire liver or the whole body as a part of

Table 3 Patterns of Recurrence in Patients with vp3 and vp4

	vp3 (n=22)	vp4 (n=15)	P value
No recurrence	2 (9.1%)	3 (20.0%)	0.463
Recurrence (initial recurrence sites)	20	12	
Liver	17 (77.3%)	10 (83.3%)	0.924
Solitary	5	4	
Multiple	10	4	
Multiple with PVTT[a]	2	2	
Lung	2 (9.1%)	1 (8.3%)	
Others	1 (4.5%)[b]	1 (8.3%)[c]	

[a] *PVTT* portal vein tumor thrombus

[b] Right adrenal gland

[c] Lymph node in one patient

systemic disease. Transplantation is an ultimate curative hepatectomy for HCC, but the result is disappointing HCC with PVTT.[7]

Another important problem after a thrombectomy is the fate of peritoneal recurrence due to tumor dissemination in the surgical field. Peritoneal recurrence usually occurs in patients with ruptured HCC,[23] and sometimes has been reported after a thrombectomy for bile duct tumor thrombus.[24,25] Inoue et al.[10] reported that peritoneal dissemination was a clinically minor problem because it was observed in only one patient undergoing a thrombectomy. This recurrence site could not be recognized during the current study period, but special attention should be taken because the postoperative observation period might have been too short to confirm a possibility of peritoneal seeding.

In this study, AFP, intrahepatic metastases, or serosal invasion, which might represent aggressive tumor behavior of HCC, were the most important prognostic factors, not the degree of PVTT. Ikai et al.[11] proposed prognostic index factors including ascites, prothrombin time, and tumor size and emphasized the significance of impaired functional hepatic reserve on the prognosis in patients with vp3 and vp4. One explanation for this difference might be due to a higher inclusion of patients with impaired hepatic function and ascites in the Ikai's series (21%). It is difficult to conclude which factors are more significant in affecting postoperative survival, and more precise information should be collected to determine useful selection criteria in the future.

Intrahepatic metastatic recurrence is inevitable and life threatening in patients with vp3 or vp4.[2,8–13,15] Managing these recurrence patterns can strongly affect postoperative survival, because multiple intrahepatic recurrences sometimes fulminantly spread to the remnant liver with tumor thrombus.[15] However, a tumor thrombus extending to the main portal trunk should not be considered to be an obstacle for a hepatectomy because there was no difference in the rate of recurrence and pattern according to the extension of tumor thrombus in the current study. Minagawa et al.[26] emphasized the usefulness of preoperative TACE in HCC patients with PVTT. However, the efficacy of preoperative TACE could not be demonstrated in the current study. Furthermore, there are two major reasons for no recommendation of preoperative TACE in patients with vp4: (1) a possibility of wide hepatic infarction when the portal vein trunk is completely occluded with tumor thrombus; (2) the rapid growth of tumor thrombus can lead to portal hypertension and acute

Table 4 The Large Series of Reports Including Hepatocellular Carcinoma and Portal Vein Tumor Thrombus in vp4

Series	Period	Setting of study	No. of patients (vp4)	5-year survival (vp4)	Mortality rate (vp4)
Wu[8]	1990–1998	HCC with invasion to portal vein (n=112)	15[a]	28.5%	0% (0/15)
Treut[9]	1998–2004	HCC with tumor thrombus to major vasculature[b] (n=108)	22	17.0%	12% (3/26)
Ikai[11]	1990–2002	HCC with PVTT vp3 and vp4 (n=78)	43	12.0%	4% (3/78)
Chen[13]	1990–2003	HCC with PVTT vp2,3,and 4 (n=438)	152	0%	3% (4/152)
Kondo[15]	1990–2008	HCC with invasion to portal vein (n=48)	5	0%	0% (0/5)
The current study	1992–2008	HCC with PVTT vp3 and vp4 (n=45)	19	20.9%	0% (0/19)

HCC hepatocellular carcinoma, *PVTT* portal vein tumor thrombus, *vp2* the tumor thrombus within the second branch of the portal vein branch, *vp3* tumor thrombus extending to the first branch of portal vein, *vp4* tumor thrombus extending to the trunk or contra-lateral first branch of portal vein

[a] Tumor thrombus in vp4 limited near bifurcation, and did not protrude into the main trunk or contra-lateral branch of portal vein. These patients did not undergo tumor thrombectomy

[b] Major vasculature involves not only portal vein but hepatic vein, including infra vena cava

liver failure. Additional postoperative TACE, intraportal venous chemotherapy, or intra-arterial infusion chemotherapy with systemic biotherapy should be considered because surgery alone cannot provide a long-term survival.[27–29]

Conclusions

A hepatectomy and thrombectomy for vp4 may make it possible to not only avoid acute portal occlusion due to tumor thrombus but also provide a comparable survival benefit with a hepatectomy for vp3. This aggressive procedure is therefore considered to be a safe and effective treatment modality in selected patients with HCC and vp4.

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2009 SSAT QUICK SHOT PRESENTATION

Predicting Major Complications after Laparoscopic Cholecystectomy: A Simple Risk Score

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Abstract
Introduction Reported morbidity varies widely for laparoscopic cholecystectomy (LC). A reliable method to determine complication risk may be useful to optimize care. We developed an integer-based risk score to determine the likelihood of major complications following LC.
Methods Using the Nationwide Inpatient Sample 1998–2006, patient discharges for LC were identified. Using previously validated methods, major complications were assessed. Preoperative covariates including patient demographics, disease characteristics, and hospital factors were used in logistic regression/bootstrap analyses to generate an integer score predicting postoperative complication rates. A randomly selected 80% was used to create the risk score, with validation in the remaining 20%.
Results Patient discharges (561,923) were identified with an overall complication rate of 6.5%. Predictive characteristics included: age, sex, Charlson comorbidity score, biliary tract inflammation, hospital teaching status, and admission type. Integer values were assigned and used to calculate an additive score. Three groups stratifying risk were assembled, with a fourfold gradient for complications ranging from 3.2% to 13.5%. The score discriminated well in both derivation and validation sets (*c*-statistic of 0.7).
Conclusion An integer-based risk score can be used to predict complications following LC and may assist in preoperative risk stratification and patient counseling.

Keywords Laparoscopic cholecystectomy · Complications · Nationwide Inpatient Sample · Outcomes

Introduction

Laparoscopic cholecystectomy (LC) has been established as the "gold standard" in the treatment of symptomatic gallbladder disease. Currently, greater than 80% of cholecystectomies are performed laparoscopically.[1] Decreased postoperative pain and ileus, earlier oral intake, decreased length of stay, improved cosmetic results, and decreased mortality are known advantages of LC over open cholecystectomy (OC).[2–5]

LC is a relatively safe operative procedure with reported mortality less than 1%.[3,5,6] Investigation of morbidity associated with LC has largely focused on comparing LC and OC, with significant variation in the number and inclusion of intraoperative and postoperative complications.[7–10] Conversion from LC to OC has been reported to occur in 2–15% of cases.[2,4,5] Utilizing preoperative factors,

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methods have been developed predicting individual patient risk for operative conversion.[4,11–14]

While perioperative mortality remains low for LC, the risk of postoperative complications for individual patients is unknown. The specific aim of this study was to develop a simple scoring system to predict the risk of developing a major postoperative in-hospital complication following laparoscopic cholecystectomy using a validated set of major complications in a large national database. The ability to accurately predict an individual patient's risk of developing a postoperative complication based on preoperative information may improve preoperative risk stratification and better facilitate patient counseling.

Materials and Methods

Database and Cohort Assembly

The Nationwide Inpatient Sample (NIS) was queried between 1998 and 2006 for patient discharges LC (International Classification of Diseases, 9th revision,[15] clinical modification (ICD-9-CM) procedure code 51.23, procedure code 51.22 with concomitant V64.4, V64.41). The NIS, a part of the Healthcare Cost and Utilization Project, is a national, all-payer discharge database containing information for approximately 7 million hospital discharges annually. This represents a stratified sample of 20% of nonfederal US community hospitals from participating states, including academic and specialty hospitals. The NIS weighting strategy facilitates population-based estimates to be drawn at the national level. All statistical analyses were performed based on these survey weights; therefore, results are reported as either unweighted (actual) or weighted (national) frequencies.[16]

We excluded patients less than 18 years of age or older than 95 years of age from further analyses (n=31,425). Patients with missing data including sex (1,234), teaching status (571), and admission type (80,577) were excluded. Patients with newborn, trauma, or other admission type were excluded (217).

Patients were categorized into two broad groups representing severity of disease by whether biliary tract inflammation or cancer was present. Biliary tract inflammation was defined by the presence of acute cholecystitis, chronic cholecystitis, cholangitis, or biliary malignancy using ICD-9 diagnoses codes (575.0, 574.00, 574.01, 575.12, 574.8, 574.6, 574.3, 576.1, 577.0, 577.1, 156.0, 156.1, 156.2, 156.8, 156.9, 155.0, 155.1, 571.6, 574.1, 575.1, 574.7, 574.4, 575.5, 576.4, 576.3) Cholelithiasis without the presence of cholecystitis (biliary colic) was defined to have no biliary tract inflammation (ICD-9 diagnoses codes; 574.2, 574.9, 574.5). Other diagnosis/indications for LC were excluded from further analyses (8,630).

Records identified for the study period were divided into two sets for score development and validation via previously described methods by Simons et al.[17,18] The development group represented a randomly selected 80% of the initial cohort, while the remaining 20% were isolated to be used as the validation set.

Outcome Measure

The primary outcome measure was development of a major postoperative in-hospital complication. The specific diagnoses and codes were chosen based on their validation as true complications rather than comorbidities using ICD-9 diagnoses and procedure codes by the work of Lawthers et al.[19] Postoperative complications were defined by secondary diagnoses including (1) postoperative infection (except wound and pneumonia) (008.45, 320.00–.99, 510.0, 510.9, 513.1, 519.2, 590.10–590.11, 590.80, 683), (2) acute myocardial infarction (410.00–410.91), (3) aspiration pneumonia (507.0), (4) deep venous thrombosis and pulmonary embolism (415.1, 451.11, 451.19, 451.2, 451.81, 453.8), (5) postoperative pulmonary compromise (514, 518.4, 518.5, 518.81, 518.82), (6) postoperative gastrointestinal hemorrhage (530.82, 531.00–.21, 531.40–.41, 531.60–.61, 532.00–.21, 532.40–.41, 532.60–.61, 533.00–.21,

Table 1 Romano Adaption of the Charlson Comorbidity Index; Points Assigned if Disease is Present

Patent comorbidity	Points
AIDS	6
Cerebrovascular disease	1
Chronic pulmonary disease	1
Congestive heart failure	1
Connective tissue/rheumatic disease	1
Dementia	1
Diabetes	
Without end organ damage	1
With end organ damage	2
Hemiplegia	2
Liver disease	
Mild–moderate	1
Severe	3
Myocardial infarction	1
Peripheral vascular disease	1
Renal disease	2
Ulcer disease	1
Cancer	2
Metastatic	6

533.40–.41, 533.60–.61, 534.00–.21, 534.40–.41, 534.60–.61, 535.01, 535.11, 535.21, 535.31, 535.41, 535.51, 535.61, 578.9), (7) reopening of a surgical site (01.23, 03.02, 06.02, 34.03, 35.93, 39.49, 54.12, 54.61), and (8) procedure-related lacerations or perforations (530.4, 569.83, 575.4, 29.51, 31.61, 33.41, 33.43, 42.82, 44.61, 46.71, 46.75, 48.71, 50.61, 51.91, 55.81, 56.82, 57.81, 58.41, 69.47).

Predictor Variables

Covariates hypothesized to affect the development of major postoperative in-hospital complications were identified prospectively. Patient sex, age (grouped as <35 years, 35–64 years, ≥65 years), comorbidities (represented by Charlson score), the presence of biliary tract inflammation, emergent versus elective hospital admission, and hospital teaching status were evaluated.

Patient comorbidities were assessed by the Romano modification of the Charlson Score.[20,21] The Charlson score uses ICD-9 diagnosis and procedure codes to give a weighted, risk-adjusted comorbidity index value for an individual patient. The Charlson score takes into consideration any comorbid conditions that occurred within a year before diagnosis and that met the inclusion criteria as defined by the index parameters. We collapsed the groups

Table 2 Univariate Analyses Comparing Patient Demographics for the 80% Development Set and the 20% Validation Set, Nationwide Inpatient Sample 1998–2006

	Development set (80%) *n* (%)	Validation set (20%) *n* (%)	*p*
Total (*n*=561,923)	449,539	112,384	
Sex			0.56
Male	141,967 (31.6)	35,389 (31.5)	
Female	307,572 (68.4)	76,995 (68.5)	
Age (years)			0.42
<35	90,953 (20.2)	22,788(24.7)	
35–64	208,487 (46.4)	52,303 (46.5)	
≥65	150,099 (33.4)	37,293 (33.2)	
Charlson score			0.46
0	306,116 (68.1)	76,529 (67.9)	
1	93,138 (20.7)	23,141 (20.6)	
2	32,032 (7.1)	8,052 (7.2)	
≥3	18,253 (4.1)	4,662 (4.3)	
Indication			0.83
No inflammation	14,062 (3.1)	3,552 (3.2)	
Inflammation	435,477 (96.9)	108,832 (96.8)	
Admission			0.87
Elective	114,293 (25.4)	28,546 (25.4)	
Emergent	335,246 (74.6)	83,838 (74.6)	
Hospital type			0.27
Nonteaching	290,614 (64.5)	72,456 (64.5)	
Teaching	158,925 (35.5)	39,928 (35.5)	
Major complication			0.70
Yes	29,170 (6.5)	7,328 (6.5)	
No	420,369 (93.5)	105,056 (93.5)	
Complication type			0.56
Postoperative infection	2,126 (0.5)	522 (0.5)	
Myocardial infarction	1,475 (0.3)	341 (0.3)	
Aspiration pneumonia	1,060 (0.2)	257 (0.2)	
Deep venous thrombosis/pulmonary embolism	1,277 (0.3)	300 (0.3)	
Pulmonary compromise	5,659 (1.3)	1,424 (1.3)	
Gastrointestinal hemorrhage	14,831 (3.3)	3,736 (3.3)	
Opening surgical site	432 (0.1)	120 (0.1)	
Laceration/perforation	2,340 (0.5)	628 (0.6)	

as follows: 0 (no pre-existing comorbidity), 1 (one comorbid condition), 2 (at least one comorbid condition), and ≥3 (more sever comorbid condition or combination of at least two lesser comorbid conditions) (Table 1).

Patients were categorized by the presence of biliary tract inflammation (inflammation present versus not present), hospital admission status (elective versus emergent), and hospital teaching status (nonteaching versus teaching).

Statistical Analysis

All analyses were performed using advanced survey methods in SAS version 9.1 (SAS Institute, Cary, NC, USA). Univariate analyses of covariates were performed using chi-square tests. Those with statistical significance at the $p<0.05$ level were combined into a logistic regression model for the outcome of major postoperative in-hospital complication; 200 bootstrap samples were then selected, and the median result for beta coefficients was then reported.

The medians for the beta coefficients from the logistic regression model were then used to develop an integer-based weighted point system for stratifying postoperative complication risk.[17] The referent for each variable was assigned a value of zero. For the remaining values of the variables, the lowest beta coefficient was given a value of 1, and the coefficients for the others were adjusted proportionately, rounding to the nearest integer. Individual scores were assigned by summing the individual risk factor points. The risk scores were stratified as follows: (1) low, 0–6; (2) medium, 7–13; and (3) high, 14–18.

Within the development set, the risk score was calculated for each patient-discharge record, and discrimination was assessed using the area under the receiver operating characteristic (ROC) curve.

For the validation of the risk score, the previously isolated 20% random sample was used. The risk score model was applied, and discrimination was assessed by ROC curve analysis. Because of the nature of the survey methods used to report information in the NIS, calibration cannot be directly assessed. In order to judge calibration overall, comparison tables were constructed to assess the observed versus expected postoperative complication rates by deciles in both the development and validation sets.

Additionally, we performed a sensitivity analysis to evaluate the 12% missing data for hospital admission type (emergent versus elective). In order to interpret missing data, we defined laparoscopic cholecystectomy performed for acute cholecystitis to be "emergent" and assigned those patient-discharge records with missing admission type accordingly. Bootstrap sampling, score assignment, and ROC curve discrimination were repeated with no significant difference in outcome.

Results

Study Cohort Characteristics

Querying the NIS for the years 1998–2006, we identified 682,827 (unweighted) patient-discharge records with a procedure code for laparoscopic cholecystectomy. Following application of previously described exclusion criteria and performing random sampling, 561,923 records remained, with 449,539 records in the 80% development set and 112,384 records in the 20% validation set. Demographics of the development and validation sets are demonstrated in Table 2, with no significant difference between the two sets. The majority of subjects were female (68%), with a mean age of 53.5 years and a Charlson score of 0 (68%). Inflammatory biliary conditions including acute and chronic cholecystitis, cholangitis, and biliary tract cancer comprised the majority of cases (97%). LC was most often performed emergently (75%) in nonteaching hospitals (65%).

The overall major postoperative in-hospital complication rate was 6.5%. The most frequent complications included gastrointestinal hemorrhage (3.3%), pulmonary compro-

Table 3 Multivariable Logistic Regression Analysis Evaluating Predictors of Complications Following Laparoscopic Cholecystectomy, Nationwide Inpatient Sample 1998–2006

	AOR (95% CI)	*p*
Sex		
Female	Referent	
Male	1.17 (1.14–1.20)	<0.0001
Age (years)		
<35	Referent	
35–64	1.51 (1.46–1.57)	<0.0001
≥65	2.07 (1.99–2.15)	<0.0001
Charlson score		
0	Referent	
1	1.75 (1.70–1.80)	<0.0001
2	2.35 (2.27–2.44)	<0.0001
≥3	2.80 (2.68–2.91)	<0.0001
Indication		
No inflammation	Referent	
Inflammation	1.21 (1.13–1.30)	<0.0001
Admission		
Elective	Referent	
Emergent	1.54 (1.50–1.59)	<0.0001
Hospital type		
Nonteaching	Referent	
Teaching	1.17 (1.14–1.19)	<0.0001

AOR adjusted odds ratio

Table 4 Integer Score Assignment Algorithm Based on Beta Coefficients from the Regression Model

Factor	Level	Point Value
Sex	Female	0
	Male	1
Age Group (years)	<35	0
	35–64	3
	≥65	5
Charlson Score	0	0
	1	4
	2	6
	≥3	7
Inflammation	No	0
	Yes	1
Admission Type	Elective	0
	Emergent	3
Hospital Type	Teaching	0
	Nonteaching	1

Available online: http://umassmed.edu/surgery/LC_complication_risk_score.aspx

mise (1.3%), postoperative infection (0.5%), and laceration/perforation (0.5%).

Prediction of Postoperative Complications

All variables hypothesized to predict the development of major postoperative in-hospital complications were included in logistic regression analyses with results shown in Table 3. Male sex (adjusted odds ratio (AOR) 1.17, 95% confidence interval (CI) 1.14–1.20), advanced age (age ≥65 years, AOR 2.07, 95% CI 1.99–2.15), the presence of increasing patient comorbidity (Charlson score 2, AOR 2.35, 95% CI 2.27–2.44; Charlson score ≥3, AOR 2.80, 95% CI 2.68–2.91), biliary tract inflammation (AOR 1.21, 95% CI 1.13–1.30), emergent surgery (AOR 1.54, 95% CI 1.50–1.59), and LC performed at a nonteaching hospital (AOR 1.17, 95% CI 1.14–1.19) were all found to be significant predictors of the developing major postoperative in-hospital complications.

The median beta coefficients from the logistic regression model were converted into integers to calculate a simple, numeric risk score estimating the risk of developing major postoperative complications. The referent groups were assigned a value of 0. Simple proportional calculations resulted in integer scores ranging from 1 to 7, with a total possible score of 18 (Table 4).

The scores were then grouped into three clinically relevant risk groups with a fourfold gradient for developing major postoperative in-hospital complications ranging from 3.2% to 13.5% (Fig. 1).

The model discriminated well with an area under the receiver operating curve of 0.7 in both the development and calibration sets. Graphical assessment of model calibration is depicted for both the development (Fig. 2) and validation sets (Fig. 3).

Discussion

We developed a simple risk score to predict individual patient risk of developing a major postoperative in-hospital complication following LC for use in the clinical setting. Factors predictive of developing complications include advanced age, higher Charlson score, male sex, biliary tract inflammation, emergent surgery, and surgery performed in a nonteaching hospital. The overall incidence of major postoperative in-hospital complications was 6.5%, ranging across the score groups from 3.2% to 13.5%.

Reported morbidity for patients undergoing LC varies widely in the literature. In a meta-analysis of mortality and complications associated with LC, Shea et al.[10] described the tremendous variability in the types of complications reported among LC case series, with some authors

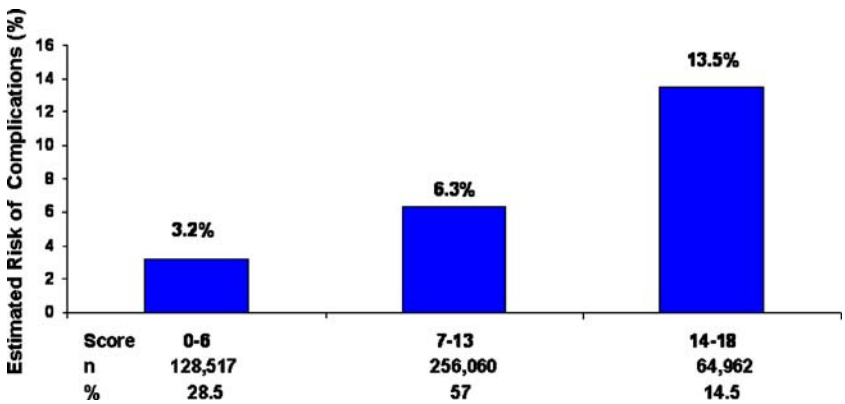

Figure 1 Estimated risk of developing a major postoperative in-hospital complication following laparoscopic cholecystectomy by score groups.

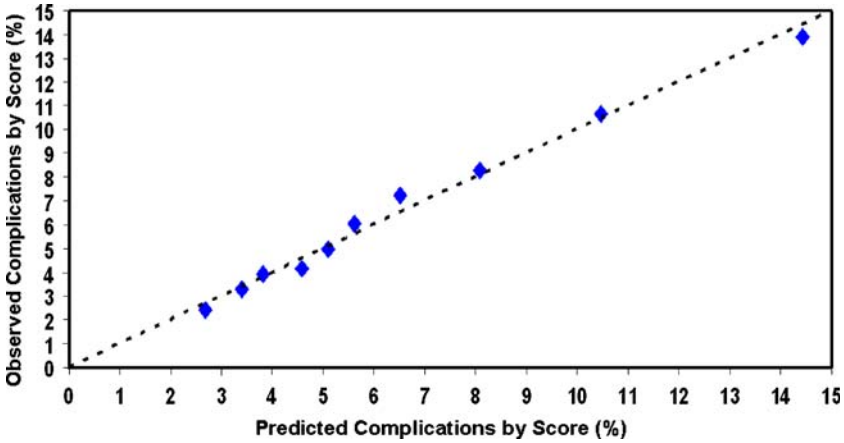

Figure 2 Observed versus expected postoperative complications following laparoscopic cholecystectomy by deciles in the 80% development set.

providing exhaustive lists,[22–24] while others focused on a more limited set.[25–27] Reporting on surgical management of acute cholecystitis using the National Hospital Discharge Survey, Csikesz et al.[2] reported morbidity associated with LC to be 17% with complications defined as bile duct injury, bleeding, accidental puncture, electrolyte abnormality, and wound infection. In a single institution retrospective review comparing LC with OC for symptomatic cholelithiasis, Brune et al.[8] reported postoperative complications to occur in 2.1% of LC and 3.7% OC with complications defined as wound hematoma, wound infection, hernia, pancreatitis, cystic-duct leak, intra-abdominal abscess, common bile duct (CBD) stone, CBD stenosis, subhepatic bile collection, hemorrhage, thrombosis, pneumonia, emboli, myocardial infarction, and renal insufficiency. Reviewing operative complications associated with LC, Shamiyeh et al.[7] reported pneumoperitoneum, bleeding (trocar sites, vascular injury, liver bed), biliary complications (spilled stone, biliary leak, bile duct injury), and bowel injury to be associated with LC.

Risk scores have been developed for use in predicting individual patient risk of operative conversion from LC to OC.[11] However, conversion to OC alone does not necessarily represent a complication but may reflect considered surgical judgment. The risk score we developed in the current study may be used in hospital settings to assess individual patient risk of developing a major postoperative complication. The clinical utility of this tool is twofold. First, it may be used in patient counseling and informed consent and, secondly, in preoperative patient risk stratification.

Prior to invasive procedures including surgery, physicians have a responsibility to educate patients on the "nature of their condition and its expected course, about the benefits and risks of the proposed treatment, and of alternative treatment or nontreatment"[28] to facilitate informed decision making. Currently for LC, general discussion of mortality and complication risk can be addressed, while individualized risk is currently unavailable. Using the simple risk score we developed, average patient risk may be quickly calculated to predict major postoperative complications. This information may be used in preoperative counseling, with patients in different risk groups (low, medium, and high) counseled accordingly.

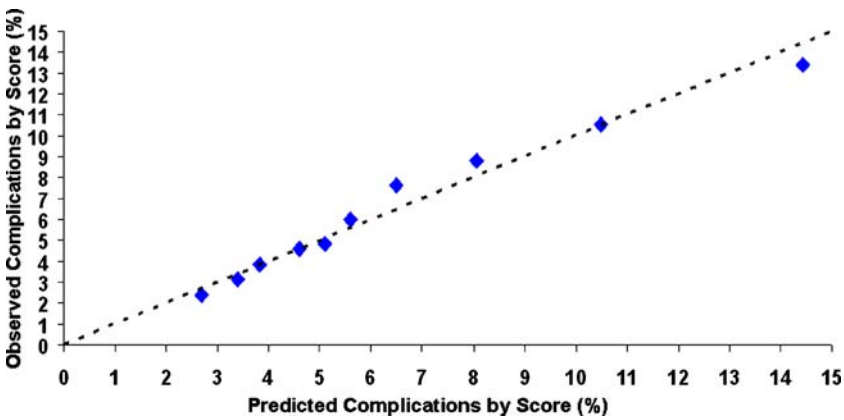

Figure 3 Observed versus expected postoperative complications following laparoscopic cholecystectomy by deciles in the 20% validation set.

Preoperative risk stratification using risk scores has been described for other gastrointestinal surgeries.[17,18,29] As the majority of patients undergoing LC present emergently with evidence of biliary tract inflammation, early surgery is generally indicated.[30] The risk score may be utilized in these patients to anticipate possible complications. Patients in the highest risk score (13.5%) may be admitted for observation, assessed more frequently postoperatively, considered for admission to telemetry floors, or possibly undergo surgery in hospitals equipped to manage major postoperative complications including intensive care unit services. The use of risk scores predicting perioperative complications after surgery may have important financial implications for hospitals in the future. Higher dollar allocation may be necessary for high-risk patients as they may require additional care and services than low-risk patients to prevent or treat complications. Additional studies investigating the cost of treating high-risk patients undergoing surgery may address this important issue.

While our risk score predicting major complications following LC may be useful clinically, its limitations must be acknowledged. The NIS is an administrative database and therefore lacks certain clinical information including patient-level factors (lab values, ultrasound results, preoperative performance status), operative data (blood loss, transfusions), and long-term follow-up/re-admission information. We evaluated the development of major postoperative in-hospital complications using a validated set of ICD-9 codes; however, the true complication rate may be underestimated, as individual medical records cannot be reviewed. Additionally, due to insufficient coding specifications in NIS, we were unable to accurately assess the important complication of bile duct injury or leak as the use of ICD-9 codes to evaluate bile duct injuries has been demonstrated to significantly underestimate the occurrence of bile duct injuries.[31,32] Future validation of the risk score in other datasets including clinical information may allow bile duct injury to be assessed as a complication. The survey methods employed by the NIS for data sampling do not allow for statistical significance testing for the score's calibration. The plots comparing the observed values and expected values must instead be inspected with clinical judgment. Surgeon and hospital volume have also not been factored in despite reports of their impact on outcomes.[33,34]

Despite these limitations, our model performs well in its discriminatory ability. The use of a nationally representative database for score development imparts generalizability over single-institution case series.[35] Our score is derived using information from all types of patients and hospitals. The simplified format, with only six preoperatively available factors included, facilitates clinic or bedside application.

Our report includes an internal validation through the use of the randomly selected 20% cohort, but future studies should be undertaken to broadly validate the score in hospital settings. In the future, we plan to prospectively validate our risk score both in our own institution and others. Additionally, we plan to use a different national database to externally validate our risk score in addition to our internal validation set. Subsequent prospective studies may be performed to assess possible changes in postoperative complications and patient satisfaction after proposed laparoscopic cholecystectomy.

Conclusion

Our results demonstrate that a simple integer-based risk score can be used to preoperatively predict major postoperative in-hospital complications following laparoscopic cholecystectomy. This score may be useful in patient education and informed consent discussions, as well as in preoperative risk stratification.

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2009 SSAT PLENARY PRESENTATION

Margin Positive Pancreaticoduodenectomy Is Superior to Palliative Bypass in Locally Advanced Pancreatic Ductal Adenocarcinoma

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Abstract
Background Pancreatic ductal adenocarcinoma is an aggressive disease. Surgical resection with negative margins (R0) offers the only opportunity for cure. Patients who have advanced disease that limits the chance for R0 surgical resection may undergo margin positive (MP) pancreaticoduodenectomy (PD), palliative surgical bypass (PB), celiac plexus neurolysis alone (PX), or neoadjuvant chemoradiation therapy in anticipation of future resection.
Objective The aim of this study was to determine if there is a difference in the perioperative outcomes and survival patterns between patients who undergo MP PD and those who undergo PB for locally advanced disease in the treatment of pancreatic ductal adenocarcinoma.
Methods We reviewed our pancreatic surgery database (January 2005–December 2007) to identify all patients who underwent exploration with curative intent of pancreatic ductal adenocarcinoma of the head/neck/uncinate process of the pancreas. Four groups of patients were identified, R0 PD, MP PD, PB, and PX.
Results We identified 126 patients who underwent PD, PB, or PX. Fifty-six patients underwent R0 PD, 37 patients underwent MP PD, 24 patients underwent a PB procedure, and nine patients underwent PX. In the PB group, 58% underwent gastrojejunostomy (GJ) plus hepaticojejunostomy (HJ), 38% underwent GJ alone, and 4% underwent HJ alone. Of these PB patients, 25% had locally advanced disease and 75% had metastatic disease. All nine patients in the PX group had metastatic disease. The mean age, gender distribution, and preoperative comorbidities were similar between the groups. For the MP PD group, the distribution of positive margins on permanent section was 57% retroperitoneal soft tissue, 19% with more than one positive margin, 11% pancreatic neck, and 8% bile duct. The perioperative complication rates for the respective groups were R0 36%, MP 49%, PB 33%, and PX 22%. The 30-day perioperative mortality rate for the entire cohort was 2%, with all three of these deaths being in the R0 group. The median follow-up for the entire cohort was 14.4 months. Median survival for the respective groups was R0 27.2 months, MP 15.6 months, PB 6.5 months, and PX 5.4 months.
Conclusions Margin positive pancreaticoduodenectomy in highly selected patients can be performed safely, with low perioperative morbidity and mortality. Further investigation to determine the role of adjuvant treatment and longer-term follow-up are required to assess the durability of survival outcomes for patients undergoing MP PD resection.

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Keywords Margin positive pancreaticoduodenectomy · Locally advanced pancreatic adenocarcinoma · Palliative surgical bypass

Introduction

Pancreatic adenocarcinoma is the fourth leading cause of cancer death in the USA. In 2008, there were an estimated 37,680 new cases diagnosed and 34,290 deaths. The overall 5-year survival rate is less than 5%.[1] Surgical resection of pancreatic adenocarcinoma is the only potentially curative therapy, and it improves the overall 5-year survival rate to 15–20%.[2,3] Unfortunately, most patients are not candidates for surgical resection at the time of diagnosis due to the presence of locally advanced disease, distant metastasis, or significant medical comorbidities.

Locally advanced pancreatic adenocarcinoma is generally defined by the presence of tumor abutment of the celiac trunk/superior mesenteric artery (SMA), or greater than 180° involvement/thrombosis of the superior mesenteric (SMV)/portal venous (PV) axis.[4–7] Preoperative evaluation of patients is in part designed to assess these anatomic factors and is successful in selecting appropriate candidates for resection 70–85% of the time.[8] High-quality contrast-enhanced multidetector computed tomography (CT) scan, magnetic resonance imaging (MRI), and endoscopic ultrasound examination are common diagnostic modalities used to determine tumor resectability. If unequivocal findings of locally advanced disease are encountered on preoperative imaging, patients are considered for neoadjuvant chemotherapy/radiation in an attempt to downstage the tumor. These patients, as well as those with distant disease, may also be candidates for palliative surgical management to alleviate tumor-related symptoms, such as gastrointestinal or biliary obstruction and refractory abdominal pain. In all, perhaps only up to 20% of patients at the time of diagnosis are eligible to undergo surgical resection, and recent evidence suggests that even this small group of potentially resectable patients is undertreated in the USA.[9,10]

Controversy remains as to the proper course of management when the patient with potentially resectable pancreatic adenocarcinoma is found in the operating room to have tumor approaching the hepatic artery (HA), SMA, or the SMV/PV axis. Intraoperative assessment in these cases is made challenging by the difficulty in distinguishing true tumor extension from peritumoral inflammation. In the past, palliative surgical bypass of the gastrointestinal and biliary tracts has been the standard course of therapy in many of these cases.[11] In recent years, with the improving safety of the Whipple procedure in many high volume centers, more of these tumors are being resected.[12,13] Proceeding with resection not only may result in complete disease removal (R0) but may also lead to microscopically positive (R1) or macroscopically positive (R2) resection margins. There are a number of factors that the surgeon must weigh before proceeding with this type of resection. Most important is safety, as the extensive dissection along the mesenteric vessels that is required to remove these tumors has the potential to cause visceral vessel injury and substantial blood loss. Other factors to consider are the potential benefit of tumor debulking upon the success of adjuvant treatment, the quality of life of the patient, and the effect of resection upon long-term survival. There is strong evidence to suggest that microscopically positive surgical margins are an important negative prognostic indicator and that the results of margin positive (MP; R1) resection are not equivalent to that of R0 resection.[14–18] However, the question remains as to how MP resection compares to palliative surgical bypass (PB) in borderline resectable disease. The objective of this study is to determine if there is a difference in the perioperative outcomes and survival patterns between patients who undergo margin positive pancreaticoduodenectomy (MP PD) and those who undergo palliative bypass for locally advanced disease in the treatment of pancreatic ductal adenocarcinoma.

Methods

We performed a retrospective review of our prospectively acquired hepatopancreatobiliary (HPB) surgery database in the Department of Surgery of Thomas Jefferson University. The database has been approved for data acquisition and query by our Institutional Review Board. Our database first began enrolling patients prospectively in January 2005, and we analyzed the data on consecutive patients explored for pancreatic ductal adenocarcinoma of the head, neck, or uncinate process of the pancreas over a 3-year period until December 2007. Four broad groups of patients were identified, those who underwent pancreaticoduodenectomy (both margin negative (R0) and MP patients were individually analyzed), those who underwent a palliative surgical bypass (including any combination of gastrointestinal or biliary bypass), and those who underwent celiac plexus neurolysis alone (PX). We analyzed patient demographics, preoperative comorbidities, operative techniques, intraoperative and postoperative variables and complications, postoperative hospital length of stay, and survival.

All of the patients included in this study underwent a standard preoperative evaluation that included a history and physical exam, standard laboratory evaluation along

with measurement of serum tumor markers (carcinoembryonic antigen (CEA) and cancer antigen (CA) 19–9), and some combination of high-quality contrast-enhanced cross-sectional imaging (CT or MRI). Many but not all patients had endoscopic ultrasound and/or endoscopic retrograde cholangiopancreatography. Patients were deemed potentially resectable and candidates for exploration if they lacked tumor involvement of the celiac axis/HA/SMA and had a patent SMV/PV with less than 180° tumor abutment and had no evidence of distant metastasis. Based upon this evaluation, patients were taken for operative exploration with the intent for a curative margin negative resection. All operations were performed at the Thomas Jefferson University Hospital by one of three experienced pancreatic surgeons (CJY, EPK, ELR).

Pancreaticoduodenectomy was performed with pylorus preservation whenever possible. The visceral vessels were routinely skeletonized, intentionally leaving no tissue behind along the SMV/PV or right lateral aspect of the SMA. A standard technique of end-to-side pancreaticojejunostomy and hepaticojejunostomy (HJ) with downstream retrocolic duodenojejunostomy was used, as has been previously described.[19] Patients were considered intraoperatively for palliative surgical bypass if they were found on exploration to have occult metastatic disease (and had evidence of impending gastrointestinal or biliary obstruction) or if their tumor was deemed locally advanced preventing an attempt at margin negative resection. The technique for gastrointestinal bypass was most commonly a two-layered hand-sewn side-to-side retrocolic isoperistaltic gastrojejunostomy (GJ). Biliary bypass was typically performed as a single-layer end-to-side Roux-en-Y hepaticojejunostomy. Celiac plexus neurolysis (nerve block) was performed by using a total volume of 40 ml of 50% ethanol, injecting 20 ml of the solution on either side of the aorta, at the level of the celiac axis. All patients with occult metastasis discovered at the time of surgery underwent celiac plexus neurolysis. Variable numbers of patients in the PD and PB groups underwent this procedure based upon patient factors such as preoperative pain, as well as surgeon preference. There was incomplete data on the number of R2 resections in the MP group, and therefore, this was not included in the results. Resected specimens underwent histopathologic evaluation for tumor size, histologic grade, lymph node involvement, lymphovascular invasion, perineural invasion, and resection margin status. Disease was staged according to the American Joint Committee on Cancer (AJCC) guidelines and meticulously reported per the College of American Pathologists guidelines.[20,21] Bile duct, pancreatic neck, and retroperitoneal soft tissue (uncinate) margins were routinely evaluated intraoperatively by frozen section analysis and were further assessed postoperatively on permanent examination of inked margins. R0 resections were considered those that lacked tumor involvement of the inked margins, whereas R1 resections had microscopically positive margins on the specimen side of the resection specimen.

Data collection was performed using information within our clinical HPB database and supplemented by reviewing patient charts and computer records. Demographic data were acquired on patient age, gender, race, social history, and body mass index (BMI). Preoperative comorbidities such as coronary artery disease, hypertension, diabetes, chronic obstructive pulmonary disease (COPD), peptic ulcer disease, cerebrovascular disease, peripheral vascular disease, and pancreatitis were evaluated. Operative variables such as estimated blood loss, transfusion requirement, and type of resection (classic Whipple vs pylorus preserving) were acquired. Postoperative complications were examined including wound infection, myocardial infarction, arrhythmia, venous thrombosis, pulmonary embolism, pneumonia, urinary tract infection, pancreatic fistula, and delayed gastric emptying, among others. Complications were graded using a system adapted from DeOliveira et al.[22] Pancreatic fistula was defined and graded by a system adapted from the International Study Group on Pancreatic Fistula (ISGPF).[23] Postoperative hospital length of stay and 30-day mortality were recorded. Hospital readmission rates included admission to the Thomas Jefferson University Hospital as well as to outside facilities. Survival was determined using time of last clinical follow-up, direct communication with patients and families, and the Social Security Database.

Statistical Methods

Demographic and clinical characteristics were summarized using means, medians, and ranges (continuous outcomes) and frequencies and percentages (categorical outcomes). Groups were compared using Wilcoxon rank-sum tests for continuous outcomes and Fisher's exact test for categorical outcomes. Survival distributions were estimated using the Kaplan–Meier method and groups were compared using the log-rank test. In addition, Cox proportional hazards regression was used to estimate the hazard ratio between groups, after adjusting for potential confounders. A propensity score model was used to adjust for factors associated with choice of procedure (age, diabetes, preoperative blood urea nitrogen, and COPD). A logistic regression model was used to calculate the probability of having a procedure given a particular preoperative profile, and this probability was

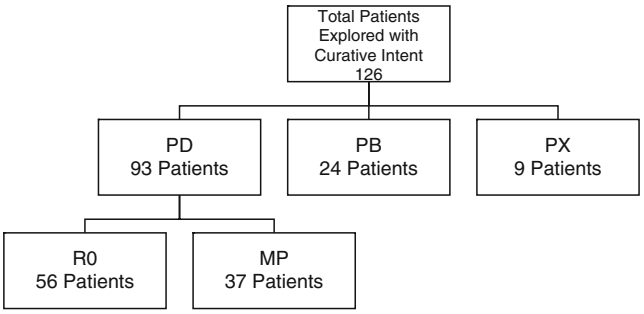

Figure 1 Patients explored with curative intent. *R0* margin negative pancreaticoduodenal resection, *MP* margin positive pancreaticoduodenal resection, *PB* palliative bypass, *PX* celiac plexus neurolysis, *PD* pancreaticoduodenectomy.

then included as a covariate in the proportional hazards model. Adjusted and unadjusted estimates of the geometric mean ratio for estimated blood loss and length of stay were calculated using linear regression. Logistic regression was used to estimate unadjusted and adjusted odds ratios for the presence of postoperative complications. Significance was accepted at the $p<0.05$ level.

Results

Demographics and Preoperative Characteristics

During the 3-year study period January 2005–December 2007, we identified 126 patients with pathologically confirmed pancreatic ductal adenocarcinoma that underwent surgical exploration with curative intent (Fig. 1). Of this cohort, 93 patients underwent pancreaticoduodenectomy; 56 of these patients had margin negative resections (R0), while 37 of these patients had margin positive resections. Twenty-four patients were deemed unresectable due to metastatic disease ($N=18$) or local tumor extension ($N=6$) and underwent a palliative surgical bypass procedure. Nine patients were found to have metastatic disease without indication for bypass and underwent celiac plexus neurolysis alone. The male/female ratio of the entire cohort was 48:52%, and the median age was 64 years (Table 1). The median preoperative serum albumin level was 4.0 g/dl and was similar between groups. Patients in the PB and PX groups had higher preoperative median CA 19–9 and CEA levels

Table 1 Demographics and Preoperative Variables

	Total *n* (%)	R0	MP	*p* value (R0 vs MP)	PB	*p* value (MP vs PB)	PX
Total, *n*	126 (100)	56	37		24		9
Gender, *n* (%)							
Male	61 (48)	28 (50)	19 (51)	NS	12 (50)	NS	2 (22)
Female	65 (52)	28 (50)	18 (49)	NS	12 (50)	NS	7 (78)
Age (years)							
Median	64	64.5	65	NS	62	NS	67
Range	35–86	41–84	35–86	–	43–80	–	51–81
Albumin (g/dl)							
Median	4.0	4.2	3.9	NS	4.0	NS	4.0
Range	2.0–5.5	2.3–5.1	2.0–5.0	–	2.6–5.5	–	3.3–4.3
CA 19–9 (U/ml)							
Median	284	167	259	NS	642	NS	852.5
Range	2–80,809	2–6,994	3–80,809	–	2–11,655	–	74–9,348
CEA (ng/ml)							
Median	2.9	2.4	2.7	NS	3.5	NS	5.45
Range	0.5–161.1	0.9–29.8	0.8–81.2	–	0.5–17.1	–	0.9–161.1
Preoperative comorbidities, *n* (%)	77 (61.0)	28 (50.0)	28 (75.6)	<0.0174	16 (66.6)	<0.0014	5 (55.5)
DM	48 (38.1)	25 (44.6)	15 (40.5)	NS	5 (20.8)	NS	3 (33.3)
Tobacco	41 (32.5)	21 (37.5)	9 (24.3)	NS	9 (37.5)	NS	2 (22.2)
BMI							
Median	25	25	25	NS	25	NS	32
Range	15–41	15–41	18–36	–	15–38	–	20–37

R0 margin negative pancreaticoduodenal resection, *MP* margin positive pancreaticoduodenal resection, *PB* palliative bypass, *PX* celiac plexus neurolysis, *BMI* body mass index, *DM* diabetes mellitus

Table 2 Perioperative Variables

	Total *n* (%)	R0	MP	*p* value (R0 vs MP)	PB	*p* value (MP vs PB)	PX
Total	126 (100)	56	37		24		9
Type of procedure							
PPPD	63 (50.0)	40 (71.4)	23 (62.2)	NS	–	–	–
Classic Whipple	30 (23.8)	16 (28.6)	14 (37.8)	NS	–	–	–
GJ + HJ	14 (11.1)	–	–	–	14 (58.3)	–	–
Gastrojejunostomy alone	9 (7.1)	–	–	–	9 (37.5)	–	–
Hepaticojejunostomy alone	1 (0.8)	–	–	–	1 (4.1)	–	–
Celiac plexus neurolysis	34 (27.0)	2 (3.6)	1 (2.7)	NS	22 (91.6)	<0.0001	9 (100)
EBL (ml)							
Median	500.0	650.0	600.0	NS	200.0	<0.0001	100.0
Range	75–1,800	200–1,500	800–1,800	–	75–500	–	100–250
Complications, *n* (%)	48 (38.1)	20 (35.7)	18 (48.6)	NS	8 (33.3)	NS	2 (22.2)
Wound infection	16 (12.7)	6 (10.7)	7 (18.9)	NS	2 (8.3)	NS	1 (11.1)
Cardiac	12 (9.5)	7 (12.5)	4 (10.8)	NS	1 (4.2)	NS	0 (0)
P. fistula	7 (7.5[a])	5 (8.9)	2 (5.4)	NS	0 (0)	NS	0 (0)
DGE	5 (4.0)	1 (1.8)	3 (8.1)	NS	1 (4.2)	NS	0 (0)
Abdominal abscess	5 (4.0)	2 (3.6)	2 (5.4)	NS	1 (4.2)	NS	0 (0)
UTI	5 (4.0)	1 (1.8)	2 (5.4)	NS	1 (4.2)	NS	1 (11.1)
C. diff. colitis	3 (2.4)	0 (0)	1 (2.7)	NS	1 (4.2)	NS	1 (11.1)
Chyle leak	3 (2.4)	0 (0)	3 (8.1)	NS	0 (0)	NS	0 (0)
DVT	3 (2.4)	1 (1.8)	0 (0)	NS	1 (4.2)	NS	1 (11.1)
Length of stay (days)							
Median	7	7	7	NS	5.5	<0.0009	5
Range	3–25	3–25	5–19	–	3–13	–	4–24
Readmissions, *n* (%)	25 (19.8)	14 (25.0)	9 (24.3)	NS	2 (8.3)	NS	0 (0)

R0 margin negative pancreaticoduodenal resection, *MP* margin positive pancreaticoduodenal resection, *PB* palliative bypass, *PX* celiac plexus neurolysis, *PPPD* pylorus preserving pancreaticoduodenectomy, *EBL* estimated blood loss, *DGE* delayed gastric emptying, *P. fistula* pancreatic fistula, *UTI* urinary tract infection, *C. diff. colitis* clostridium difficile colitis, *DVT* deep venous thrombosis

[a] Calculated based on pancreaticoduodenectomy patients only

than either the R0 or MP groups. Preoperative comorbidities were observed in 61% of all the patients and in the respective groups were R0 50%, MP 76%, PB 67%, and PX 56%. The PX group had a higher median BMI (32) than the other groups (25). Five of the patients in the series received neoadjuvant chemoradiation therapy. Of these patients, four underwent R0 resection and one underwent a MP resection.

Operative Management

Of the 93 pancreaticoduodenectomies performed in this series, 63 were pylorus preserving and 30 were classic Whipple resections (Table 2). Two patients in the series underwent portal venous resection and reconstruction. Of the 24 patients who underwent palliative surgical bypass, 58% underwent GJ plus HJ, 38% underwent GJ alone, 4% underwent HJ alone, and 92% of these patients received a concomitant celiac plexus block. Of these 24 PB patients, 25% underwent

Table 3 Modified Clavien Classification of In-hospital Postoperative Surgical Complications

	R0, *n* (%)	MP, *n* (%)	PB, *n* (%)
Total patients	56	37	24
Complications			
Total	20 (35.7)	18 (48.6)	8 (33.3)
Type I	4 (7.1)	3 (8.1)	0 (0)
Type II	10 (17.9)	10 (27.0)	5 (20.8)
Type IIIa/b	5 (8.9)	5 (13.5)	2 (8.3)
Type IVa/b	0 (0)	0 (0)	1 (4.1)
Type V	1 (1.8)	0 (0)	0 (0)

Adapted from DeOliveira et al.[22]

Grade I any deviation from normal postoperative course without pharmacological/surgical treatment, *Grade II* requiring pharmacological treatment with drugs, *Grade III* requiring surgical/radiological/endoscopic intervention, *Grade IV* life-threatening complication requiring ICU management, *Grade V* death of patient, *R0* margin negative pancreaticoduodenal resection, *MP* margin positive pancreaticoduodenal resection, *PB* palliative bypass

Table 4 Pathology in Resected Patients

	Total PD, *n* (%)	R0, *n* (%)	MP, *n* (%)	*p* value (R0 vs MP)
Total, *n*	93	56	37	
T stage[20]				
T1	9 (9.7)	7 (12.5)	2 (5.4)	NS
T2	23 (24.7)	19 (33.9)	4 (10.8)	<0.05
T3	61 (65.5)	30 (53.5)	31 (83.7)	<0.05
T4	0 (0)	0 (0)	0 (0)	–
Differentiation				
Poor	15 (16.1)	10 (17.9)	5 (13.5)	NS
Moderate	71 (76.3)	40 (71.4)	31 (83.7)	NS
Well	7 (7.5)	6 (10.7)	1 (2.7)	NS
Lymph nodes				
Positive nodes, *n* (%)	61 (65.6)	32 (57.1)	29 (78.4)	<0.05
Resected median (mean)	12 (13.2)	11 (12.6)	14 (14.0)	NS
Positive median (mean)	1 (2.8)	1 (1.8)	4 (4.3)	<0.05
(+) Lymphovascular invasion	34 (36.6)	16 (28.6)	18 (48.6)	NS
(+) Perineural invasion	77 (82.8)	43 (76.8)	34 (91.8)	NS

R0 margin negative pancreaticoduodenal resection, *MP* margin positive pancreaticoduodenal resection, *PB* palliative bypass, *PD* pancreaticoduodenectomy

the procedure for borderline resectable or locally advanced disease and 75% for metastatic disease. Celiac plexus neurolysis alone was performed in nine patients, and in each such case, the patient was well palliated by a biliary endoprosthesis and there was no impending tumor encroachment on the duodenum, reflecting the lack of need for gastrojejunostomy. All patients in the PX group had metastatic disease. Median estimated blood loss for the entire cohort was 500 ml and was higher in the R0 and MP groups (650 and 600 ml, respectively) than it was in the PB and PX groups (200 and 100 ml, respectively; $p<0.05$).

Perioperative Morbidity and Mortality

The postoperative morbidity rate for the entire cohort was 38% (Table 2), reflecting differing rates per group: R0 36%, MP 49%, PB 33%, and PX 22%. The most common complications identified were wound infection 13%, cardiac 10%, pancreatic fistula 8%, delayed gastric emptying 4%, intraabdominal abscess 4%, urinary tract infection 4%, pneumonia/pleural effusion 3%, Clostridium difficile infection 2%, chyle leak 2%, and deep venous thrombosis 2%. The pancreatic fistula rate in the R0 group was 9% and in the MP group was 5%. Of these pancreatic fistulae, 43% were type A, and 57% were type B, as defined by the ISGPF. There were no type C pancreatic fistulae. Median postoperative length of hospital stay was 7 (5–25) days for patients undergoing pancreaticoduodenectomy (R0 and MP) and 5 (3–24) days for patients undergoing PB or PX ($p<0.05$). The readmission rate to either Thomas Jefferson University Hospital or outside hospitals for the entire cohort was 20%, 25% for patients undergoing pancreaticoduodenectomy, and 8% for those undergoing PB. The 30-day perioperative mortality for the entire cohort was 2%. All three of these deaths were in the R0 group (5%). Two of these patients died suddenly at home 6 and 3 days after discharge, having been progressing well both in-hospital and at home. The cause of death was not clear in either case, and they were suspected to be due to pulmonary embolus or cardiac arrhythmia. The third patient died in the hospital on postoperative day 3 when he became bradycardic and hypotensive following an episode of massive emesis with aspiration.

Table 3 shows complications classified by a system adapted from DeOliveira et al.,[22] comparing the R0, MP, and PB groups. All three groups had similar rates of high-grade types III and IV complications, while the MP group had the highest rate of type II complications.

Pathology and Surgical Margins

Of the patients who underwent surgical resection of their tumors, the patients with MP resections had significantly

Table 5 Location of Positive Margins in MP Patients ($n=37$)

Positive margin	No. of patients (%)
Retroperitoneal soft tissue	21 (57)
More than 1 positive margin	7 (19)
Pancreatic neck	4 (11)
Bile duct	3 (8)
Circumferential	2 (5)

Retroperitoneal soft tissue represents the pancreatic soft tissue adjacent to the SMV/PV ventrally and the SMA dorsally. Circumferential = soft tissue that lies dorsal to the pancreatic head and uncinate which is bounded by the inferior vena cava, aorta, and left renal vein

Table 6 Survival

	Total (n=126)	R0 (n=56)	MP (n=37)	p value (R0 vs MP)	PB (n=24)	p value (MP vs PB)	PX (n=9)
Median survival (months)	14.8	27.2	15.6	NS	6.5	<0.05	5.4
1-year survival (%)	58.7	71.5	64.9	NS	29.2	<0.05	12.5

R0 margin negative pancreaticoduodenal resection, *MP* margin positive pancreaticoduodenal resection, *PB* palliative bypass, *PX* celiac plexus neurolysis

higher percentages of T3 tumors (84% vs 54%, $p<0.05$) and lymph node involvement (78% vs 51%, $p<0.05$) and higher rates of perineural and lymphovascular invasion as compared to the R0 patients (Table 4). For the MP group, the distribution of positive margins on permanent section (Table 5) was 57% retroperitoneal soft tissue (uncinate), 19% with more than one positive margin, 11% pancreatic neck, 8% bile duct, and 5% circumferential. Of the patients with more than one positive margin, the retroperitoneal soft tissue (uncinate) was involved 86% of the time.

Survival

The median follow-up period for the entire cohort was 14.4 months. The median survival times estimated from the Kaplan–Meier curves for the respective groups were 27.2 months for the R0 group, 15.6 months for the MP group, 6.5 months for the PB group, and 5.4 months for the PX group (Table 6; Fig. 2). One-year survival rates for the groups were R0 72%, MP 65%, PB 29%, and PX 13%. When comparing the MP group with the subgroup of PB patients with locally advanced disease (PB-L; $N=6$), the median survival times were 15.6 vs 13.2 months, and the 1-year survival rates were 65% vs 50%, respectively (Table 7).

Although multivariate regression analysis did not reveal many statistically significant differences (Table 8), as would be expected, patients with smaller tumors (hazard ratio (HR), 0.78; 95% confidence interval (CI), 0.28–2.17; $p=0.63$) and those who underwent R0 resection (HR, 0.91; 95% CI, 0.50–1.68; $p=0.76$) tended to have longer survival. Patients who had positive resected lymph nodes (HR, 1.30; 95% CI, 0.59–2.87; $p=0.51$), perineural invasion (HR, 2.30; 95% CI, 0.96–5.50; $p=0.06$), and lymphovascular invasion (HR, 1.70; 95% CI, 0.92–3.14; $p=0.09$) tended to survive for shorter periods of time than if those factors were absent. Patients who underwent PB had a significantly increased likelihood of death as compared to those that underwent MP resection (HR, 2.52; 95% CI, 1.37–4.65; $p=0.003$). Additionally, for the subset of patients who underwent PB-L as compared to MP resec-

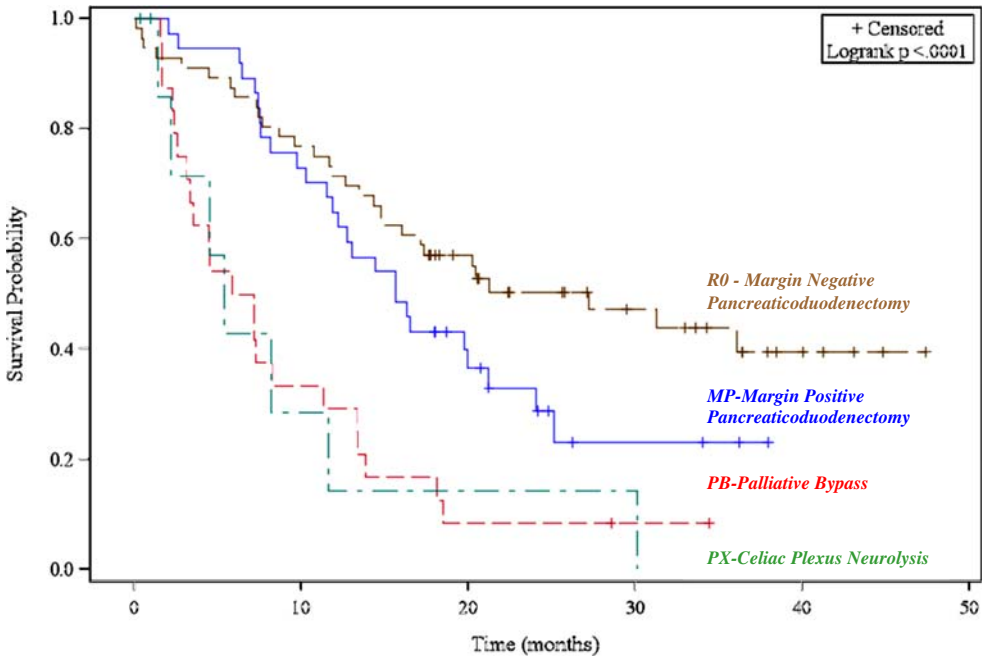

Figure 2 Kaplan–Meier survival curves for the four groups. For the R0 pancreaticoduodenectomy group, the median survival was 27.2 months, and the 1- and 2-year survival rates were 71.5% and 50.4%, respectively. For the MP pancreaticoduodenectomy group, the median survival was 15.6 months, and the 1- and 2-year survival rates were 64.9% and 32.9%, respectively. For the PB group, the median survival was 6.5 months, and the 1- and 2-year survival rates were 29.2% and 8.3%, respectively. For the PX group, the median survival was 5.4 months, and the 1- and 2-year survival rates were both 12.5%.

Table 7 MP vs PB Local Disease Survival

Survival	MP (n=37)	PB local disease (n=6)	p value
1 year (%)	64.9	50.0	0.65
2 years (%)	32.9	16.7	0.53
3 years (%)	23.0	–	–
Median (months)	15.6	13.2	0.4736

MP margin positive pancreaticoduodenal resection, *PB* palliative bypass

tion, there was a trend toward shorter survival (HR, 1.62; 95% CI, 0.64–4.13; p=0.31).

Discussion

Although careful preoperative evaluation of patients with pancreatic adenocarcinoma is designed to identify candidates for whom R0 resection is possible, during operative exploration, one is often confronted with a tumor that appears more advanced than previously thought. In some circumstances, this is because time has elapsed between high-quality CT or MR imaging and exploration, and in other cases, the imaging may have underrepresented the proximity of the tumor to the major visceral vessels. In such instances, the surgeon must decide whether to perform a resection with the possibility of microscopically positive margins or to leave the tumor in place and perform a palliative surgical bypass. The factors that the surgeon must consider in performing such a resection include safety, as well as the effects of tumor debulking upon adjuvant treatment, quality of life, and long-term survival. In this retrospective review of patients undergoing exploration with curative intent, we sought to compare the outcomes of patients undergoing MP resection with those who underwent PB for locally advanced disease in the treatment of pancreatic ductal adenocarcinoma.

In this study, we found that of the patients explored for pancreatic adenocarcinoma, 74% ultimately underwent pancreaticoduodenectomy. Of these, 39.8% were classified with careful pathologic assessment as margin positive resections, which falls within the range (14–60%) reported in the literature.[15,24] In the MP group, the most common site of margin positivity was the retroperitoneal soft tissue (uncinate) margin, representing 73% of all of the margin positive cases. These findings are consistent with results reported in previous studies[25] and are not surprising, as this margin is typically the most difficult to clear. It represents the pancreatic soft tissue adjacent to the superior mesenteric vein and portal vein ventrally and the superior mesenteric artery dorsally. In our hands, every effort is made to resect this tissue from the right lateral aspect of the superior mesenteric artery during the initial separation of the specimen from the visceral vessels. Thus, further resection in this area for a positive margin is not typically possible without performing an arterial resection.

The data from our study suggest that margin positive pancreaticoduodenectomy can be performed safely, with low perioperative morbidity and mortality. The postoperative complication rates were similar between the R0 and MP groups, 36% and 49%, respectively, and were only slightly higher than in the PB group, 33%. Patients in the MP group tended to have a higher rate of minor Clavien types I and II complications when compared to the PB group (especially wound infections, 19% vs 8%), while more serious types III and IV complications were equally distributed between groups. The median postoperative length of hospital stay showed only a 1.5-day difference between patients undergoing resection (R0, MP 7 days) and those undergoing palliative bypass (5.5 days). This is likely because a traditional palliative double bypass, which includes a Roux-en Y hepaticojejunostomy as well as a gastrojejunostomy, involves three separate anastomoses and, aside from the risk of pancreatic fistula, has a similar complication profile to pancreaticoduodenectomy. Perioperative mortality for the entire cohort was only 2% and was confined to the R0 group. There was no perioperative mortality in the MP or PB groups.

Table 8 Multivariate Regression Analysis: Factors Affecting Survival

Parameter	Hazard ratio	95% Hazard ratio confidence limits		p value
R0 vs MP	0.910	0.495	1.675	0.7629
Stage (I vs II)	0.779	0.279	2.173	0.6332
Positive lymph nodes (>0 vs 0)	1.302	0.590	2.871	0.5135
Perineural invasion (yes vs no)	2.298	0.961	5.500	0.0615
Lymphovascular invasion (yes vs no)	1.704	0.924	3.144	0.0881
PB vs MP	2.521	1.366	4.653	0.0031
PB local vs MP	1.624	0.639	4.128	0.3083

R0 margin negative pancreaticoduodenal resection, *MP* margin positive pancreaticoduodenal resection, *PB* palliative bypass, *PB local* palliative bypass locally advanced disease

Some recent published reports have suggested that margin status does not independently affect disease recurrence or survival.[26,27] There are several theories that attempt to explain this finding. First, many patients will harbor silent local or distant metastases at the time of surgery, making the status of surgical resection margins less important than might generally be considered. This would explain the high rates of recurrence even in patients with disease thought to be completely resected. Secondly, because margin positivity is defined by the presence of microscopic tumor cells present on the specimen side of the margin, one might expect that a certain percentage of margin positive patients do not harbor further disease on the retained side, allowing their outcomes to more closely approximate the R0 group. Despite these theories, our data show the expected trend toward increased survival in R0 patients compared to MP patients, with 1-year survival (72% vs 65%) and median survival (27.2 vs 15.6 months) both favoring the R0 group, though these results did not reach significance. As would be expected, patients with MP resections had larger tumors and higher rates of lymph node involvement and lymphovascular and perineural invasion as compared to the R0 group.

A number of authors have suggested a role for margin positive resection by demonstrating that margin positive pancreaticoduodenectomy is associated with better survival than palliative bypass procedures.[28–30] In a recent study performed in the Royal Free and University College Medical School, median survival was significantly longer after MP resection than after PB (18 vs 9 months).[24] Our data are similar to this with 1-year survival rates between the groups of 65% and 29% and median survival of 15.6 and 6.5 months in the MP and PB groups, respectively. When looking at the subgroup of PB-L, patients who most closely approximate the MP group, there is a trend toward increased median survival in the MP group in absolute terms, though given the small sample size of the PB-L group, this difference was not significant. One-, 2-, and 3-year survival rates in the MP group were higher than in the PB-L group at 65% vs 50%, 33% vs 17%, and 23% vs 0%, respectively. The presence of a small number of long-term survivors in the MP group that we have found in this study is consistently identifiable in a number of major surgical series. This MP survival percentage tends to be fairly consistent between series and approximates 20% survival at 3 years.[24,27,31] The same cannot be said in examining series on PB, where the median survival length tends to be short (6 to 9 months) and 3-year survival rates approach 0–1%.[32–36] Although our sample size was too limited to reach statistical significance, the trends demonstrate that patients in the MP group behave more like the R0 group than the PB-L group. This suggests that in intraoperative decision making in a highly selected group of patients, it would be reasonable to lean more toward resection than bypass.

The limitations of this study must be acknowledged. It used a retrospective design and had a relatively short follow-up period, and there were insufficient numbers to reach statistical significance for many of the identifiable trends. Moreover, there was incomplete data on adjuvant treatment which has been shown to impact survival[37] although not nearly to the extent as resection. Also, this study does not specifically deal with the issue of attempted resection for tumors that approach the visceral vessels vs delaying surgery in favor of neoadjuvant treatment. Furthermore, this study lacks quality of life assessment in the groups, an important aspect of pancreatic cancer therapy, decision making, and outcomes.

Conclusions

Margin positive pancreaticoduodenectomy for pancreatic ductal adenocarcinoma in a highly selected group of patients can be performed safely with low perioperative morbidity and mortality. Patients who undergo MP resection have outcomes more closely aligned with patients undergoing R0 resection as compared to patients undergoing PB for locally advanced disease. A small group of long-term survivors exist in the MP group that are not present in the PB for locally advanced disease group. Further work to determine the role of adjuvant treatment and longer-term follow-up are required to assess the durability of survival outcomes for patients undergoing MP resection.

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Discussant

Dr. Attila Nakeeb (Indianapolis, IN): Clearly your group has again shown that achieving an RO resection margin is the most important factor in the management of pancreatic cancer. I have got a couple of questions regarding your philosophy and strategy in regards to these patients.

When you compare the palliative bypass group to the patients undergoing positive margin resection, almost 75% of your palliative bypass patients actually were bypassed in the setting of metastatic disease and not for locally advanced disease. I would like to get a feeling for your thoughts of whether surgical bypass and palliation are actually necessary in patients with metastatic disease. Do you employ any additional staging such as laparoscopy in patients with suspected metastatic disease, especially in patients with elevated CA 19–9 levels, because those have a much higher incidence of requiring palliative bypass.

Secondly, what is your approach to patients with borderline resectable tumors at Jefferson? Are those patients being taken to the operating room immediately with the plan for venous resection, or are they all going for neoadjuvant therapy?

Finally, in those patients that are not able to have an R0 resection, if you compare your margin positive Whipples to the palliative bypass patients, is there any difference in the number of patients that actually receive adjuvant therapy postoperatively or in the time it takes to start therapy?

Closing Discussant

Dr. Harish Lavu (Philadelphia, PA): Your first question asked about whether or not we routinely perform diagnostic laparoscopy given the high percentage of patients with metastatic disease. The answer is that in the majority of patients, we do not. We rely heavily on the CAT scan to help us differentiate these patients, and what we have found is that the majority of unresectable patients ultimately require some sort of palliative bypass, whether it be to the biliary tree or the gastrointestinal tract. We generally believe palliative bypass to be superior to endoscopic management in terms of quality of life in those patients who undergo exploration, so we do not routinely perform laparoscopy.

Your second question was regarding patients with borderline resectable disease, and how we select patients for neoadjuvant treatment? Patients who have superior mesenteric vein or portal vein occlusion or who have a greater than 180° encasement of these vessels with significant stenosis of the vein, or patients who have superior mesenteric artery or celiac axis abutment of tumor. Those are the kinds of patients that we routinely send for neoadjuvant treatment.

Your third question on adjuvant treatment, unfortunately I cannot answer. Many of our patients do not receive their adjuvant treatment at our facility and so it is difficult for us to get a good handle on who was receiving treatment and who was not and when.

Discussant

Dr. L. William Traverso (Seattle, WA): I would like to congratulate the Thomas Jefferson group—with the plethora of great research coming out of Philadelphia on this disease. We look forward to many more contributions.

I am trying to think now not as a surgeon but as a medical oncologist. I note that the 13 months in survival time for the nonresected group outstrips that of the literature, which is about 9 months. You have already made progress there. In Seattle, it is 18 months for the nonresected group, higher than your margin positive Whipple group. Part of this may be experience to choose which chemotherapy will allow a response so it is no longer as much empiric but targeted therapy, somewhat.

I wonder if you might consider the following study—a patient totally managed endoscopically with stents, screened with prechemo laparoscopy (the latter will removed 28% of the patients as they will have positive peritoneal cytology), and then targeted therapy. Therefore, you have the perfect group to compare to the margin positive resected group. I expect in the next 5 years that we will observe 3- or 4-year survivors without any surgery, as we have seen in Seattle. Would you consider that study?

Closing Discussant

Dr. Harish Lavu (Philadelphia, PA): I think we would consider that. It is a very interesting point that you bring up. I would say that there are a number of studies now that are questioning the difference in outcomes between R0 resection and margin positive resections, specifically R1 resections, in terms of how it affects survival and to what time frame does it affect survival?

We know that surgical resection is superior to any adjuvant treatment that is commonly used today. So I think that if there are breakthroughs in adjuvant treatment in the future, there may develop a more aggressive philosophy toward taking patients for surgical resection.

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2009 SSAT PLENARY PRESENTATION

Plectin-1 is a Biomarker of Malignant Pancreatic Intraductal Papillary Mucinous Neoplasms

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Abstract
Introduction Pancreatic intraductal papillary mucinous neoplasms (IPMN) are now identified with increasing frequency. The detection of carcinoma in IPMN is difficult and suffers from high false-positive and false-negative rates, often resulting in inappropriate treatment decisions. Improved detection of malignancy using novel biomarkers may therefore improve diagnostic accuracy. One such promising novel biomarker is Plectin-1 (Plec-1).
Methods Using immunohistochemistry, Plec-1 expression was assayed in benign (low and moderate dysplasia, $n=6$) as well as malignant IPMN (high-grade dysplasia and invasive carcinoma, $n=31$) and lymph node metastases from carcinoma arising in IPMN ($n=12$). Furthermore, cyst fluids from benign ($n=3$) and malignant IPMN ($n=4$) were evaluated for Plec-1 expression.
Results and discussion Twenty-six of 31 malignant IPMN and all 12 lymph node metastases were Plec-1 positive. In contrast, only one of six benign IPMN expressed Plec-1. The specificity of Plec-1 in distinguishing malignant IPMN from benign IPMN was 83% and its sensitivity 84%. Furthermore, all (four out of four) cyst fluids from malignant IPMN, but none of the three benign IPMN, were Plec-1 positive. These data support Plec-1 as an excellent biomarker for the early detection of carcinoma arising in IPMN.

Keywords Plectin-1 · Biomarker · Malignant IPMN · Benign IPMN

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Introduction

Over the past decades, intraductal papillary mucinous neoplasms (IPMN) of the pancreas have been identified with increasing frequency; they now account for up to 20% of all resected pancreatic specimens in large referral centers.[1–6] IPMNs are thought to progress from low-grade dysplasia (adenoma) to high-grade dysplasia (carcinoma in situ) and invasive carcinoma through moderate-grade dysplasia (borderline malignancy). Invasive carcinoma in IPMN is present in 12.5–57% of all IPMN cases.[3,4,7,8] The 5-year survival rate of all patients with surgically resected malignant IPMN is up to 70%[3,4,7,8] and thus much better than the corresponding rate for pancreatic ductal adenocarcinoma, which is less than 20%.[9–11]

Clinically, IPMNs are classified as lesions of the main pancreatic duct (MD-IPMN), the branch ducts (BD-IPMN),

or both (combined-type IPMN).[5] MD-IPMN and combined-type IPMN have a high risk of malignancy (57–92%) and frequently of invasive carcinoma (23–57%), and surgery is therefore recommended.[5,6,12,13] BD-IPMN has a much lower risk of malignancy, 6–46%[5,6,12,13] and indications for surgery are less clear. The only way to stratify the risk of malignancy in IPMNs at present is by clinical symptoms, high-resolution cross-sectional abdominal imaging, endoscopic ultrasound with fine needle aspiration biopsies (FNA), cytology, and cyst fluid analysis for carcinoembryonic antigen and carbohydrate antigen 19.9.[14–19] Current international consensus management guidelines[5] recommend following small BD-IPMN (<3 cm) in asymptomatic patients with high-resolution cross-sectional abdominal imaging studies. Mural nodules on radiologic studies, a dilated main pancreatic duct (>6 mm), positive cytology, or a cyst size larger than 3 cm all correlate with malignancy, and resection is therefore recommended when these signs and symptoms are present. However, the predictive value of these guidelines to correctly distinguish benign from malignant cysts remains low, as not all small and asymptomatic BD-IPMN are nonmalignant[20] and up to 85% of surgically treated patients have no malignancy despite the presence of the aforementioned signs and thus undergo unnecessary resection.[21]

Recently, in an effort to improve diagnostic accuracy, analyses of genetic changes in cyst fluid have been used, but these suffer from limited clinical validation and high cost.[22] No other reliable biomarkers are presently available to clearly distinguish malignant from nonmalignant IPMN.

Novel biomarkers that improve the accuracy of detection of malignancy in IPMN, especially in BD-IPMN, are therefore much needed. One such novel and promising biomarker may be Plectin-1 (Plec-1). Plec-1 was initially identified in a phage display screen for unique markers of pancreatic ductal adenocarcinoma[23] and found to be highly specific and sensitive for early and invasive cancer. Plec-1 expression intensity increases during pancreatic carcinogenesis; strong and specific staining has been identified in 60% of PanIN-3 lesions (ductal carcinoma in situ; Bausch et al., manuscript in preparation). Based on these findings, the aim of this study was to evaluate whether Plec-1 is a potential specific biomarker for malignant IPMNs, whether Plec-1 expression can be exploited to identify metastatic foci in lymph nodes, and whether cyst fluid analysis for Plec-1 allows the discrimination of malignant from benign cysts.

Material and Methods

Tissue Samples

All tissues and biologic samples were collected with the approval and in accordance with the requirements of the Institutional Review Board of the Massachusetts General Hospital, Boston, MA, USA. Paraffin-embedded tissue samples were obtained from the files of the Department of Pathology of the Massachusetts General Hospital, Boston, MA, USA. All specimens had an established diagnosis at the time of assessment. A total of 37 cases of IPMN were obtained, six benign cases and 31 malignant IPMN.

The six benign IPMN were low- and moderate-grade dysplasia (adenoma and borderline malignancy). Ten of the malignant cases were high-grade dysplasia (carcinoma in situ); six of the invasive carcinomas arising in IPMN were of colloid and 15 of ductal phenotype. Three colloid and nine ductal adenocarcinomas were noted to have lymph node metastases and were also analyzed for Plec-1 expression. Cyst fluids were from benign (n=3) and malignant (n=4) IPMN and were also analyzed for Plec-1 expression.

Immunostaining

Paraffin-embedded sections were deparaffinized, hydrated with Tris-buffered saline (TBS) and blocked with H_2O_2. Antigen retrieval was achieved by boiling tissue in Retrievit (BioGenex, San Ramon, CA, USA). After blocking with avidin/biotin (Vector Laboratories, Burlingame, CA, USA) and 5% goat serum in TBS, slides were incubated overnight at 4°C with 1:250 Plec-1 antibody (Abcam). Sections were washed three times in TBST, followed by incubation with biotinylated antirabbit goat secondary antibody (Vector Laboratories, Burlingame, CA, USA), then developed using 3,3′-diaminobenzidine tetrachloride (Invitrogen, Carlsbad, CA, USA), and counterstained with hematoxylin.

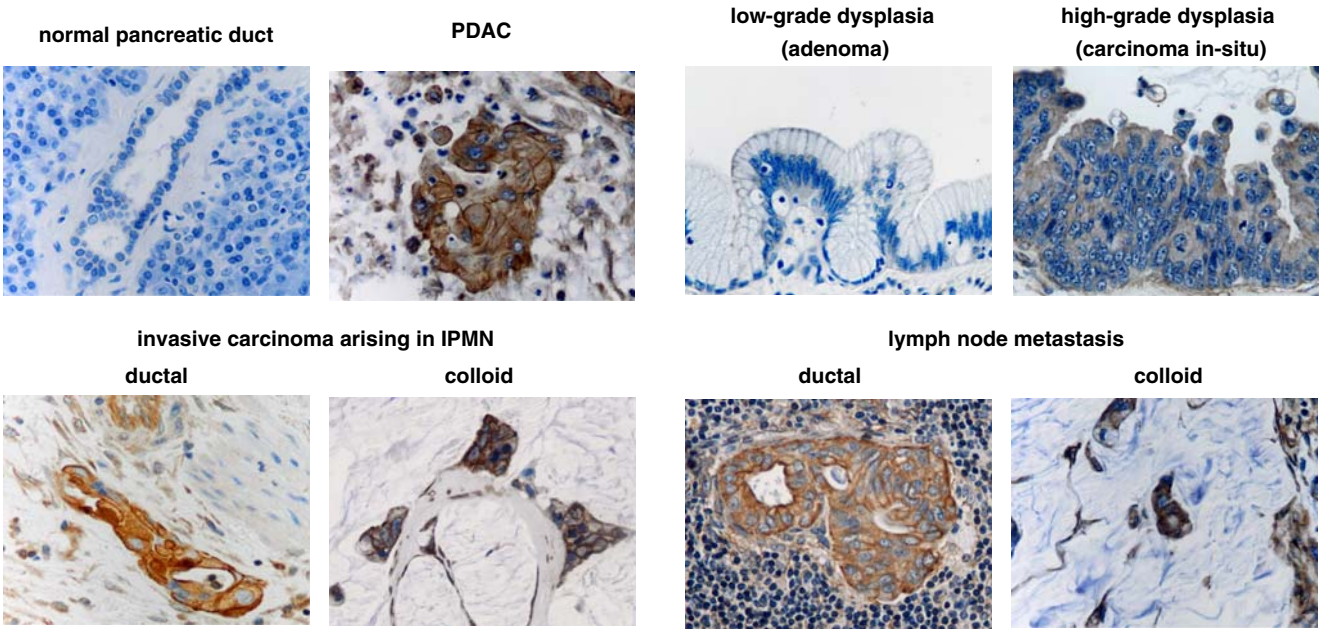
Histological Assessment

Nerves were noted to have moderate staining intensity for Plec-1 and were present on all slides. Expression of Plec-1 in nerves within each slide was therefore used as a staining control and reference for staining intensity. Staining intensity was recorded by two independent observers and, in case of discrepant results, evaluated by a third observer. Specific focal staining of abnormal epithelial cells was considered positive if it was noted to be at least as strong as nerves.

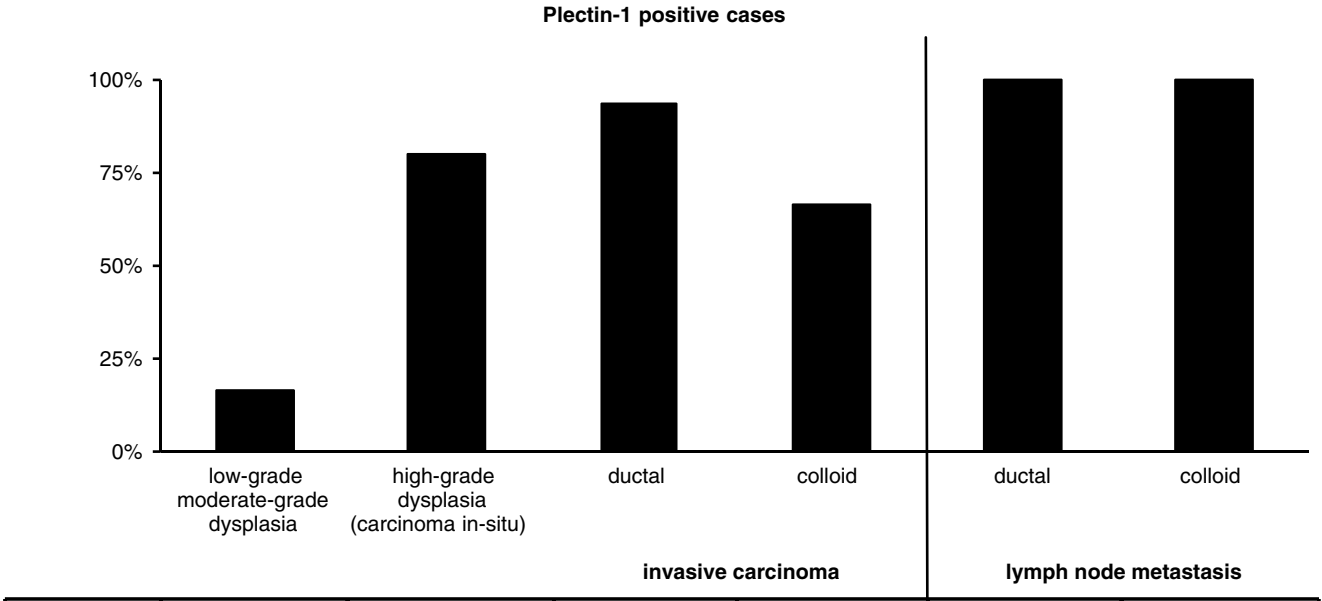
Immunoprecipitation and Western Blot Analysis of Cyst Fluids

Plec-1 expression was evaluated by Immunoprecipitation and Western Blot analysis of cyst fluids from nonmalignant and malignant IPMN. After the addition of Triton X-100 to a final concentration of 1% (v/v) in combination with a protease inhibitor cocktail (Halt™, Thermo Scientific,

a



b



positive	n=1	n=8	n=14	n=4	n=9	n=3
negative	n=5	n=2	n=1	n=2	n=0	n=0

Figure 1 Plectin-1 immunohistochemistry. **a** Representative images of evaluated normal pancreata, PDAC, low-grade and high-grade dysplasia IPMN, ductal and colloid carcinoma arising in the background of IPMN as well as lymph node metastases from ductal and colloid carcinoma arising in the background of IPMN. Normal pancreas and the majority of benign IPMN do not express Plec-1. PDAC, as well as most high-grade dysplasia IPMN, ductal and colloid carcinomas arising in the background of IPMN and their lymph node metastases, are Plec-1 positive. **b** Distribution of staining for Plec-1 in the specimens. The majority of benign IPMN are Plectin-1 negative. Most high-grade dysplasia IPMN, ductal and colloid carcinomas arising in the background of IPMN, as well as their lymph node metastases, are Plec-1 positive.

Rockford, IL, USA), fluids were incubated at 4°C and cleared by centrifugation. 0.1 to 1 ml of fluid was incubated together with 10 μg mouse monoclonal antibody against human Plec-1 (Santa Cruz Biotechnology, La Jolla, CA, USA) and 50 μl protein G Sepharose (Amersham Biosciences, NJ, USA). The beads were then washed thrice with washing buffer (20 mM Tris, pH 7.4, 137 mM NaCl, 1% Triton X-100). Bound protein was eluted by boiling in sodium dodecyl sulfate (SDS) sample buffer. Proteins were separated via SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. Antigen detection was performed using a rabbit monoclonal antibody against human Plec-1 (Abcam, Cambridge, MA, USA). The secondary antibody was an horseradish peroxidase-coupled goat antirabbit polyclonal antibody (Sigma-Aldrich, St. Louis, MO, USA). Bands were visualized with enhanced chemiluminescence. Rat brain lysate (Santa Cruz Biotechnology, La Jolla, CA, USA) was used as positive control.[24]

Results

Plec-1 was identified in 84% (26 out of 31) of malignant IPMNs. Eighty percent (eight out of ten) of the high-grade dysplasia (carcinoma in situ) samples expressed Plec-1 and 86% (18 out of 21) of the invasive carcinomas were Plec-1 positive. Two distinct types of invasive carcinoma occur in IPMN: colloid carcinoma (CC) and ductal adenocarcinoma (DA). DA is characterized histologically by infiltrating small tubular units and a marked desmoplastic host reaction, while CC is characterized by dissecting nodules of mucin that contain scant numbers of malignant cells. Fifteen of the 21 invasive carcinomas were classified as DA; the remaining six were classified as CC. Fourteen of the 15 DA samples (93%), but only 66% (four out of six) of CC samples, expressed Plec-1 (Fig. 1a, b). In contrast to malignant IPMNs, only one out of six of the benign IPMN was Plec-1 positive (Fig. 1a, b). The positive benign IPMN was identified as moderate-grade dysplasia.

Taken together, the specificity of Plec-1 in distinguishing malignant from benign IPMN was 83% and its sensitivity was 84%. Sensitivity for high-grade dysplasia (in situ carcinoma) was 80% and for invasive carcinoma was 86%.

Plec-1 was also reliably identified in lymph node metastases. Twelve of the 31 malignant IPMN evaluated had metastases to lymph nodes. Nine were of DA and three of CC differentiation. Metastases in all nine lymph node metastases deriving from DA-IPMN and all three lymph nodes from CC-IPMN stained for Plec-1 (Fig. 1a, b).

To determine whether there is sufficient Plec-1 present in IPMN cyst fluids to allow the detection of malignancy, Plec-1 immunoprecipitation of cyst fluids from benign and malignant IPMN was performed. Plec-1 was found in 100% (four out of four) of the cyst fluids from malignant IPMN. Three of the Plec-1-positive cyst fluids were from DA and one was from CC. In contrast, cyst fluid from all three benign IPMN contained no detectable Plec-1 (Fig. 2).

Discussion

Presently, there are no available biomarkers to assist accurately in distinguishing benign from malignant IPMN.

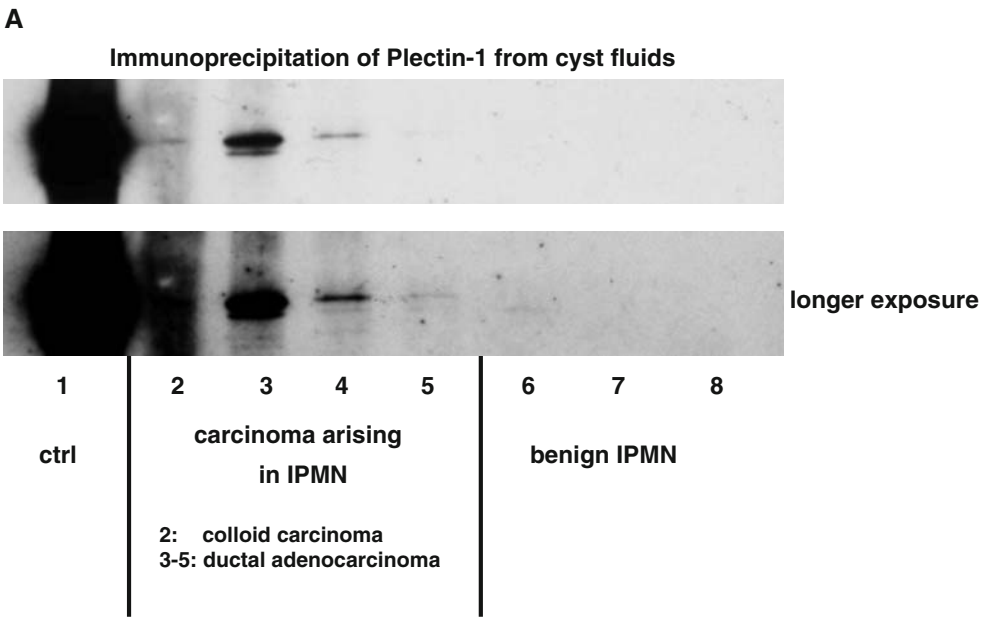


Figure 2 Plectin-1 immunoprecipitation. **a** Plec-1 in cyst fluid alone is sufficient to distinguish malignant from benign IPMN. Plec-1 was found in 100% (four out of four) of the cyst fluids from malignant IPMN, whereas the cyst fluid from all three benign IPMN did not contain detectable amounts. The Plec-1-positive malignant IPMN cyst fluids were from three ductal adenocarcinomas and one colloid carcinoma.

Here, we identify Plec-1 as a potential novel biomarker for carcinoma arising in IPMN. Plec-1 was specifically expressed in the vast majority of carcinomas arising in IPMN as well as in its lymph node metastases, whereas most benign IPMN did not express the protein. In addition to these findings, we were able to demonstrate specific Plec-1 detection in cyst fluid from malignant IPMN. This allows the incorporation of Plec-1 expression analysis into the routine clinical analysis of cyst fluid as an additional screening measure for cancer.

Two distinct types of invasive carcinoma occur in IPMN, CC and DA. It is estimated that 25% to 50% of carcinomas arising in IPMNs are CC,[1,4,8,25–27] and in our series, CC accounted for 29% of the invasive carcinomas. In contrast to DA, where Plec-1 was almost always (93%) expressed, CC showed somewhat fewer Plec-1-positive cases (66%). This may be due in part to difficulty in detecting Plec-1 in the abundant intracellular mucin content of CC cells. However, genetic differences between DA and CC may also account for the lower expression rate of Plec-1 in CC compared to DA.

IPMN follow a classical adenoma–carcinoma sequence, progressing from low-grade and moderate-grade dysplasia to carcinoma in situ (high-grade dysplasia) and finally to invasive carcinoma. Based on our data, Plec-1 expression is acquired during the transition from moderate-grade dysplasia to carcinoma in situ (high-grade dysplasia). A small fraction of moderate-grade dysplasia IPMN (17%) expressed Plec-1, while the majority of carcinoma in situ cases (80%) were Plec-1 positive. Invasive DA had an even higher rate of positive samples (93%). It appears that Plec-1 overexpression may begin to appear at the stage of moderate dysplasia even before histological progression becomes evident.

Overall, Plec-1 expression analysis offers improved specificity over present methods of detecting malignancy in IPMN. In one series of 84 patients, the overall sensitivity of the international consensus guidelines[5] for predicting malignancy in BD-IPMN was 97.3%; however, their specificity was only 29.8%.[14]

FNA with cytology can identify malignancy with high specificity and sensitivity when a relevant tissue sample can be obtained.[17,19] However, the reliability of this diagnostic method depends on an experienced gastrointestinal cytopathologist, and its utility for risk stratification and therapeutic approach is limited.[28]

In contrast, Plec-1 in this study of 37 patients had a sensitivity of 84% and specificity of 83% in distinguishing malignant from benign IPMN and was thus equivalent to or better than other diagnostic approaches. The sensitivity of Plec-1 staining for high-grade dysplasia was a somewhat lower (80%), but it was excellent for invasive carcinoma (86%), especially the more common DA (93%).

Evaluation of cyst fluid from FNA for Plec-1 may greatly improve diagnostic accuracy and add valuable additional information to the clinical diagnostic panel at little additional cost. Additionally, in contrast to cytology, it is technically easy to perform and not operator dependent. The technical feasibility of cyst fluid analysis for Plec-1 was successfully demonstrated in this study, in which the protein was detectable in all cyst fluids from malignant IPMN. Validation in a larger data set is in progress.

We conclude that Plec-1 is a sensitive and specific biomarker for the early detection of malignant IPMN. Plec-1 expression analysis can be easily incorporated into routine clinical cyst fluid analyses and holds promise of contributing substantially to improving diagnostic accuracy for the detection of malignancy arising in IPMN.

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Discussant

Dr. Edward Whang (BWU, Boston): Congratulations for a nice study, and congratulations on picking an excellent mentor with whom to work.

I have some tough questions, but I am sure you will be able to handle them.

First, some methodology questions: How did you pick the cases for inclusion in the study? Surely this is a very small subset of IPMNs available to study at MGH.

Also, I noticed the results you presented today are somewhat different from what you wrote in the abstract. In the abstract, you wrote that one of the colloid carcinomas expressed Plectin-1, whereas today, you said four of them expressed Plectin-1. Is there difficulty in interpretation of the immunohistochemistry in the examples that account for that difference?

Now for some philosophical questions: The sensitivity and specificity associated with using Plectin-1 expression status as a basis for distinguishing benign and malignant IPMNs are each less than 85%. Are those performance characteristics sufficient for clinical application?

Lastly, why is it important to differentiate benign from malignant IPMNs preoperatively? Is not the goal of surgery to prevent cancer from developing? Maybe what you really should seek is a biomarker that differentiates benign IPMNs that are destined to become cancer from benign IPMNs that are destined to remain benign for the remainder of the patient's life.

Closing Discussant

Dr. Dirk Bausch: Invasive carcinoma arising in IPMN is a rare occurrence. Therefore, only a limited number of cases were available to us and included in the study. Benign

IPMN and noninvasive malignant IPMN are much more common. For the purpose of the study, an equal number of main duct and branch duct IPMN were assayed. The relatively small number of benign cases compared to malignant cases assayed is a limitation of the study.

Colloid carcinoma cells contain a high amount of mucin, replacing most of the cytoplasm where Plectin-1 is normally identified. This made the evaluation of these samples exceedingly difficult. To accommodate for these difficulties, two independent observers evaluated all slides.

Sensitivity and specificity of Plectin-1 to detect malignant IPMN were about 85%. Currently employed screening methodology to detect malignancy in branch duct IPMNs, such as the international consensus criteria, have a sensitivity of only about 30%. Therefore, the clinical use of Plectin-1 as an additional screening modality may improve the sensitivity to detect malignancy in IPMN substantially.

The distinction between benign and malignant IPMN is important in the case of branch duct IPMNs, which have a relatively low risk of malignancy. Here, the risk associated with surgical therapy can outweigh the risk of malignancy, especially in the elderly population with small IPMNs.

However, it is important that a biomarker for IPMN identifies preinvasive carcinoma in situ, i.e., high-grade dysplasia lesions, whose prognosis is excellent after surgical resection. Plectin-1 identified about 80% of these cases.

Discussant

Dr. Joe Hines (UCLA): Let me ask the goal of this would be to take cyst aspirate to determine if it the cyst benign or malignant?

Closing Discussant

Dr. Dirk Bausch: The aim is to determine if a cyst is benign or malignant by assessing Plectin-1 expression in a cyst fluid aspirate. The goal is to exclude the operator dependency cytology suffers from and to substitute or augment it with an objective assay for Plectin-1.

Discussant

Dr. Joe Hines (UCLA): But my question is, is Plectin-1 shed into the fluid or does the analysis actually require cells? Because, as you said, the ability to access cytologic aspects for these types of lesion is highly unreliable.

Closing Discussant

Dr. Dirk Bausch: In this study, we used cyst fluids obtained from surgical specimens. Since all cyst fluids were centrifuged before being assayed, they should not contain cells. Therefore, Plectin-1 is most likely shed into the fluid itself. However, Plectin-1 content is very low in cyst fluid. Therefore, enrichment by immunoprecipitation was required prior to detection.

Discussant

Dr. Marc Basson (Michigan State University, East Lansing, MI): Do you think that you would you improve on your sensitivity and specificity if you combined the Plectin-1 reactivity with the other clinical criteria that are already in use. Have you gone back and looked at the numbers? I realize they are small.

Closing Discussant

Dr. Dirk Bausch: No such comparison was made in the current study. Our long-term goal is to improve overall sensitivity and specificity by using Plectin-1 together with clinical criteria.

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2009 SSAT PLENARY PRESENTATION

CC Chemokine Receptor 9 Enhances Proliferation in Pancreatic Intraepithelial Neoplasia and Pancreatic Cancer Cells

Xiaoming Shen · Brian Mailey · Joshua D. I. Ellenhorn · Peiguo G. Chu · Andrew M. Lowy · Joseph Kim

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Abstract
Introduction Chemokine receptors may regulate the progression and metastasis of invasive malignancies. There are little data, however, regarding their role in premalignant lesions. Our objective was to determine the role of CC chemokine receptor 9 (CCR9) in pancreatic intraepithelial neoplasia (PanIN).
Methods Human and murine formalin-fixed paraffin-embedded (FFPE) PanIN specimens were assessed for CCR9 expression. The established murine PanIN, invasive pancreatic cancer (5143PDA) and liver metastasis (5143LM) cell lines, and human pancreatic cancer cell line (PANC-1) were obtained to verify CCR9 expression and function.
Results Immunohistochemistry of FFPE specimens demonstrated CCR9 expression in both murine and human PanIN lesions. CCR9 expression in murine and human cell lines was verified by Western blot assay, immunofluorescence, and flow cytometry. CCR9 function was demonstrated by in vitro exposure to CCL25, the selective CCR9 ligand, which resulted in significantly increased cell proliferation in PanIN and pancreatic cancer cell lines.
Conclusions This is the first report of chemokine receptor CCR9 expression in murine and human PanIN tissues. Our results demonstrate enhanced PanIN and pancreatic cancer cell proliferation with activation of CCR9 by its selective ligand CCL25. CCR9 may prove to be a novel therapeutic target for PanIN and its progression to invasive cancer.

Keywords Chemokine receptor · CCR9 · CCL25 · PanIN · Pancreatic cancer

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Introduction

Clinical trials for pancreatic cancer have primarily focused on patients with locally advanced or metastatic disease, since most patients are ineligible for curative surgical resection. However, despite recent advances in biologic and targeted cancer therapies, none has provided durable survival benefit in pancreatic cancer.[1–4] Regrettably, pancreatic cancer remains a deadly disease with few effective treatment options. An improved understanding of the pathogenesis, rather than metastasis of pancreatic cancer, may provide better insight to develop more effective therapeutic agents. To that end, the recent characterization of pancreatic intraepithelial neoplasia (PanIN),[5,6] the accepted precursor lesion to invasive duct cancer, may provide the means. The lack of appropriate in vitro and in vivo PanIN models for research investigation has been partially addressed by the development of a murine PanIN model that faithfully recapitulates the human disease.[7]

Using the established murine PanIN model, our group previously investigated the expression of chemokine receptor CXCR4 in PanIN.[8] We reported a progressive increase in CXCR4 expression with advancing degrees of PanIN and demonstrated that activated CXCR4 increased the growth and proliferation of cultured PanIN cells. To investigate other potential signaling pathways that may contribute to PanIN growth and proliferation, we assessed other chemokine receptors and selected CC chemokine receptor 9 (CCR9) for further investigation.

CCR9 is a G-protein-coupled receptor originally described in the development of the thymus.[9] T cells that express CCR9 are recruited to discrete locations within the thymus through chemoattraction by thymus-expressed chemokine (TECK), the selective CCR9 ligand.[10,11] TECK, now known as CCL25, is predominantly expressed by most thymic epithelial and dendritic cells; and it is also abundantly expressed in epithelial cells lining the small intestine, but not the large intestine.[12] Additionally, a small subset of human CCR9 (+) peripheral T cells exists and appears endowed with gut-homing properties.[13,14] As such, the CCR9/CCL25 axis may also function in the selective homing of CCR9 (+) memory intestinal T cells or lymphocytes to the small intestine. Interestingly, a recent report suggests that selective release of CCL25 by the small intestine may account for the metastasis of a subgroup of cutaneous melanomas to the small intestine.[15]

Here, our objective was to determine whether chemokine receptor CCR9 is expressed in murine and human PanIN and pancreatic cancer tissues. We used an established murine PanIN model and human pancreatic cancer clinical specimens to assess CCR9 expression patterns. Our secondary objective was to establish whether activated CCR9 altered the growth and proliferative properties of PanIN and pancreatic cancer cells.

Materials and Methods

Reagents

Recombinant murine and human CCL25 were obtained from R&D Systems (Minneapolis, MN, USA). Immunohistochemistry (IHC) and Western blot assay were performed with goat polyclonal anti-CCR9 and rabbit polyclonal anti-CCR9 antibody (Abcam; Cambridge, MA, USA), respectively. Flow cytometric analysis was performed with primary rabbit monoclonal anti-CCR9 antibody (Abcam; Cambridge, MA, USA) and a secondary phycoerythrin (PE)-conjugated antirabbit antibody (Santa Cruz).

Cell Culture

The murine PanIN cell line from the established Pdx1-cre/LSL-K-ras^{G12D} murine PanIN model[7] and corresponding invasive pancreatic cancer (5143PDA) and liver metastasis (5143LM) cell lines, derived from the Pdx1-cre/LSL-K-ras^{G12D}/$p53^{R172H}$ mouse model of pancreatic cancer,[16] were utilized in this research investigation. These cell lines were selected to evaluate potential changes in CCR9 expression during the progression from PanIN to invasive pancreatic duct cancer to pancreatic cancer liver metastasis. The normal human pancreatic duct epithelial line (HPDE)[17] was kindly provided by Dr. M.S. Tsao and was also used for this investigation. The murine cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Hyclone; Logan, UT, USA) supplemented with 10% fetal bovine serum, 2% penicillin/streptomycin, and 1% nonessential amino acids. The established pancreatic cancer cell line PANC-1 was selected to verify CCR9 expression in human pancreatic cancer. It was obtained from the American Type Culture Collection (Manassas, VA, USA) and grown in DMEM at 37°C with 5% CO_2. None of these cells has been previously assessed for CCR9 expression.

Immunohistochemistry

IHC for CCR9 was performed on sections prepared from formalin-fixed paraffin-embedded (FFPE) tissues. Specimens were obtained from patients (n=10) who underwent curative resection for pancreatic ductal adenocarcinoma at City of Hope. Institutional Review Board approval was obtained to assess these specimens. FFPE specimens were also obtained from Pdx1-cre/LSL-Kras^{G12D} mice (n=6).

Tissue blocks were sectioned (5 μm), placed on slides, and deparaffinized in xylene. Specimens were then blocked with 3% hydrogen peroxide and treated to promote antigen retrieval in a citrate buffer solution (pH 6.0). Slides were incubated in Protein Block for 20 min then overnight at 4°C with an anti-CCR9 antibody at 1:50 dilution. The next day, slides were washed in buffer, incubated with chromogen diaminobenzidine tetrahydrochloride, counterstained with hematoxylin, and mounted. CCR9 immunostaining was assessed by the intensity of staining under high-power magnification.

Western Blot Assay

CCR9 expression in PanIN, 5143PDA, 5143LM, PANC-1, and HPDE cells was assessed by Western blot assay. Cells were washed twice with cold phosphate-buffered saline and then harvested by a radioimmunoprecipitation assay lysis buffer (25 mM Tris–HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate

(SDS); Pierce; Rockford, IL, USA) with the addition of protease and phosphatase inhibitors (Pierce) according to the manufacturer's instructions. Fifty micrograms of protein was separated on 12% SDS-polyacrylamide gel and transferred onto polyvinylidene fluoride membranes (Millipore; Bedford, MA, USA). The membranes were blocked for 1 h in Tris buffered saline (TBS)-Tween containing 5% nonfat milk, 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, and 0.05% Tween and probed overnight at 4°C with primary antibodies. After washing three times with TBS-Tween, the membranes were labeled for 1 h at room temperature with horse-radish-peroxidase-conjugated secondary antibodies (Bio-Rad; Hercules, CA). The blots were developed with enhanced chemiluminescent substrate (Thermo; Rockford, IL, USA) and imaged.

Immunofluorescence

Cells were seeded on culture slides and grown to confluence. Cells were fixed in formalin, permeated with 0.1% Triton for 5 min at room temperature, and blocked with Tyramide Signal Amplification (TSA) reagent (PerkinElmer; Shelton, CT, USA) for 30 min. Cells were then incubated with an anti-CCR9 antibody at 4°C overnight. Slides were processed with the TSA Fluorescein Systems (PerkinElmer) according to the manufacturer's instructions. Images were captured using a fluorescence microscope (Olympus AX70; Center Valley, PA, USA).

Flow Cytometry

Flow cytometry was performed to further verify CCR9 expression on PanIN, 5143PDA, 5143LM, and PANC-1 cells. Cells were detached, washed, and stained with anti-CCR9 antibody for 30 min, followed by PE-labeled secondary antibody for 40 min. Fluorescent cells were analyzed by FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA). Isotype-matched immunoglobulins were used as negative controls.

Cell Proliferation Assay

PanIN, 5143PDA, 5143LM, and PANC-1 cells were exposed to CCL25 (400 ng/ml) in order to determine functional consequence of activated CCR9. A proliferation assay (CellTiter-Glo; Promega; Madison, WI, USA) based on the quantification of ATP was used according to the manufacturer's instructions. In brief, cells were plated in 96-well plates at a density of 5×10^3 cells per well. After cell adherence, the media was changed to a serum-free formula. Cells were exposed to CCL25 (400 ng/ml) for 5 days. For detection of the luminescent signal, CellTiter-Glo reagent was added, and the plates were incubated for 10 min. Light was measured on a luminometer (PerkinElmer; Shelton, CT, USA). At least three independent cell proliferation assays were performed. The mean absorbance±one standard deviation was plotted for each treatment group.

Results

CCR9 Expression in FFPE Specimens

Representative murine and human PanIN lesions were assessed by IHC for CCR9 expression (Fig. 1). The pattern of immunostaining was heterogeneous with predominant cytoplasmic staining and minor cell surface staining for CCR9 in the murine PanIN lesions (Fig. 1a–c). In contrast, CCR9 immunostaining was more homogeneous with more intense cytoplasmic staining in human PanIN lesions (Fig. 1d–f).

CCR9 Expression in Cell Lines

PanIN, 5143PDA, 5143LM, PANC-1, and HPDE cells were assessed for CCR9 expression. Immunoblotting demonstrated CCR9 protein expression in all five cell lines (Fig. 2). Staining levels were low in PanIN and HPDE cells (Fig. 2a, b) and relatively higher in 5143PDA and PANC-1 cells (Fig. 2c, d). CCR9 protein expression was confirmed by immunofluorescence (Fig. 3). Flow cytometric analysis verified comparable CCR9 expression levels in the cell lines (Fig. 4). These studies establish CCR9 expression in PanIN, 5143PDA, 5143LM, and PANC-1 cells.

Activated CCR9 Is Functional and Enhances Cell Proliferation

To determine whether the CCR9 receptor regulates downstream cellular function in our cell lines of interest, we performed a proliferation assay. PanIN and PANC-1 cells were exposed to CCL25 (400 ng/ml) over a period of 5 days. A significant increase in cell proliferation was observed for both PanIN and PANC-1 cell lines (Fig. 5).

Discussion

Despite advances in the development of biologic and targeted therapies, pancreatic cancer remains difficult to treat. This underscores the urgency to identify novel signaling pathways and targets to better understand and treat pancreatic cancer. The recent characterization of PanIN[5,6] may provide additional clues to identify and investigate potential novel therapeutic targets. Our previous

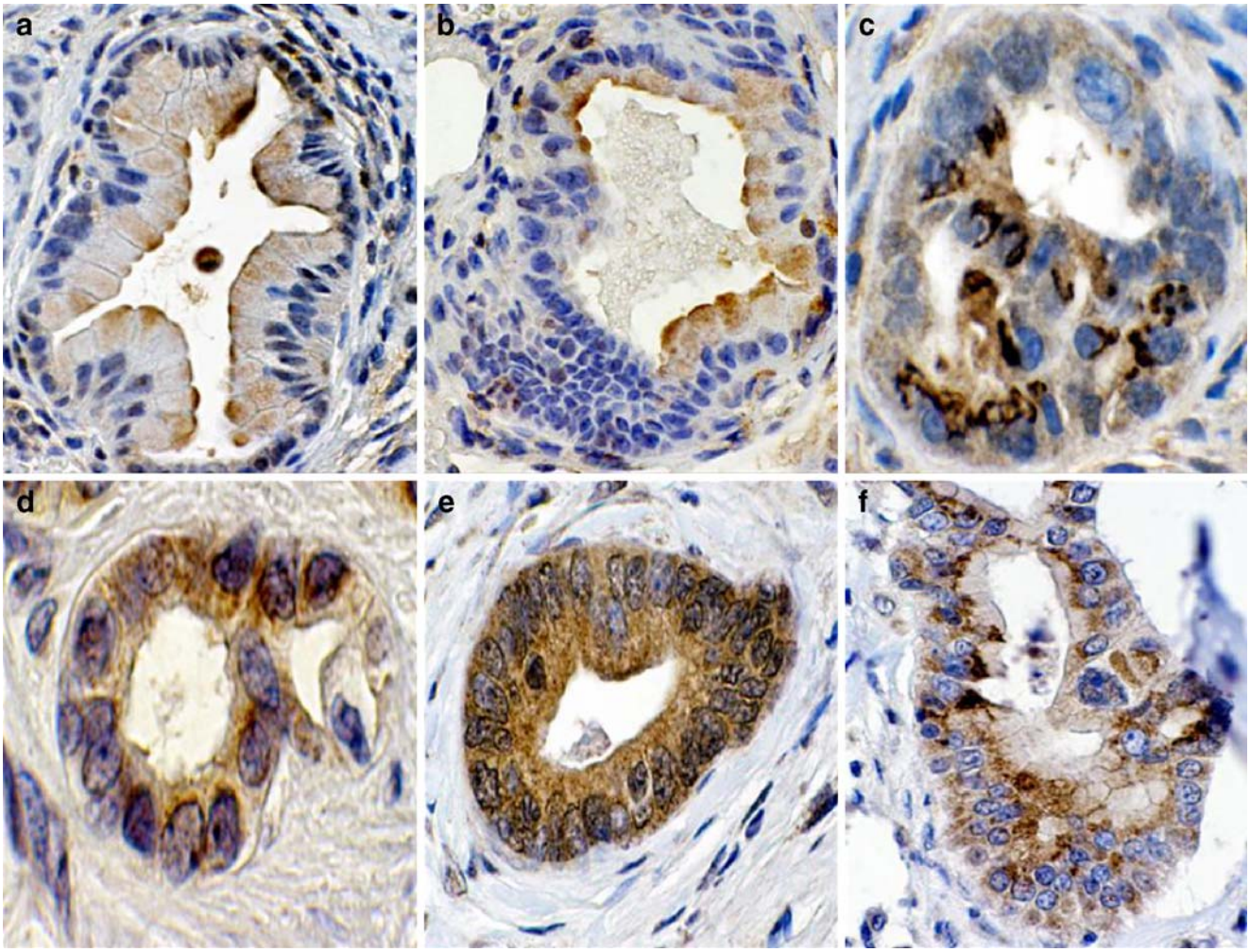

Figure 1 Immunostaining for CCR9 expression was performed in murine (**a**–**c**) and human (**d**–**f**) PanIN lesions. Cytoplasmic and cell surface staining was generally heterogeneous in murine PanIN-1 (**a**), PanIN-2 (**b**), and PanIN-3 (**c**) lesions. In comparison, CCR9 staining was more homogeneous in human PanIN-1 (**d**), PanIN-2 (**e**), and PanIN-3 (**f**) lesions. Light microscopy was performed at ×400–800 magnification.

studies suggested chemokine receptor CXCR4 to be a promising target to prevent PanIN or its progression to pancreatic cancer.[8] We are currently conducting in vivo studies to further test our hypothesis. However, successful therapeutic cancer regimens (e.g., in breast cancer[18] and colorectal cancer[19]) have highlighted the need to abrogate or antagonize multiple signaling pathways for effective treatment responses. To this end, we have examined additional chemokine receptors in PanIN and pancreatic cancer aside from chemokine receptor CXCR4. This initial report verifies that chemokine receptor CCR9 is not only expressed in PanIN but that its activation contributes to PanIN and pancreatic cancer growth and proliferation. Taken together, our results suggest a potential role for CCR9 in the progression of PanIN.

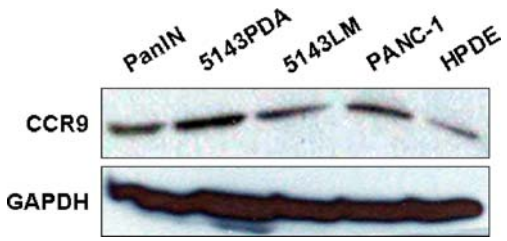

Figure 2 Western blot assay for CCR9 expression demonstrated expression in PanIN, 5143PDA, 5143LM, PANC-1, and HPDE cells. CCR9 immunoblotting was minor for PanIN and HPDE cells compared to 5143PDA and 5143LM cell lines. GAPDH served as a loading control.

Chemokine receptors have gained great interest during the past decade because of their potential role in the metastatic process of cancers.[20,21] For example, chemokine receptor CXCR4 has been implicated in the metastasis of over 20 different cancers to select distant

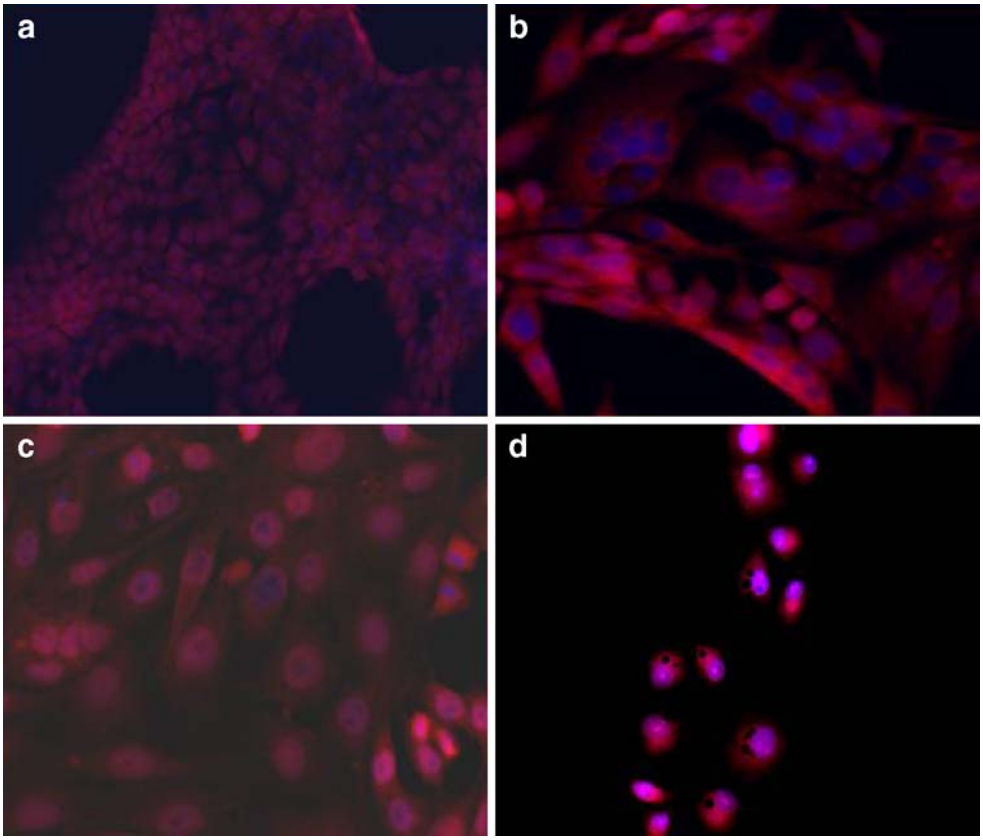

Figure 3 Immunofluorescence demonstrated CCR9 expression in PanIN (**a**), 5143PDA (**b**), 5143LM (**c**), and PANC-1 (**d**) cell lines.

organs.[20] Cancer cells have appropriated the traditional chemoattractive properties of chemokine receptors to engender a metastatic aggressive phenotype. Directional migration/metastasis of CCR9 bearing cells to regions with high release of CCL25 may account for the unique metastasis of select melanoma cells to the small intestine.[15] Our results show that PanIN and pancreatic cancer cells may also employ chemokine receptors to support their initial growth and progression.

Our secondary objective was to demonstrate that CCR9 receptors serve downstream function in PanIN and pancreatic cancer. Previous reports have suggested that some chemokine receptors have no function in the cells harboring them.[22,23] Accordingly, we chose to examine proliferation in PanIN. Growth and proliferation are more relevant and important than migration or metastasis in PanIN which is a preneoplastic lesion. Our proliferation data demonstrate a clear significant increase in PanIN and pancreatic cell proliferation in vitro when exposed to the selective CCR9 ligand, CCL25. An approximate 32% increase in PanIN proliferation was observed, which is similar to the enhanced proliferation observed when CXCR4 was activated in PanIN cells.[8] We are currently exploring whether there is a synergistic effect on proliferation when both CCR9 and CXCR4 are activated in PanIN. Our results demonstrating improved proliferation in PanIN and pancreatic cancer proliferation are consistent with a published report on enhanced proliferation with chemokine receptor activation.[24]

A previous investigation of CCR9 in melanoma and breast cancer cells showed that these cell lines do not express CCR9.[25] Another study suggested that cancer cells that do not metastasize to the small bowel have minimal to no CCR9 expression.[15] In contrast, our studies clearly show that preneoplastic PanIN and invasive pancreatic cancer cells, which rarely metastasize to the small intestine, express CCR9. Our initial rationale to investigate CCR9 was secondary to the unique and selective expression of its ligand CCL25 in the small intestine. Our results verify that CCR9 is expressed by PanIN and invasive pancreatic cancer and liver metastasis cells, and the activation of CCR9 enhances cell proliferation. We hypothesize that paracrine release of CCL25 from the adjacent small intestine to stimulate CCR9 in PanIN and pancreatic cancer lesions accounts for enhanced proliferation. Future studies will determine whether there is a paracrine or even autocrine stimulatory network for the CCL25/CCR9 axis.

In summary, this is the first report of CCR9 in PanIN and pancreatic cancer, and we show that in vitro activation

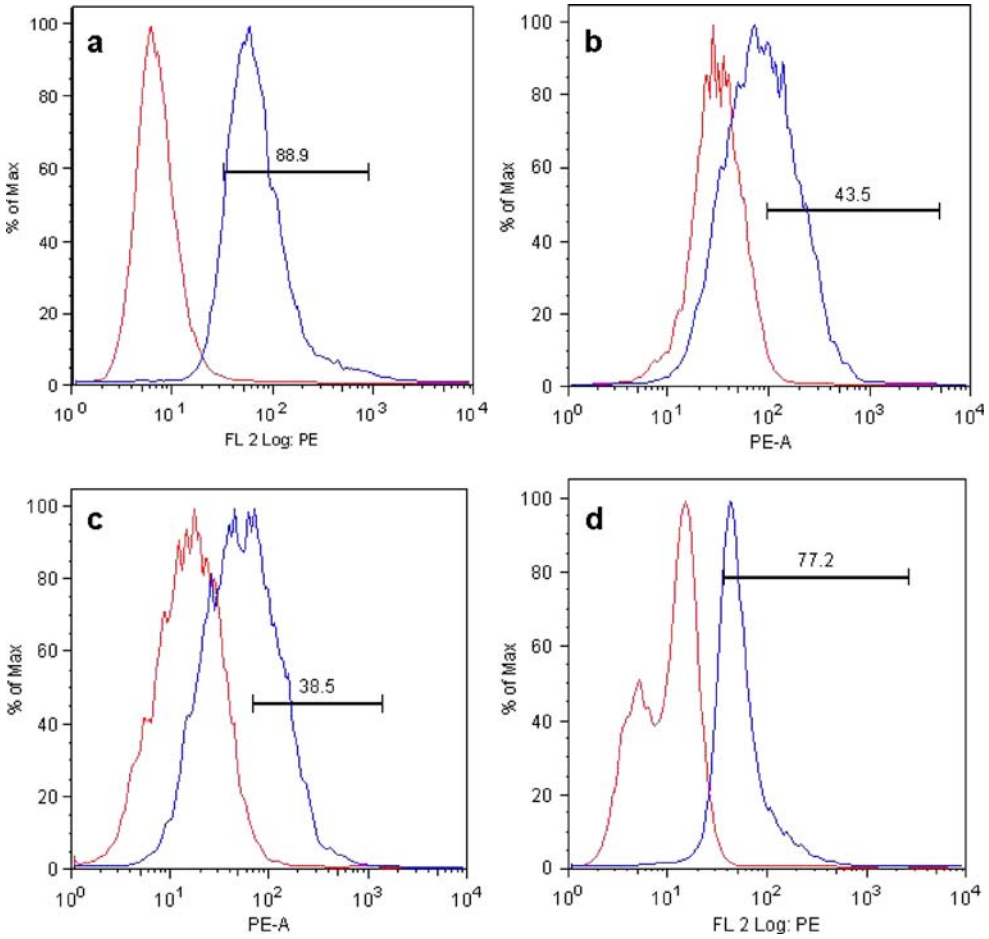

Figure 4 Flow cytometric analysis demonstrated that 39–89% of PanIN (**a**), 5143PDA (**b**), 5143LM (**c**), and PANC-1 (**d**) cells stained positive for CCR9 with similar mean fluorescence intensities.

of CCR9 results in enhanced PanIN and pancreatic cancer proliferation. As we further characterize the potential clinical significance of CCR9 signaling in PanIN and pancreatic cancer, we are preparing in vivo studies to determine whether CCR9 antagonism can abrogate the growth or progression of PanIN. We will also perform investigations in invasive pancreatic cancer cells to determine whether CCR9 signaling may have different roles in malignancy in comparison to PanIN. These results may provide the first direct demonstration that chemokine receptor CCR9 is an appropriate therapeutic target for patients with pancreatic cancer.

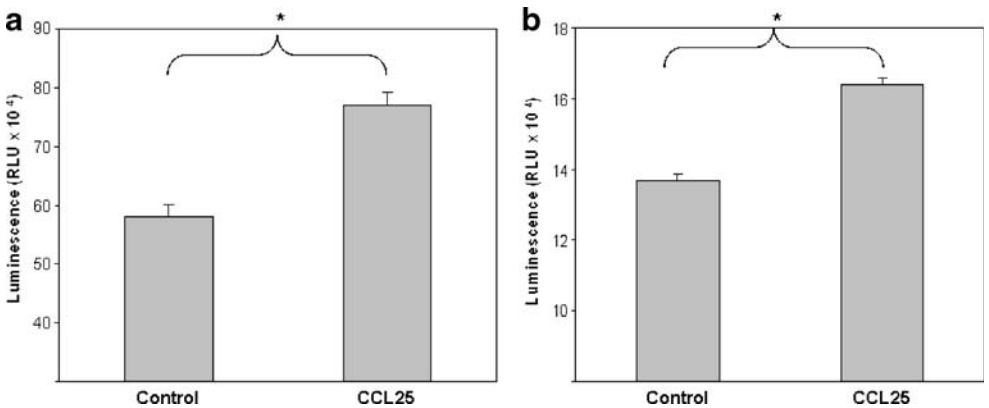

Figure 5 A cell proliferation assay was performed for PanIN and PANC-1 cells. Significant increases of 32% and 19% in proliferation were observed for PanIN (**a**) and PANC-1 (**b**) cells, respectively, following exposure of cells to CCL25, the specific CCR9 ligand.*$p<0.001$.

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Discussion

Dr. Brian A. Mailey, Presenter (City of Hope, Duarte, CA, USA)

Discussant

Dr. Syed Ahmad (Cincinnati, OH, USA): Your group has previously presented on CXCR4 in PanIN cells and demonstrated advancing degree of expression as PanIN progresses from 1 to 3 and that activation of CXCR4 increased growth and proliferation. And in this current study, you've demonstrated that CCR9 is present in PanIN cells. With that background, I have a few questions.

Were you able to demonstrate a similar finding in terms of differential degree of expression from PanIN 1 to PanIN 3? Was there a difference in expression in primary pancreatic cancers versus liver metastases? Have you looked at that or is that something you are planning to do?

The second question I have is, how do you explain the presence of CCR9 on normal ductal epithelial cells? In fact in the western that you demonstrated, it appeared that the expression of CCR9 was higher in normal cells than in PanIN cells. Have you done any assays to see if ductal cells proliferate when exposed to the ligand for CCR9?

Finally, the last question I have is, if you are proposing this as a mechanism of progression, how do you explain that mechanism? Where does the ligand come from? And do you see this as more of a chemo preventive target or a chemotherapeutic target?

Closing Discussant

Dr. Brian Mailey: We are very interested in the CCR9 receptor, but we are still in the early stages of investigation. The question that you raised about the increase in progression of expression in CXCR4 and what we have seen in CCR9, is that it is not exactly the same. We don't see a progression in CCR9 expression from PanIN 1 to PanIN 3, although there are pending studies which may help further define this.

The second question about the HPDE cell line; we did demonstrate that CCR9 is present in normal pancreatic ductal cells. The function of CCR9 in these cells? We can speculate on what it may potentially do, although we don't have a firm answer for that yet. I think that the western blot demonstrates that CCR9 expression is at least as high in PanIN as it is in HPDE. It will be interesting to determine if CCR9 does play a function in normal cells, and if the downstream signaling functions differently in pathologic cells.

(Questioner from the floor not using a microphone.)

The ligand is one of the most interesting aspects of this receptor, which really stimulated our interest in investigating it further when we discovered CCR9 on the microarray analysis. It is produced by the small bowel as I mentioned, and this was part of our hypothesis, that the ligand may be released in a paracrine manner to stimulate pancreatic cancer cells.

(Questioner from the floor not using a microphone.)

It may be a possible explanation for the extremely poor survival in pancreatic cancer, even for patients with early stage lesions. I think that chemo preventive versus therapeutic measures are still forthcoming. I think it's a little bit early for us to tell.

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2009 SSAT PLENARY PRESENTATION

Readmission After Pancreatectomy for Pancreatic Cancer in Medicare Patients

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Abstract
Objective The objective of this study was to use a population-based dataset to evaluate the number of readmissions and reasons for readmission in Medicare patients undergoing pancreatectomy for pancreatic cancer.
Methods We used Surveillance, Epidemiology, and End Results–Medicare linked data (1992–2003) to evaluate the initial hospitalization, readmission rates within 30 days (early), and between 30 days and 1 year (late) after initial discharge and reasons for readmission in patients 66 years and older undergoing pancreatectomy.
Results We identified 1,730 subjects who underwent pancreatectomy for pancreatic cancer. The in-hospital mortality was 7.5%. The overall Kaplan–Meier readmission rate was 16% at 30 days and 53% at 1 year, accounting for 15,409 additional hospital days. Early readmissions were clearly related to operative complications in 80% of cases and unrelated diagnoses in 20% of cases. Late readmissions were related to recurrence in 48%, operative complications in 25%, and unrelated diagnoses in 27% of cases. In a multivariate analysis, only distal pancreatic resection (P=0.02) and initial postoperative length of stay ≥10 days (P=0.03) predicted early readmission. When compared to patients not readmitted, patients readmitted early had worse median survival (11.8 vs.16.5 months, P=0.04), but the 5-year survival was identical (18%). Late readmission was associated with worse median and 5-year survival (19.4 vs. 12.1 months, 12% vs. 21%, P<0.0001).
Conclusions Our study demonstrates overall 30-day and 1-year readmission rates of 16% and 53%. The majority of early readmissions were related to postoperative complications but not related to patient and tumor characteristics. Complications causing early readmission are a cause of early mortality and are potentially preventable. Conversely, late readmissions are related to disease progression and are a marker of early mortality and not the cause.

Keywords Readmission · Pancreatic resection · Kaplan–Meier · Operative complications

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Introduction

Over the last three decades, the operative mortality and lengths of stay have decreased following pancreatic resection,[1–6] which can be attributed to increasing regionalization of care,[7–10] improved perioperative and critical care,[11–13] improved prevention and management of complications, implementation of critical pathways,[14,15] and improved post-hospital inpatient and outpatient care. Despite the improvements in mortality and lengths of stay, the morbidity rates, usually defined as the occurrence of any complication in the postoperative period, remain high with reported rates in excess of 30% even at major centers.[3,4,6,16–20] Readmission, a good measure of morbidity, is rarely reported. In addition, when reported, the focus is on readmissions within the first year.

There are three previous studies evaluating readmission following pancreatic surgery.[16,21,22] Two studies are single-institution studies, both of which included pancreaticoduodenectomy for benign and malignant disease.[16,21] Neither study reported readmissions within 30 days of discharge. Emick and colleagues[16] reported a 19% readmission rate in the year after surgery in 1,643 patients undergoing pancreaticoduodenectomy. van Geenen and colleagues[21] reported an overall 1 year readmission rate of 38% in 283 patients undergoing pancreaticoduodenectomy. Given the single-institution nature of these studies, readmissions to other facilities may not be identified, so the reported rates may not reflect national readmission rates.

A population-based study using the California tumor registry and hospital discharge data reports a 59% readmission rate in the year after pancreaticoduodenectomy in patients with pancreatic cancer.[22] They also report decreased long-term survival in the group requiring readmission. The majority of readmissions were related to disease progression. As such, they are a marker of early mortality and not the cause. None of the above studies evaluated readmissions using a time-to-event analysis and therefore potentially underestimated readmission rates.

The goals of our study were to use a population-based data set [Surveillance, Epidemiology, and End Results (SEER)–Medicare-linked data] to evaluate the readmission rates using time-to-event methods. We also evaluate the reasons for readmission within 30 days of pancreatic resection (early readmission) and between 30 days and 1 year (late readmissions). We hypothesize that early readmissions are related to operative complications, contribute to early mortality, and are potentially preventable. Conversely, late readmissions are associated with disease progression and are a marker, rather than a cause, of early mortality. Therefore, it is critical to analyze 30-day readmissions separately. We also determine the patient and tumor factors associated with early readmission and perform a survival analysis to determine the effect of early and late readmission on survival.

Methods

This study was approved by the Institutional Review Board at the University of Texas Medical Branch at Galveston. A Data Use Agreement for the use of SEER–Medicare data has been signed.

Data Source

We used data from the SEER–Medicare Linked Data Project (SMLDP) for the analysis. The SEER tumor registry is a National Cancer Institute (NCI) program, which tracks the incidence of cancer in the USA. The SEER database contains information on patient demographics, tumor characteristics, first course of treatment, and survival data (obtained via linkage to the National Death Index). From 1992–1999, SEER was comprised of 14 registries, 12 of which participated in the SEER–Medicare linkage. After 2000, SEER had 18 registries, 16 of which participated in the SEER–Medicare linkage.[23,24]

The SMLDP includes the SEER program, the NCI, and the Centers for Medicare and Medicaid Services). Ninety-three percent of all SEER patients older than age 65 are matched with Medicare enrollment files. In addition to the variables available in SEER, claims data for hospital stays, physician services, and hospital outpatient visits are included. The data used in this proposal include SEER subjects through 2002 and their Medicare claims through 2003.

Patient Cohort Selection

Using the SEER–Medicare-linked data, the following subjects were included in the study: (1) patients with International Classification of Diseases (ICD)-O-3 histology codes consistent with adenocarcinoma to eliminate other pancreatic tumor types such as neuroendocrine and acinar cell cancers, (2) patients diagnosed between 1992 and 2002, (3) patients with a pancreatic cancer as their first primary cancer, (4) patients enrolled in both Medicare Parts A and B without HMO for 12 months before their cancer diagnosis and for 1 year after their diagnosis, (5) patients aged ≥66 (to ensure available Medicare claims data for a full year prior to diagnosis), and (6) patients undergoing pancreatic resection (complete resection of the primary tumor). Pancreatic resection was identified by searching MEDPAR inpatient claims files for ICD-9 CM codes for total pancreatectomy, radical pancreaticoduodenectomy, proximal pancreatectomy, distal pancreatectomy, radical subtotal pancreatectomy, or other partial pancreatectomy (codes shown in Table 1). Patients diagnosed at autopsy only or patients diagnosed by death certificate only were excluded.

Assessment of Readmissions and Diagnoses

We defined readmission as the number of patients who were discharged from an acute care hospital and readmitted to the hospital within (1) 30 days (early) or (2) between 30 days and 1 year (late) from the date of discharge from the index admission for pancreatic resection. To account for a decrease in the number of patients at risk in each time period as a result of tumor- and operative-related deaths, a Kaplan–Meier analysis modeling the time to readmission was used to obtain accurate readmission rates. We cannot directly identify patients that were transferred from one

Table 1 ICD-9 Procedure and Diagnosis Codes

Procedure	ICD-9 procedure codes
Total pancreatectomy	52.7
Radical pancreaticoduodenectomy	52.6
Proximal pancreatectomy	52.51
Distal pancreatectomy	52.52
Radical subtotal pancreatectomy	52.53
Other partial pancreatectomy	52.29
Upper endoscopy with or without intervention	44.13, 44.12, 44.14, 44.19, 44.12, 44.22, 44.43, 45.13, 45.16, 45.22, 45.23, 45.24, 45.25, 45.28, 45.29, 45.30, 54.59
Biliary drainage via percutaneous, endoscopic, or operative approach	51.98, 51.87, 51.10, 51.11, 51.86, 51.85, 57.84, 51.1, 51.31, 51.51, 51.59, 51.3, 51.32, 51.42, 51.37, 51.34, 51.43, 51.49, 87.51
Diagnosis	ICD-9 Diagnosis Codes
Operative complications	998, 998.0, 998.11, 998.12, 998.13, 998.3, 998.30, 998.31, 998.32, 998.4, 998.51, 998.59, 998.6, 998.83, 998.89, 998.9, 997.4, 997.5, 997.9
Metastatic disease	197, 197.0, 197.1, 197.2, 197.3, 197.4, 197.5, 197.6, 197.7, 197.8, 197.9, 198, 198.0, 198.1, 198.2, 198.3, 198.4, 198.5, 198.6, 198.7, 198.8, 198.81, 198.82, 198.89, 196.1, 196.2, 196.3, 196.4, 196.5, 196.6, 196.7, 196.8, 196.9, 199, 199.0, 199.1, 789.5
Dehydration	276, 276.0, 276.2, 276.4, 276.5, 276.50, 276.51, 276.52
Gastric outlet obstruction/delayed gastric emptying	537, 537.0, 537.3, 537.89, 537.9, 536.3, 536.8, 536.9
Venous thromboembolism/ pulmonary embolism	453.8, 444.21, 444.42, 453.1, 453.2, 453.40, 453.4, 453.42, 453.9, 415.19
Pneumonia	480.0, 480.1, 480.2, 480.3, 480.8, 480.9, 481, 481.0, 482.0, 482.1, 482.2, 482.30, 482.31, 482.32, 482.39. 482.40, 482.41, 482.42, 482.49, 482.81, 482.82, 482.83, 482.84, 482.89, 482.9, 483.0, 483.1, 483.8, 484.1, 484.3, 484.5, 484.6, 484.7, 484.8, 487.0, 486, 485
Cholangitis	576.1, 576.2, 576.8, 576.9, 572
Small bowel obstruction	560.2, 560.81, 560.89, 560.9
Gastritis/duodenitis/gastric ulcer/ duodenal ulcer	531.00, 531.01, 531.10, 531.11, 531.20, 531.21, 531.30, 531.31, 531.40, 531.41, 531.50, 531.51, 531.60, 531.61, 531.70, 531.71, 531.90, 531.91, 532.00, 532.01, 532.10, 532.11, 532.20, 532.21, 532.30, 532.31, 532.40, 532.41, 532.50, 532.51, 532.60, 532.61, 523.70, 532.71, 532.90, 532.91, 533.00, 533.01, 533.10, 533.11, 533.20, 533.21, 533.30, 533.31, 533.40, 533.41, 533.50, 533.51, 533.60, 533.61, 533.70, 533.71, 533.90, 533.91, 534.00, 534.01, 534.10, 534.11, 534.20, 534.21, 534.30, 534.31, 534.40, 534.41, 534.60, 534.61, 534.70, 534.71, 534.90, 534.91, 535.00, 535.01, 535, 535.0, 535.10, 535.11, 535.20, 535.21, 535.30, 535.31, 535.40, 535.41, 535.50, 535.51, 535.60, 535.61, 535.70, 535.71
Incisional hernia	553.20, 553.2, 553.21, 553.29, 552.2, 552.20, 552.21, 552.29, 551, 551.10, 551.00)
Pancreatic pseudocyst	577.2
Acute myocardial infarction	410, 410.0, 410.00, 410.01, 410.02, 410.10, 410.11, 410.12, 410.20, 410.21, 410.22, 410.30, 410.40, 410.41, 410.42, 410.50, 410.51, 410.52, 410.60, 410.61, 410.62, 410.70, 410.71, 410.72, 410.80, 410.82, 410.90. 410.91, 410.92
Cerebrovascular accident	435, 435.0, 435.1, 435.2, 435.3, 435.8, 235.9, 436, 437.1
Bile leak	576.4
Hip fracture	820, 820.0, 820.00, 820.01, 820.02, 820.03, 820.09, 820.10, 820.11, 820.12, 820.13, 820.19, 820.20, 820.21, 820.22, 820.30, 820.31, 820.32, 820.8, 820.9, 821.00, 821.10

acute care hospital to another acute care hospital or rehabilitation facility. Therefore, we excluded patients who were readmitted on the same day they were discharged, assuming these represented hospital to hospital transfers. In many cases, patients were readmitted more than one time over the 1-year time period.

A record was created for each readmission including the first seven discharge diagnosis codes and the first seven procedure codes for each hospitalization. Many patients were readmitted more than one time. On examining the reasons for readmissions, it was clear that reasons for readmission were clustered among multiple readmissions in the same patient. Over 95% of multiple readmissions in a single patient were for the same or related diagnoses. For this reason, we evaluated only the first readmission. For example, if a patient was readmitted for the first time in the early time period and again in the late time period, only the readmission in the early time period was reexamined. We also evaluated late readmission using a conditional repeated events analysis, and the conclusions did not change. We report the former.

Each readmission record was independently reviewed by two authors. After examining the diagnosis and procedure codes, each readmission was assigned a primary reason for the admission. The proportion of readmissions clearly related to postoperative complications was reported. However, these were subject to the reviewers' interpretation. Therefore, we also report the frequency of specific diagnoses based on the appearance of the ICD-9 diagnosis code anytime during admission. Each readmission record was queried to identify the incidence of several specific diagnoses and procedures present. The incidence of each diagnosis or procedure in each time period was calculated by identifying the frequency of the ICD-9 codes for each respective diagnosis or procedure and dividing this number by the number of first readmissions during the same time period. These diagnoses need only be present in the readmission diagnosis codes but did not need to be the primary reason for readmission. As such, they do not add up to 100%.

The ICD-9 codes used to identify specific procedures and diagnoses are shown in Table 1. Specific procedures evaluated included upper endoscopy with or without intervention and biliary drainage via a percutaneous, endoscopic, or operative approach. Specific diagnoses evaluated included operative complications, metastatic disease, dehydration, gastric outlet obstruction/delayed gastric emptying, venous thromboembolism (VTE) and/or pulmonary embolus (PE), pneumonia, cholangitis, small bowel obstruction, gastritis/duodenitis/gastric ulcer/duodenal ulcer/marginal ulcer, incisional hernia, pancreatic pseudocyst, acute myocardial infarction, cerebrovascular accident, bile leak, and hip fracture.

Statistical Analysis

SAS version 9.1.3 (Cary, NC) was used for all statistical analyses. Descriptive statistics were reported for the patient demographics, operative details, the in-hospital and 30-day mortality, the number of patients requiring readmission (total, within 30 days, and between 30 days and 1 year), number of total readmissions in each time period, the primary reason for each readmission, and the incidence of specific diagnoses during readmissions. The diagnoses during readmission were compared between the early and late readmission groups using univariate statistics (chi-square, Fisher's exact test).

Kaplan–Meier survival curves were used to determine readmission rates over the first year. Deaths related to pancreatic cancer were censored at their time of death if death occurred within the first year of discharge and prior to any readmission. Within 30 days of discharge, there were only 13 deaths (11 occurring without a previous readmission), so death was not a significant competing event. Beyond 30 days, the number of deaths increased with time, and death became a significant competing cause. We used a Cox proportional hazards model with deaths treated as censored values to assess patient-level predictors of readmission within 30 days. Between 30 days and 1 year, we excluded patients who died or were readmitted within the first 30 days. We then used two separate Cox models: the first treated deaths within the first year as censored and the second treated deaths as a competing cause. The patient-level factors determining late readmission in each model were analyzed. The assumption of proportionality was tested using Schoenfeld residuals. Significance was accepted at the $P<0.05$ level.

Results

Overall Cohort

Between 1992 and 2003, 1,730 subjects met the inclusion criteria for the study. The demographic data, type of procedure, number of in-hospital deaths, postoperative length of stay, 30-day mortality rate (including in-hospital deaths), and stage of disease for the overall cohort are shown in Table 2. The mean age of the study population was 72.6±6.4 years. Forty-eight percent of patients were male and 82% were white. The location of the tumor dictated the type of procedure performed. Pancreaticoduodenal resections were performed in 76% of patients, distal pancreatectomy in 18% of patients, total pancreatectomy in 3% of patients, and 3% underwent pancreatectomy not otherwise specified. The median postoperative length of stay for all patients was 14 days (25th percentile=10 days, 75th percentile=21 days).

Table 2 Demographics of Overall Cohort (N=1,730)

	Number of patients	Percent
Age at surgery	1,730	72.6±6.4 years
Gender		
Male	822	48%
Female	908	52%
Race		
White	1,427	82%
Black	132	8%
Hispanic	74	4%
Other	97	6%
Marital status		
Married	1,044	60%
Single	250	14%
Widowed	380	22%
Unknown	56	3%
Charlson comorbidity score		
0	1,108	64%
1	411	24%
2	138	8%
3 or more	73	4%
Type of procedure		
Pancreaticoduodenal resection	1,309	76%
Distal pancreatectomy	311	18%
Total pancreatectomy	62	3%
Pancreatectomy, not otherwise specified	48	3%
Stage of disease		
Locoregional	1,493	86%
Distant/unknown	237	14%
Postoperative length of stay	1,730	17.5±11.6 days
In-hospital mortality	130	7.5%
30-day mortality	143	8.3%

Among the 1,730 patients, there were 130 in-hospital deaths (7.5%) following surgery, leaving a total of 1,600 patients with the potential for readmission. The 30-day mortality was 8.3% (including in-hospital mortalities); 13 patients died after the first discharge but within 30 days.

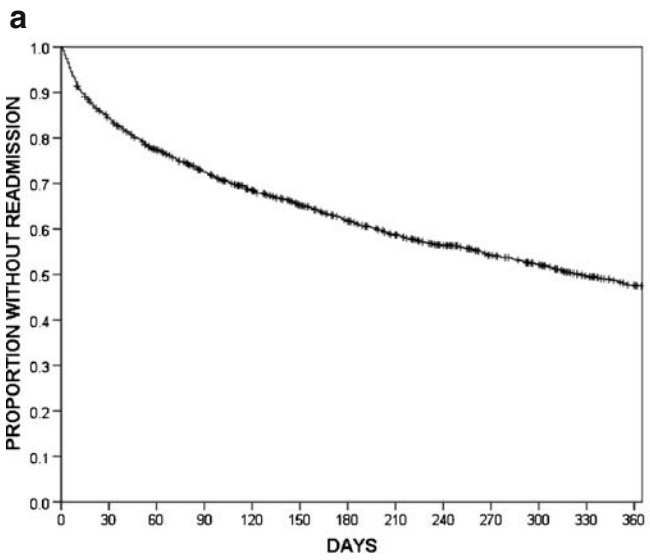
Overall Readmissions

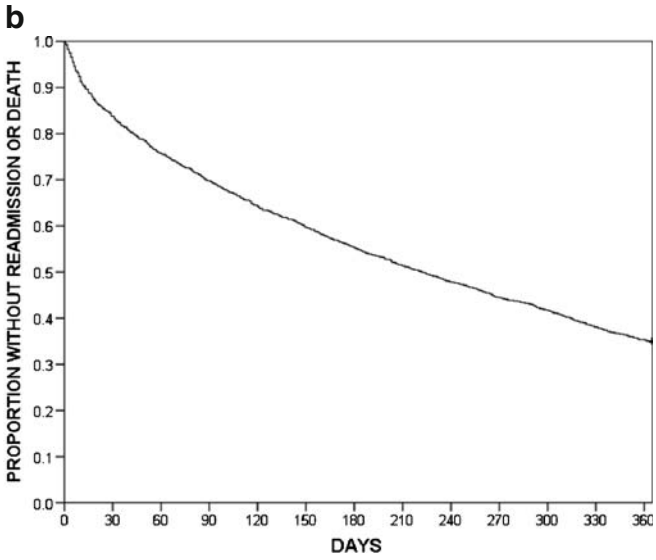
Of the 1,600 patients with the potential for readmission, 784 patients were readmitted a total of 1,766 times within the first year of discharge. Three hundred twenty-six patients were readmitted once, 211 patients were readmitted twice, 117 patients were readmitted three times, and 130 patients were readmitted four or more times. Figures 1a and 1b are Kaplan–Meier curves showing the time to readmission over the first year following discharge. Operative deaths are censored in Fig. 1a and treated as competing events in Fig. 1b. In addition to the survival curve, Fig. 1c depicts the number at risk in each time period, the cumulative readmissions at the end of each time period, the cumulative deaths without readmission at the end of each time period, and the Kaplan–Meier estimates for readmission rates at the same time points. The Kaplan–Meier curve shows a 15.5% readmission rate at 30 days and a 52.6% readmission rate at 1 year.

The mean duration for readmission was 8.8±9.7 days (median=6 days). Readmissions accounted for 15,409 additional hospital days in the 784 patients.

Early Readmissions

Within 30 days after discharge, 248 patients (16%) who survived the initial hospitalization were readmitted a total of 320 times; 190 patients were readmitted once, 46 patients were readmitted twice, and 12 patients were readmitted three or more times. When evaluating the individual reason for each readmission (first readmission only), 80% were related to operative complications and





c

Interval after Discharge	Patients at Risk at Beginning of Period	Cumulative Readmissions by End of Period	Cumulative Deaths without Readmission by End of Period
	Number (Percent)	Number (Percent)	Number (Percent)
1 – 29 days	1600 (100.0)	248 (15.5)	11 (0.7)
30 – 59 days	1341 (83.8)	358 (22.4)	29 (1.8)
60 – 89 days	1213 (75.8)	434 (27.1)	50 (3.1)
90 – 179 days	1116 (69.8)	593 (37.1)	130 (8.1)
179 – 364 days	887 (55.4)	784 (49.0)	266 (16.6)
>= 365 days	560 (35.0)		

Figure 1 Kaplan–Meier curves showing the time to readmission over the first year following initial discharge after pancreatectomy in Medicare patients. **a** Kaplan–Meier curve for time to readmission with deaths treated as censored. **b** Kaplan–Meier curve for time to readmission with deaths treated as a competing event. **c** The table shows the patients at risk at five different time intervals over the first year following discharge, the cumulative readmissions, and the cumulative deaths for five time periods, and the Kaplan–Meier estimates of readmission rates as well as combined readmission and death rates.

included abscess, sepsis, hemorrhage, probable pancreatic fistula, GI bleed, UTI, pneumonia, respiratory failure, and VTE/PE. When looking for specific ICD-9 codes, the most common diagnosis codes noted during readmission were for operative complications (27.4%), dehydration (27.8%), and gastric outlet obstruction/delayed gastric emptying (8.1%). Of the patients, 19.4% had a diagnosis code for metastatic disease, but this was often not the primary reason for admission. A complete list of diagnoses noted during early readmission is shown in Table 3.

Late Readmissions

There were 688 patients readmitted a total of 1,446 times between 30 days and 1 year following the initial pancreatectomy. Of the 688 patients, 152 were also readmitted within the first 30 days following surgery, leaving 536 patients in the late group at risk for first readmission. Late readmission was related to recurrence in 48%, operative complications in 25%, and unrelated diagnoses in 27% of cases. The diagnoses recorded during readmission in this time period differed significantly from the reasons observed in the first 30 days following discharge (Table 3). The most common diagnoses during late readmissions were metastatic disease (44.0%), dehydration (23.3%), and VTE/PE (9.1%).

Comparison of Reasons for Early and Late Readmissions

We compared the incidence of specific diagnoses during readmission in the early and late readmission groups. These

Table 3 Comparison of Reasons: Early vs. Late

	Early (*N*=248) Number (%)	Late (*N*=536) Number (%)	*P* value <0.0001
Operative complications	68 (27.4)	20 (3.7)	<0.0001
Metastases	48 (19.4)	236 (44.0)	<0.0001
Dehydration	69 (27.8)	125 (23.3)	0.17
Gastric outlet obstruction/delayed gastric emptying	20 (8.1)	21 (3.9)	0.02
VTE/PE	12 (4.8)	49 (9.1)	0.04
Pneumonia	<11	32 (6.0)	0.10
Cholangitis	14 (5.6)	32 (6.0)	0.86
Small bowel obstruction	11 (4.4)	35 (6.5)	0.24
Gastritis/duodenitis/gastric ulcer/duodenal ulcer/marginal ulcer	15 (6.0)	34 (6.3)	0.87
Incisional hernia[a]	0 (0%)	<11 (<2.1%)	0.09
Pancreatic pseudocyst	<11 (<4.4%)	<11 (<2.1%)	0.21
Acute myocardial infarction	<11 (<4.4%)	<11 (<2.1%)	0.91
Cerebrovascular accident	<11 (<4.4%)	<11 (<2.1%)	0.93
Bile leak[b]	<11 (<4.4%)	0 (0%)	0.04
Hip fracture[a]	0 (0%)	<11 (<2.1%)	0.03
Esophagogastroduodenoscopy and related procedures	22 (8.9)	71 (13.3)	0.08
Biliary drainage (endoscopic, percutaneous, or operative)	15 (6.1)	49 (9.1)	0.14

[a] Both groups <11 patients but significantly higher incidence of incisional hernias and hip fractures in the late readmission group.

[b] Both groups <11 patients but significantly higher incidence of bile leaks in early readmission group.

diagnoses may not have been the primary reason for readmission. When compared to patients in the late readmission group, patients readmitted early were more likely to be readmitted with a diagnosis of postoperative complications, gastric outlet obstruction/delayed gastric emptying, and pancreatic pseudocyst. They were less likely to be readmitted with metastatic disease, VTE/PE, and hip fractures (Table 3). The incidence of dehydration was similar between the two groups; however, in the late group, this diagnosis is more related to metastatic disease and failure to thrive, whereas in the early group, it is related postoperative complications.

Cox Proportional Hazards Model: Factors Predicting Early Readmission

The year of surgery, age, race, sex, marital status, income, education, Charlson comorbidity score, type of operation, complications recorded in the billing records for the initial hospitalization, postoperative length of stay (<10 days or ≥10 days), tumor stage, and nodal status were included in all multivariate models. For early readmissions, only the type of operation and the postoperative length of stay (LOS) predicted readmission. Patients undergoing distal pancreatectomy had a hazard ratio (HR) for readmission of 1.66 (95% CI, 1.19–2.33) when compared to those undergoing pancreaticoduodenectomy. Patients undergoing total pancreatectomy and pancreatectomy not otherwise specified had the same likelihood of readmission as patients undergoing pancreaticoduodenal resection. Patients with an initial postoperative LOS of ≥10 days had a HR for readmission of 1.46 (95% CI, 1.04–2.05). A recorded diagnosis code for operative complications during initial hospitalization (see methods) did not predict survival. The final model is shown in Table 4.

For late readmissions, deaths due to pancreatic cancer became a significant competing cause. Figure 1b shows a Kaplan–Meier curve where death and readmission are both treated as events. The rate of readmission or death within the first year was 35% (Fig. 1c).

We ran multivariate Cox proportional hazards models, the first with deaths as censored values at the time of death and the second with deaths as a competing event (Table 5). Considering deaths as censored creates informative censoring, since the same factors that influence cancer deaths likely influence late readmissions. As a result, in the first model, treating deaths as censored, only Hispanic race and a Charlson score of 3 or more (Table 4) predicted readmission. In the model with death as a competing event, the presence of distant disease at the time of surgery, positive nodal status, a Charlson score of 3 or more, and an initial length of stay ≥10 days predicted readmission (Table 5).

Survival Analysis

Patients readmitted within 30 days of discharge had worse median survival (median=11.8 months; 5-year survival,

Table 4 Cox Proportional Hazards Model: Factors Associated with Early Readmission

Factor (reference group)	HR	95% CI	Type 3, *P* value
Length of stay ≥10 days (<10 days)	1.46	1.04–2.05	0.03
Operation (pancreaticoduodenectomy)			0.02
Distal pancreatectomy	1.66	1.19–2.33	
Total pancreatectomy	1.29	0.67–2.46	
Other pancreatectomy	0.76	0.31–1.88	
Operative complications initial stay (yes)	0.98	0.71–1.34	0.91
Age (per year of age)	1.00	0.98–1.02	0.78
Year of surgery (per year)	1.04	1.00–1.08	0.07
Gender (male)	0.87	0.66–1.14	0.30
Race (non-Hispanic white)			0.20
Non-Hispanic black	1.03	0.62–1.70	
Hispanic	0.29	0.09–0.91	
Marital status (married)			0.92
Single	1.05	0.71–1.56	
Widowed	1.08	0.77–1.51	
Highest income quartile (1=lowest)			0.18
2	0.86	0.58–1.26	
3	0.67	0.47–1.04	
4	0.61	0.38–0.99	
Highest education quartile (1=lowest)			0.25
2	1.28	0.85–1.93	
3	1.31	0.84–2.03	
4	1.67	1.01–2.75	
Charlson comorbidity score (0)			0.39
1	1.06	0.77–1.44	
2	1.45	0.95–2.23	
3 or more	1.01	0.58–1.93	
Tumor stage (distant)	0.82	0.56–1.18	0.28
Nodal status (negative)	0.98	0.74–1.30	0.98

18%) than patients not requiring readmission (median= 16.5 months; 5-year survival, 18%, p=0.04, Fig. 2, N= 248). From the curves, you can see that this difference in mortality is early and likely attributable to postoperative complications. Patients surviving the insult of the postoperative complications have similar 5-year survival rates to those who did not suffer complications.

Readmission between 30 days and a year was associated with worse median survival (12.1 vs. 19.4 months) and 5-year survival (12% vs. 21%, p<0.0001, Fig. 3, N=536) when compared to those not requiring late readmission. As a quarter of late readmissions are related primarily to progression of disease requiring readmission, this is expected.

Multiple readmissions in the early time period were not correlated with survival. Multiple readmissions in the late time period were correlated with worse survival, presumably from recurrent disease requiring rehospitalization, whereas those who remained disease-free did not require hospital admission.

Discussion

Our study demonstrates an overall readmission rate of 53% and an early (within 30 days) readmission rate of 16% after pancreatectomy for pancreatic cancer in Medicare patients. The overall readmission rate, calculated using at time-to-event analysis, is similar to the 59% readmission rate in a previous population-based study[22] and higher than the reported rates in previous single-institution studies.[16,21] The higher rates in population-based studies more likely represent true readmission rates in the general population. Moreover, Yermilov and colleagues[22] found that 47% of readmissions were not to the hospital performing the primary surgery. It is likely that the single-institution studies did not capture readmissions to outside hospitals and may grossly underestimate readmission rates even in their own patients. In addition, the non-time-dependent methods used in previous studies will inflate the denominator or number at risk in a given period, decreasing the observed readmission rates.

Table 5 Cox Proportional Hazards Model: Factors Associated with Early Readmission

Factor (reference group)	Model with deaths censored HR (95% CI)	Model with deaths as competing cause
Race (non-Hispanic white)		
Non-Hispanic black	1.21 (0.88–1.67)	1.13 (0.85–1.50)
Hispanic	0.42 (0.23–0.75)	0.69 (0.46–1.01)
Charlson comorbidity score (0)		
1	1.12 (0.91–1.35)	1.09 (0.91–1.29)
2	1.06 (0.74–1.50)	0.98 (0.73–1.32)
3 or more	1.78 (1.18–2.68)	1.48 (1.04–2.12)
Tumor stage (distant)	0.82 (0.62–1.08)	0.71 (0.57–0.88)
Nodal status (negative)	1.19 (0.98–1.45)	1.30 (1.11–1.53)
Length of stay ≥10 days (<10 days)	1.14 (0.91–1.44)	1.23 (1.02–1.49)
Operation (pancreaticoduodenectomy)		
Distal pancreatectomy	1.07 (0.83–1.38)	1.16 (0.95–1.43)
Total pancreatectomy	1.11 (0.68–1.81)	1.12 (0.75–1.66)
Other pancreatectomy	1.34 (0.83–2.17)	1.46 (0.98–2.18)
Operative complications initial stay (yes)	1.09 (0.87–1.37)	1.07 (0.88–1.28)
Age (per year of age)	0.98 (0.97–1.00)	1.00 (0.99–1.01)
Year of surgery (per year)	0.99 (0.97–1.02)	1.01 (0.98–1.03)
Gender (male)	0.84 (0.70–1.01)	0.99 (0.80–1.09)
Marital status (married)		
Single	0.98 (0.75–1.28)	0.93 (0.74–1.16)
Widowed	1.08 (0.55–1.36)	1.03 (0.85–1.25)
Highest income quartile (1=lowest)		
2	0.83 (0.64–1.08)	1.01 (0.80–1.26)
3	0.80 (0.60–1.07)	1.01 (0.79–1.29)
4	0.69 (0.50–0.95)	0.91 (0.70–1.20)
Highest education quartile (1=lowest)		
2	1.27 (0.96–1.66)	1.10 (0.87–1.38)
3	1.19 (0.89–1.60)	1.02 (0.80–1.29)
4	1.19 (0.84–1.67)	1.00 (0.76–1.33)

Deaths or readmissions within 30 days were excluded. The first model treats deaths within the first year as censored. The second treats death as a competing event

As hypothesized, the length of the time period elapsed since undergoing pancreatectomy determined the reason for readmission. Early readmissions were more commonly associated with postoperative complications, while late readmission after 30 days was more likely to be due to disease progression (metastases or recurrence).

Dehydration occurred in approximately one quarter of patients in both the early and late groups. When evaluating diagnosis codes concurrent with dehydration, dehydration was more commonly related to surgical complications in the early readmission group while dehydration in the late group was more commonly related to chemotherapy or recurrence of pancreatic cancer and general failure to thrive. Likewise, the nature of postoperative complications differed between the early and late groups. Postoperative complications requiring early readmission most commonly included sepsis, abscess, anastomotic leak, and acute hemorrhage, whereas late complications included small bowel obstruction, incisional hernias, biliary strictures, and cholangitis. A diagnosis of delayed gastric emptying or gastric outlet obstruction was seen in 8% of early readmissions and 4% of late readmissions. Similar to dehydration, the reasons for the delayed gastric emptying or gastric outlet obstruction differed between the early and late groups. Early delayed gastric emptying following pancreaticoduodenectomy has been reported in 10–20% of patients immediately following pancreatic resection[3,25] and accounts for the majority of delayed gastric emptying or gastric outlet obstruction in the early group. In the late group, however, this diagnosis was associated with gastric outlet obstruction secondary to tumor recurrence.

Only the initial length of stay and the type of resection predicted early readmission. Those who had an initial length of stay of ten or more days were more likely to require early

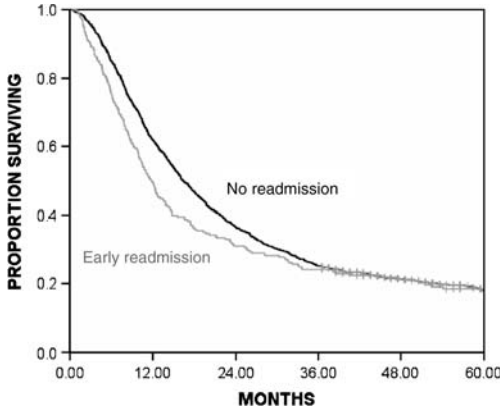


Figure 2 Kaplan–Meier actuarial survival curves showing comparing survival in patients who did not require readmission and those who were readmitted early (within 30 days, N=248). When compared to patients not readmitted, patients readmitted early had worse median survival (11.8 vs.16.5 months, P=0.04), but the long-term survival was identical (18%).

readmission. A diagnosis code for operative complications did not predict readmission. This suggests two things: first, not all operative complications are noted during initial admission and second, not all complications lead to readmission. A prolonged initial length of stay does not cause readmission; rather, it is likely a marker of serious postoperative complications, the most common diagnosis during early readmission. In addition, longer lengths of stay predispose patients to developing additional iatrogenic infections, as well as VTE/PE and atelectasis associated with prolonged immobility, which typically occur in a hospital setting. There was no association of age or patient comorbidities with early readmission.

This is the first study to demonstrate that patients undergoing distal pancreatectomy have an increased risk of readmission. This is unexpected as pancreaticoduodenectomy is a more complex procedure and thought to be fraught with more complications. However, pancreatic fistula rates have been reported to be higher following distal pancreatectomy than pancreatic head resection.[26–28] This fact, coupled with the fact that distal pancreatectomy is less likely to be performed at high-volume centers by experienced surgeons,[8] likely contribute to this finding.

In the first year after initial discharge, deaths due to pancreatic cancer became a significant competing event. Considering deaths as censored creates informative censoring, since the same factors that influence cancer deaths, likely influence late readmissions. As a result, the multivariate model evaluating the factors associated with late readmission, which treated deaths as censored, does not demonstrate the same predictive factors as the model that treats death as a competing event. By treating deaths a censored, patients with advanced tumor stage (distant disease and positive nodes) are removed from the at risk cohort. However, these factors are related to recurrence, the most common reason for readmission, and would likely have led to readmission in the absence of death.

While the median survival was lower in patients requiring early readmission compared to those who did not, the long-term survival was identical at 18%, suggesting that operative complications increase early deaths. However, survivors of these complications can expect similar survival to their counterparts who had an uncomplicated postoperative course. Late readmission is more commonly due to recurrence and is a marker of early mortality. As expected, it was associated with significantly worse median and long-term survival as shown previously.[22]

This study has several limitations, mostly related to the use of administrative data. The reported reasons for readmission in Table 3 were based on identification of specific ICD-9 diagnosis codes both in the primary discharge diagnosis and additional diagnoses provided for the same discharge. We also individually reviewed each readmission record and looked at the diagnosis and procedure codes and gave each readmission a primary reason for the admission. The results were similar using the two methods, in that early readmissions were related to surgical complications and late readmissions were related to recurrence; however, these were subject to the reviewers' interpretation.

It is often difficult to identify specific complications commonly reported after pancreatic surgery using administrative data, including pancreatic fistula and bile leak. For example, there are codes for postoperative complications and anastomotic leak, but they are not specific. In addition, the administrative data is used for billing purposes, so diagnosis codes mandating reimbursement may be more likely to be coded. While we were able to look at nodal status, data were not available on margin status to evaluate its effect on early and late readmission.

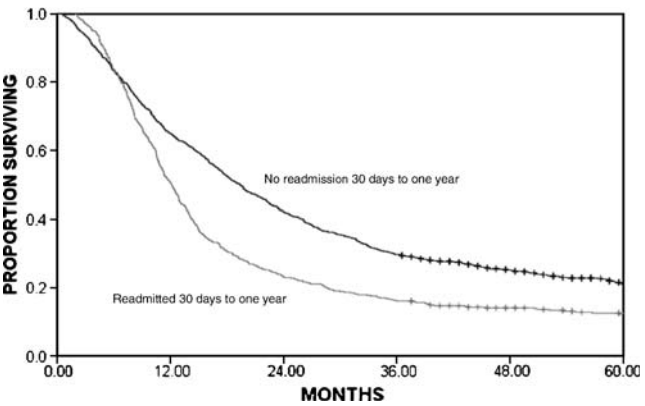


Figure 3 Kaplan–Meier actuarial survival curves showing comparing survival in patients who did not require readmission and those who were readmitted late (between 30 days and 1 year, N=536). Late readmission was associated with worse median and long-term survival (19.4 vs. 12.1 months, 21% vs. 12%, P<0.0001).

In summary, this study demonstrates the rates and the most frequent causes of early and late readmissions and identifies predictors of hospitalization during these time periods after initial discharge following pancreatectomy for pancreatic cancer. These findings reinforce the finding that readmission rates in the general population following pancreatectomy occur in over 50% of patients and are underreported in single-institution studies. Additionally, this study delineates the factors contributing to early and late readmissions. It demonstrates that early readmission related to complications shortens median but does not affect long-term survival if the patient survives the operative complication. Late readmissions are a marker of early mortality. Death due to cancer is a competing event with late readmission. As such, the factors influencing late readmission are similar to those that predict early mortality. The 15% of readmissions related to operative complications are, therefore, potentially preventable. The reasons for early readmissions need to be studied further to identify individual factors and operative techniques that decrease these preventable readmissions.

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Deepthi Martha Reddy, Presenter (University of Texas, Galveston, TX medical student)

Discussant

Dr. Sharon Weber (Madison): First, I have to congratulate you as a medical student in presenting this work. This is a very timely paper and I am really happy to see it presented here at the SSAT. As many of you know, CMS plans to use readmission as a quality of care indicator in the future because the estimated cost of readmissions has been estimated at about $17 billion. We know we have underestimated the rate of readmission when utilizing single-institution studies because of readmission at other hospitals. Using the SEER-Medicare database is a great way to obtain the actual rate of readmission, so I congratulate you on this work.

I have questions surrounding two main points. First, "how can we impact this?" and the second question surrounding this issue—"Are these findings real?"

To address the first question, clearly, the mortality of almost 8% in-house and 23% at 30 days is not acceptable. In addition, the readmission rate of 16% at 30 days is also very high, considering that the median length of stay was 14 days.

Your group has presented some of the seminal work looking at hospital volume, and I am wondering if you did not look at that here. Is hospital volume one area where we may be able to impact the rate of readmission and mortality? Were high-volume hospitals less likely to have higher numbers of readmissions? In addition, was there any difference in geographic patterns for readmissions?

Secondly, a recent publication by Coleman, in the *New England Journal* in April 2009, examined readmissions for Medicare patients using claims data. Of those 800,000 patients who underwent both small and larger surgical procedures, the readmission rates at 30 days and 1 year were almost identical to yours. Thus, this leads to the question, "do your findings represent a real phenomenon—that the readmission is higher after pancreatectomy, which is clearly a more complex operation than the average surgical procedure?" Or do these findings just imply that the Medicare population has a higher rate of readmission overall, perhaps because of increased age?

Closing discussant

Deepthi Martha Reddy: Thank you, Dr. Weber. We did not include data on hospital volume. We did not do so because some of the hospitals at which patients might go to undergo pancreatic resection may not be included in the SEER regions. As a result, they may falsely appear as low-volume hospitals.

When we evaluated hospital volume excluding hospitals not in SEER regions, hospital volume predicted mortality, but not readmission.

For the second question, I would like to refer to my mentor Dr. Riall.

Closing discussant

Dr. Taylor S. Riall (Galveston, TX): We did not include hospital volume because some of the hospitals at which patients might undergo pancreatic resection may not be included in the SEER regions. As a result, they may falsely appear as low-volume hospitals. For instance, Johns Hopkins is a high volume hospital, but it is not in a SEER region.

If you take patients who live in New Jersey, which is a SEER region, they may travel to Baltimore to have their surgery done at Johns Hopkins. In the database, we would be able to identify Johns Hopkins as an individual hospital, but it would not appear as a high volume hospital, since we would be calculating volume based only on the number of patients living in SEER regions who had their surgery done there.

In addition, you are looking at Medicare volume and not total pancreatic resection volume. Therefore, there are inherent problems with looking at hospital volume. When we evaluated hospital volume including only hospitals in the SEER regions, hospital volume was a predictor of mortality and increased length of stay, but not readmission.

With regard to your question regarding Medicare readmission rates compared to readmission rates for pancreatectomy specifically, I think you make a good observation. Even in the single institution studies, the readmission rates are high. Therefore, I think this is actually real and not simply the readmission rates for the Medicare population. Readmission is common following pancreatectomy, and we need to evaluate the reasons for readmission and areas for improvement in a multi-institutional setting. This can be increasingly important in this pay-for-performance era.

Discussant

Dr. Keith D. Lillemoe (Indianapolis): Again, I would just echo that the medical students here put us all to shame. Great presentation.

There is a bit of a disconnection. The 8.3%, 30-day mortality is high. Obviously it is not a high-volume center, tertiary center, or teaching hospital. It is a national database. Regardless, it is still too high.

However, the 15% readmission rate is very acceptable. Why is there such a disconnection? Is it the fact that these people are dying before they get readmitted? I do not quite understand your data because 15% readmission is about as good as you are going to see from any of the best of institutions, whereas an 8.3% mortality is unacceptably high.

Could you explain that disconnection to me? Is it something related to the data analysis or the database that you are using?

Closing discussant

Deepthi Martha Reddy: The early the 15% readmission rate was related to postoperative complications. This rate is likely lower than reported readmission rates since most studies look at 1 year readmission rates and not 30-day readmission rates.

Discussant

Dr. Keith Lillemoe (Indianapolis): However, do you not anticipate that the 8.3% operative mortalities are dying of postoperative complications? Those are not tumor progression for Whipples or pancreatic resections in 30 days.

Discussant

Dr. Charles Vollmer (Boston): I would like to shift gears and take it from the administrative level back down to the practice level. And the one thing that really struck me was the fact that if you are in the hospital for greater than 10 days, you have a very high chance of being readmitted soon thereafter.

These are cases where there is a deviation in the standard progression of the postoperative recovery period. And I wonder if we as surgeons can find a way to impact on that readmission rate by figuring out what we are doing wrong, or what is going on with the patient, in that first 10-day period or first stay.

Therefore, in other words, what could be predictive factors from the in-house recovery period that would say this person should not be sent home at this point? Maybe we are doing a disservice in trying to cut the length of stay days down, on some of these patients when we could tidy them up and solve the problems by keeping them in the hospital longer. Any thoughts?

Closing discussant

Dr. Taylor S. Riall: I personally think we are seeing these readmissions when we do not recognize postoperative complications. When you look at the readmission rates before and after initiation of critical pathways, you see decreasing length of stay and the readmission rates actually go down.

Therefore, I do not think the answer is to keep those people there longer to prevent the complications but, as you suggest, to identify the ones who have occult problems and need to stay. I think the patients that get readmitted are the occult complications that we do not recognize. For example, we might miss a pancreatic fistula that did not show up in the drains, so the patient appears to be "on the pathway." Then, we send them home, and they develop an abscess. I am not sure we are going to be able to reduce our readmission rates to zero, but I think it would be beneficial for high-volume centers to pool our data and identify factors predictive of readmission. This could potentially cut down readmissions and cost significantly.

I think one way to do it is to continue to centralize pancreatic resection at high volume centers. We could incorporate these predictive factors into our pathways.

Discussant

Dr. Henry Pitt (Indianapolis, IN): We have the NSQIP data from 2005–2007 on 2,000 pancreatectomies, and the mortality is less than 3% in that data base.

Closing discussant

Dr. Taylor S. Riall: This is Medicare data, and I suspect the higher observed operative mortality is expected. Increased mortality following pancreatic surgery in elderly patients has been well documented, so I would expect a higher rate in this data set than NSQIP, which includes patients of all ages and resections done for benign disease.

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2009 SSAT QUICK SHOT PRESENTATION

Role of Vagal Innervation in Diurnal Rhythm of Intestinal Peptide Transporter 1 (PEPT1)

Hisham G. Qandeel · Fernando Alonso · David J. Hernandez · Judith A. Duenes · Ye Zheng · Jeffrey S. Scow · Michael G. Sarr

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Abstract
Background Protein is absorbed predominantly as di/tripeptides via H^+/peptide cotransporter-1 (PEPT1). We demonstrated previously diurnal variations in expression and function of duodenal and jejunal but not ileal PEPT1; neural regulation of this pattern is unexplored.
Hypothesis Complete abdominal vagotomy abolishes diurnal variations in gene expression and transport function of PEPT1.
Methods Twenty-four rats maintained in a 12-h light/dark room [6AM–6PM] underwent abdominal vagotomy; 24 other rats were controls. Four weeks later, mucosal levels of mRNA and protein were measured at 9AM, 3PM, 9PM, and 3AM (n=6 each) by quantitative real-time PCR and Western blots, respectively; transporter-mediated uptake of dipeptide (Gly–Sar) was measured by the everted-sleeve technique.
Results Diurnal variation in mRNA, as in controls, was retained post-vagotomy in duodenum and jejunum (peak at 3PM, $p<0.05$) but not in ileum. Diurnal variations in expression of protein and Gly–Sar uptake, however, were absent post-vagotomy ($p>0.3$). Similar to controls, maximal uptake was in jejunum after vagotomy (V_{max}, nmol/cm/min: jejunum vs. duodenum and ileum; 163 vs. 88 and 71 at 3AM; $p<0.04$); K_m remained unchanged.
Conclusions Vagal innervation appears to mediate in part diurnal variations in protein expression and transport function of PEPT1, but not diurnal variation in *mRNA expression* of PEPT1.

Keywords PEPT1 · Diurnal rhythm · Vagotomy · Rat small intestine · Protein absorption

Introduction

The extrinsic nervous innervation regulates and modulates many physiologic functions of the small bowel, including both intestinal motility and absorption.[1–6] The cellular and molecular mechanisms of this neural control, however, remain largely unknown. Insights into these mechanisms will advance our understanding of the physiologic and pathophysiologic changes expected after certain forms of operative denervation, such as abdominal vagotomy and small-bowel transplantation, leading potentially to improvements in clinical practice.

Enormous interest has focused in recent years on the mechanisms regulating nutrient absorption from the lumen of the gut. In our laboratory, we characterized previously the gene expression and transport function of several mucosal nutrient transporters in the rat small intestine, such as hexose transporters[7–10] and, most recently, the H^+/peptide cotransporter-1 "PEPT1."[11,12] Being the exclusive peptide transporter in the apical membrane of enterocytes, PEPT1 mediates the uptake of essentially all di/tripeptides (the major protein digestion products) in addition to certain peptide-like drugs (e.g., β-Lactam antibiotics, ACE inhibitors) from the lumen.[13–15] Similar to several other mucosal transporters,

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PEPT1 exhibits diurnal variations in gene expression and transport function in rodents, especially in the proximal intestine (i.e., duodenum and jejunum); this diurnal pattern occurs in temporal association with their nocturnal feeding behavior.[11,16–19] Various factors appear to regulate this diurnal rhythm, such as luminal peptide substrates, hormones, and clock genes;[20–22] however, the role of extrinsic innervation to the gut in control of PEPT1 diurnal rhythmicity, as occurs with the hexose transporters, is unexplored.

The vagus nerve represents one of the primary extrinsic innervations to the gut. Because we and others have shown that vagal innervation appears to mediate in part the diurnal variations in the expression of other mucosal proteins (e.g., hexose transporters),[8,23] our aim was to determine whether neural mechanisms mediated by the vagus nerves mediate diurnal variation in gene expression (mRNA, protein) and transport function of the peptide transporter PEPT1. We hypothesized that total abdominal vagotomy would abolish diurnal variations of gene expression and transport function of PEPT1 in the rat small intestine.

Methods

Handling of animals, surgical procedures, and conduct of experiments were performed only after approval from the Mayo Clinic Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guidelines for the Humane Use and Care of Laboratory Animals. Male Lewis rats weighing 200–250 g (Harlan, Indianapolis, IN) were acclimatized to a 12-h photoperiod room (lights on only from 6AM to 6PM) with free access to water and standard rat chow (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO). Twenty-four rats underwent subdiaphragmatic, total abdominal vagotomy (see below, "Abdominal Vagotomy"); another 24 rats served as normal, unoperated controls. Daily weights of each rat and the chow consumed separately per light and dark hours were tabulated for 4 weeks postoperatively. To determine the presence or absence of diurnal rhythmicity in the expression and function of PEPT1, six rats at each of four time points (9AM, 3PM, 9PM, 3AM) were killed, and the levels of mRNA, protein, and transport activity for PEPT1 were measured in duodenum, jejunum, and ileum.

Abdominal Vagotomy

Rats were anesthetized initially using 2% inhaled isoflurane followed by intraperitoneal injection of 50 mg/kg sodium pentobarbital. A midline incision (3–5 cm) was performed, and the gastroesophageal junction was identified using blunt and sharp dissection as necessary. Using ×2 to ×3 optical magnifications, both anterior and posterior vagus nerves were identified, lifted off the esophagus, and ligated with 6-0 silk sutures, and 1-cm were sections excised. Homeostasis was achieved as needed with electric cauterization or by topical pressure. The abdominal wall was closed in two layers using a running 5-0 polyglactin suture. Postoperatively, all rats were maintained in a 12-h light/dark facility (one rat per cage) and allowed free (yet monitored) access to chow and water containing acetaminophen for the first 48 h postoperatively.

Tissue Harvest

At the time of tissue harvest, rats were anesthetized with inhaled isoflurane and intraperitoneal pentobarbital. After celiotomy, successful vagotomy was confirmed by visual observation of the distended stomach.[8] The duodenum was then cannulated just distal to the pylorus, and the small intestine was flushed with cold (4°C) Ringer's solution. The small intestine was excised and placed immediately in cold (4°C), oxygenated (95% O_2/5% CO_2) Ringer's solution. The proximal duodenum was used for the experiments of transport function using everted sleeves (see below, "Uptake Function"), and the distal duodenum was used for measurements of mRNA and protein. Similarly, mid-jejunum and mid-ileum were studied. The mucosa was scraped bluntly using a glass slide into cold, phosphate-buffered saline. Samples for mRNA analysis were placed in RNA stabilization buffer (RNALater, Qiagen, Valencia, CA) and stored immediately at −80°C. The samples for protein analysis were collected separately, placed in cold radioimmunoprecipitation assay (RIPA) buffer containing Halt protease inhibitors (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 of each anatomic segment were pinned on a support and fixed in 10% buffered formalin.

mRNA Measurement

Real-time, reverse transcription, polymerase chain reaction (RT-PCR) was used to quantitate mRNA levels of PEPT1 as described previously.[11] 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 cDNA using the Super Script III kit (Invitrogen, Carlsbad, CA); cDNA levels of PEPT1 and the stably expressed housekeeping gene glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were then determined 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. Within each major group (control and vagotomy), all samples underwent RT-PCR simultaneously using the same reverse transcription kit to minimize the possibility of error and variability within the same group.[24,25] Moreover, to enable direct comparisons between individual groups (i.e., anatomic segments at each time point) across the two major groups (control vs. vagotomy), we ran a further set of analyses of RT-PCR ensuring quantification of copy numbers using cDNA from simultaneous reverse transcription for all compared individual groups.

Protein Measurement

Levels of total cellular protein for PEPT1 were measured semi-quantitatively using our well-characterized technique with Western blots.[11] Tissue samples stored in RIPA buffer containing Halt protease inhibitors and PMSF were thawed on ice; the presence of protease inhibitors was used in attempt to minimize protein degradation. Samples were homogenized using a Kontes Pellet Pestle (Fisher Scientific, Pittsburg, PA). 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% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel (Bio-Rad, Hercules, CA) and transferred electrically to a polyvinylidene fluoride 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, visualized with a colorimetric reaction using Opti-4CN Substrate kits (Bio-Rad) for GAPDH and amplified Opti-4CN for PEPT1, were scanned, and 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 amount of protein per enterocyte.

Uptake Function

We measured transporter-mediated uptake of the dipeptide Glycyl–Sarcosine (Gly–Sar), a non-hydrolyzable substrate for PEPT1,[12,21] using a modified everted sleeve technique as we described previously.[9] Intestinal segments (1 cm) were everted over a pre-grooved steel rod and secured with silk ties, thereby exposing the mucosal surface externally. The intestinal "sleeves" were kept in chilled (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 mM: 129 NaCl, 5.1 KCL, 1.4 $CaCl_2$, 1.3 NaH_2PO_4, and 1.3 Na_2HPO_4; pH 6.0)[11,19] for 5 min bubbled with 95% O_2/5% CO_2 and stirred at 1,200 rpm. Then, the sleeves were placed in 8 ml of 38°C incubation medium containing 0.02, 1, 5, 20, or 40 mM Gly–Sar maintaining iso-osmotic conditions with replacement with appropriate amounts of NaCl. One µCi 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 containing 1 ml of tissue solubilizer (Perkin-Elmer, Boston, MA), and kept in a 50°C water bath for 3 h. After complete solubilization, 15 ml of scintillation counting cocktail (Opti-Flour, Perkin-Elmer, Waltham, MA) was added, and disintegrations per minute (DPMs) of ^{14}C were determined using liquid scintillation.

Transporter vs. non-transporter-mediated uptake The method of estimating transporter- vs. non-transporter-mediated uptake of Gly–Sar was described previously.[9] To calculate transporter-mediated uptake, total uptake needed to be corrected for passive diffusion and mucosal adherence (non-transporter-mediated "uptake"). Non-transporter-mediated uptake at lesser concentrations is best estimated from observed uptake at markedly greater concentrations.[11,26] 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 (0.02, 1, 5 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 tissues from both groups (control and vagotomy) 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. Measurements from each specimen were made on at least six slides with at least three measurements per slide.

Statistical Analysis

All levels of mRNA and protein 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 nmol/cm/min with Lineweaver–Burke plots used to calculate V_{max} and K_m. Data are reported as median±interquartile range (IQR). Kruskal–Wallis analysis was used to compare nonparametric 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 groups (control and vagotomy) displayed a nocturnal-based feeding pattern through the entire 4-week period (until tissue harvest); greater than 70% of chow intake occurred between 6PM and 6AM ($p<0.001$). During the first postoperative week (days 1–6), vagotomized rats consumed lesser amounts of food (as total daily intake) compared to controls ($p\leq0.003$; Fig. 1); however, by the end of the first week (day 7), there was no difference between both groups either in total daily intake or in night/day ratio of food consumption per rat ($p>0.1$). During the 4-week interval, the rats in the control group gained a median weight of 103 g (IQR, 97–110 g), while vagotomized rats gained a median weight of 95 g (IQR, 70–109 g) ($p>0.2$).

mRNA Expression

Diurnal Patterns of Expression Similar to the pattern exhibited by controls, mRNA levels of PEPT1 varied diurnally in the duodenum and jejunum of vagotomized rats (peak at 3PM, trough at 3AM; $p<0.05$; Fig. 2a, b), whereas ileal mRNA levels had no diurnal variations either in controls or in the vagotomy group ($p=0.2$). The median relative fold changes (peak over minimum levels) in the duodenum and jejunum of control rats were 5- and 4-fold, respectively. Similarly, median fold changes in vagotomized rats were 4- and 3-fold in the duodenum and jejunum, respectively.

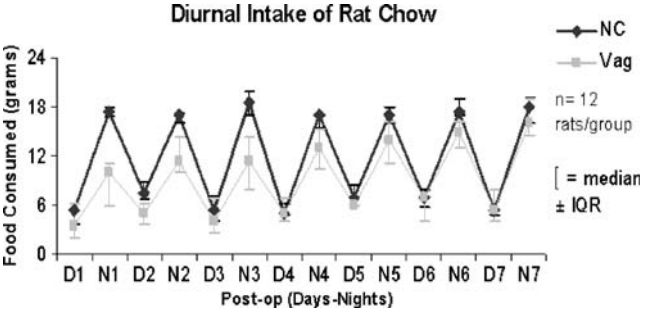

Figure 1 Pattern of food intake of normal control rats (*NC*) and vagotomized rats (*Vag*) for the first postoperative week; thereafter, food intake was virtually identical (data not shown).

Segmental Anatomic Expression In controls, there were no differences between the anatomic segments at any time point in the relative expression levels of mRNA per enterocyte ($p>0.2$); after vagotomy, however, this lack of difference between anatomic segments was retained only at

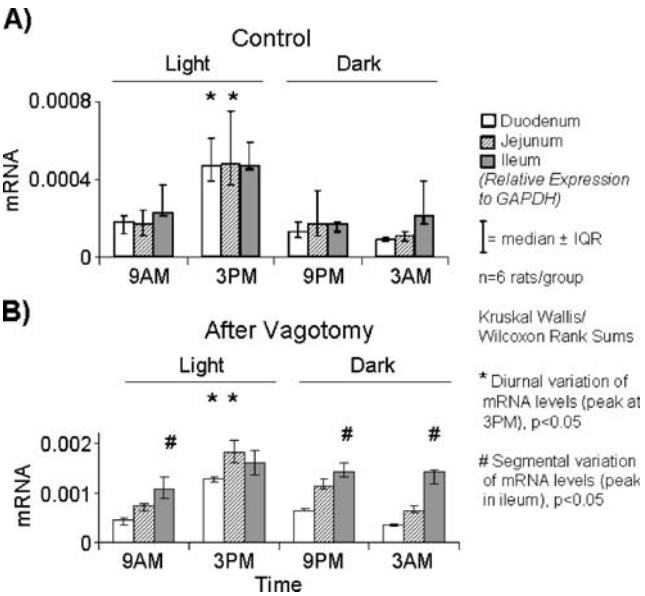

Figure 2 Relative expression levels of mRNA in **a** control and **b** vagotomized rats for PEPT1 in the three anatomic segments at four time points (each major group was studied separately using two different sets of analysis of RT-PCR). Levels of mRNA varied diurnally and peaked at 3PM in both groups. In controls, there was no difference between the intestinal segments in relative expression of mRNA (per enterocyte) at each time point; however, relative expression of mRNA varied between intestinal segments of vagotomized rats (peak in ileum).

3PM (when mRNA levels peak), but at other time points (9AM, 9PM, 3AM.), transcription of mRNA (per enterocyte) was greater aborally in the small bowel of vagotomized rats (ileum > jejunum > duodenum; $p<0.01$). Furthermore, when site-specific segments from both groups were compared individually (control vs. vagotomy for a given segment at each time point), vagotomized rats had greater mRNA levels than controls at 9PM in duodenum and 3PM in jejunum ($p<0.02$; Fig. 3a, b); there were no differences in ileal mRNA levels between any of the groups at all time points ($p>0.1$; Fig. 3c).

Protein Expression

Diurnal Patterns of Expression Levels of total cellular protein for PEPT1 showed a slight, albeit statistically significant, increase during the dark phase (9PM and 3AM) in the jejunum and ileum of control rats (<1.5-fold changes across time points; $p<0.05$; Fig. 4a). After vagotomy, however, these diurnal variations in total cellular protein seen in controls were abolished in all anatomic segments of vagotomized rats ($p>0.2$; Fig. 4b).

Segmental Anatomic Expression In both groups (control and vagotomy), there were no measurable differences between the anatomic segments in total cellular protein for PEPT1 ($p>0.3$). Moreover, when comparing each anatomic segment between groups (control vs. vagotomy at each time point), no differences were seen between any site-specific segments in levels of total protein (for PEPT1) per enterocyte ($p>0.1$).

Transporter-Mediated Uptake of Gly–Sar

Diurnal Pattern of Uptake Uptake of Gly–Sar in both control and vagotomy groups demonstrated saturation kinetics in all three anatomic segments consistent with transporter-mediated uptake. In control rats, uptake of Gly–Sar varied diurnally in duodenum and jejunum with values of Gly–Sar uptake (nmol/cm/min) being greater at 3PM and 9PM compared to 9AM for all concentrations ($p<0.05$; Fig. 5a, b); in the ileum, no significant diurnal variation was noted in Gly–Sar uptake ($p>0.5$; Fig. 5c).

In contrast, in vagotomized rats, all diurnal variations in transport function of PEPT1 (dipeptide uptake) were abolished completely; when measured at 4 weeks post-vagotomy, no diurnal variation in Gly–Sar uptake was noted in duodenum, jejunum, or ileum ($p>0.1$; Fig. 6a–c).

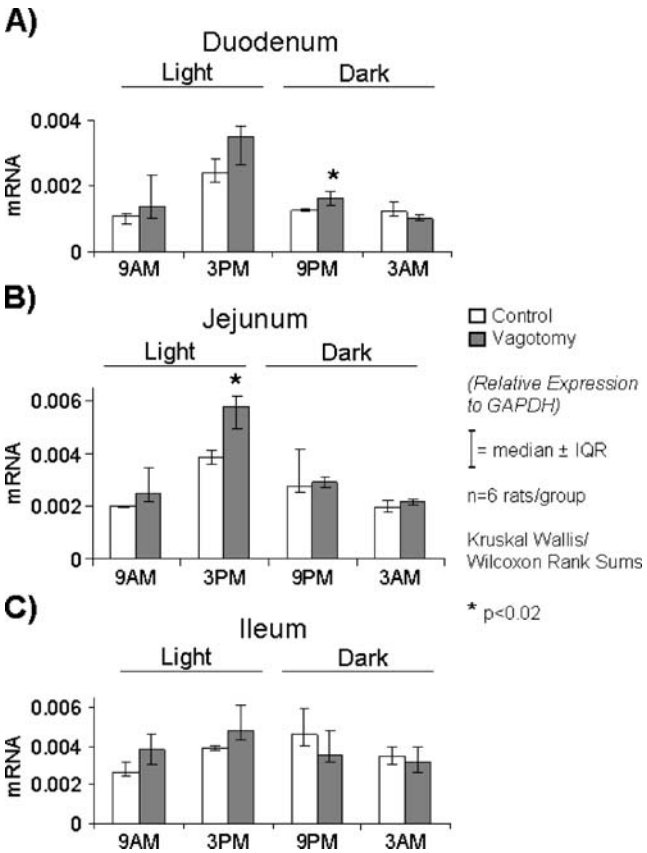

Figure 3 Variations in levels of mRNA between both groups (control and vagotomy) in **a** duodenum, **b** jejunum, and **c** ileum at four time points during the day (individual groups from both major groups were compared directly using additional sets of analysis of RT-PCR). mRNA expression was greater in vagotomized rats (compared to controls) in duodenum (at 9PM) and jejunum (at 3PM).

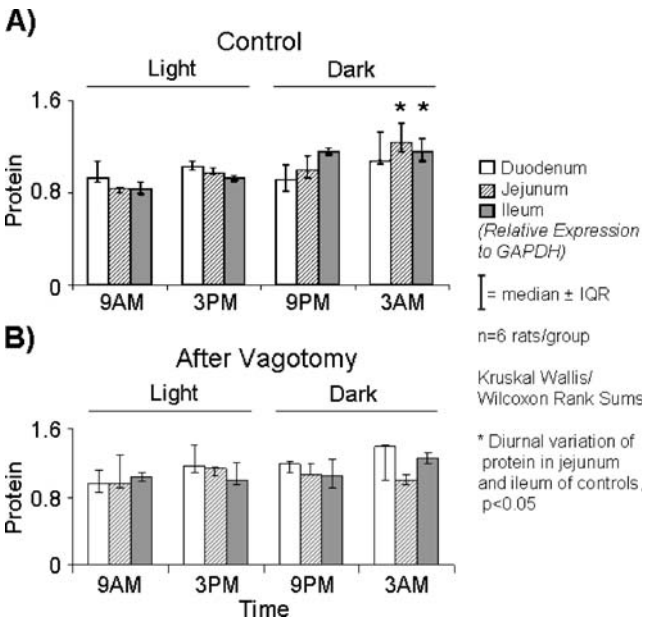

Figure 4 Variations in levels of protein in **a** controls and **b** vagotomized rats. Small but significant ($p<0.05$) diurnal changes were noted in protein levels in the jejunum and ileum of controls but not in vagotomized rats.

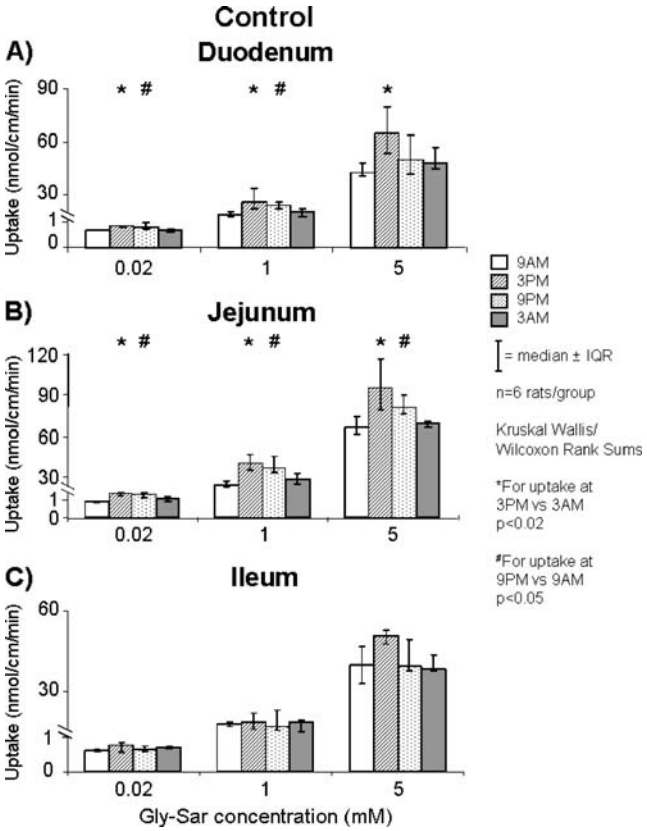

Figure 5 In control rats, transporter-mediated uptake of Gly–Sar at three concentrations in **a** duodenum, **b** jejunum, and **c** ileum at four time points. Gly–Sar uptake varied diurnally in duodenum and jejunum but not in ileum.

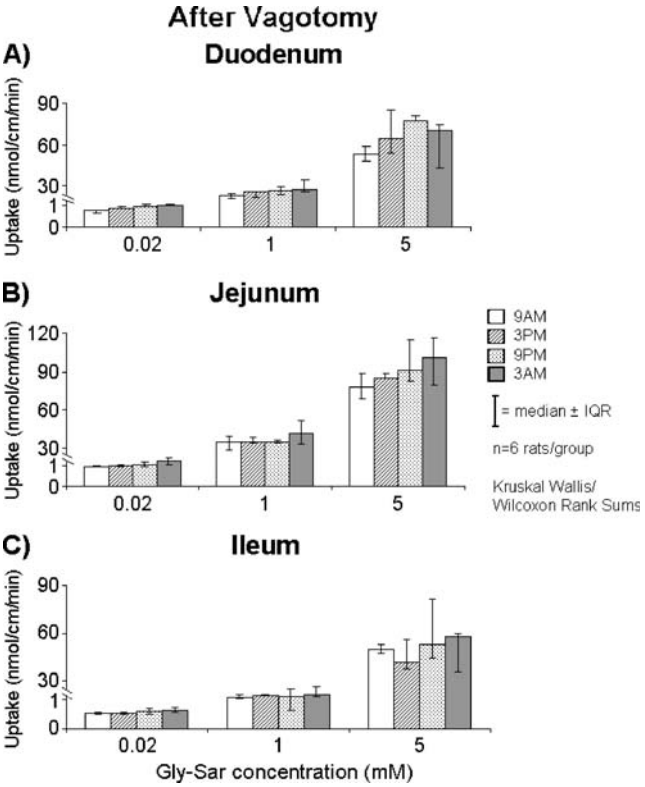

Figure 6 In vagotomized rats, transporter-mediated uptake of Gly–Sar at three concentrations in **a** duodenum, **b** jejunum, and **c** ileum at four time points. Gly–Sar uptake did not vary diurnally in any intestinal segment.

Uptake Kinetics The calculated V_{max} (in nmol/cm/min, a function of the number of apical transporters participating actively in uptake) varied diurnally in the proximal intestine of control rats (3PM vs. 9AM: 104 vs. 62 in duodenum and 185 vs. 101 in jejunum, $p<0.03$; Fig. 6a), while V_{max} remained unchanged in the ileum. In contrast, after vagotomy, V_{max} remained unchanged across time points at all anatomic segments (3PM vs. 9AM: 83 vs. 73 in duodenum and 138 vs. 137 in jejunum, $p>0.2$; Fig. 7b). K_m did not differ among the segments at all time points for both groups ($p>0.1$, Fig. 8a, b). When comparing V_{max} values between controls and vagotomized rats at each time point in each anatomic segment, no differences were present in duodenal and ileal segments ($p>0.2$); however, in the jejunum, vagotomized rats had a lesser V_{max} value compared to controls at 3PM (when uptake peaks in controls) but greater values than controls at 9AM and 3AM ($p<0.5$).

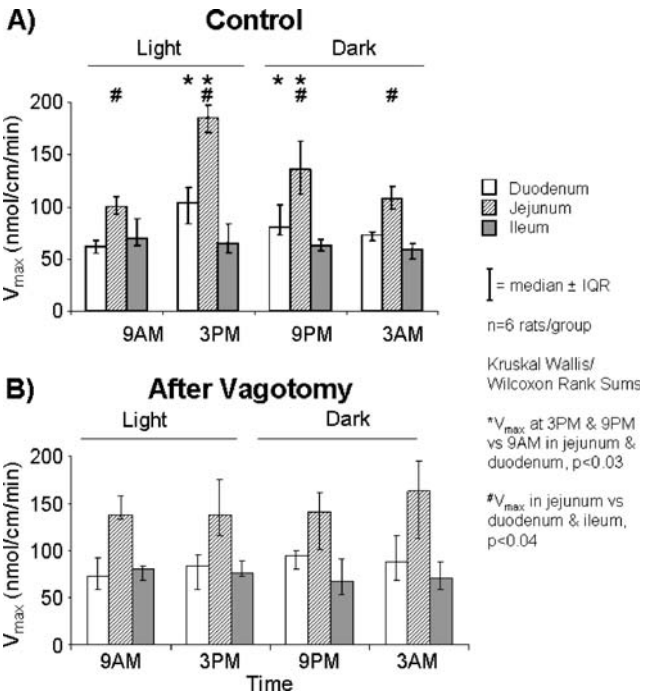

Figure 7 Values for V_{max} in each intestinal segment at four time points in **a** control rats and **b** vagotomized rats. In control rats, V_{max} varied diurnally in duodenum and jejunum but not in ileum. There was no variation of V_{max} in rats after vagotomy.

Villous Height

In both groups as expected, median villous height was greater in duodenum and jejunum compared to ileum (0.47

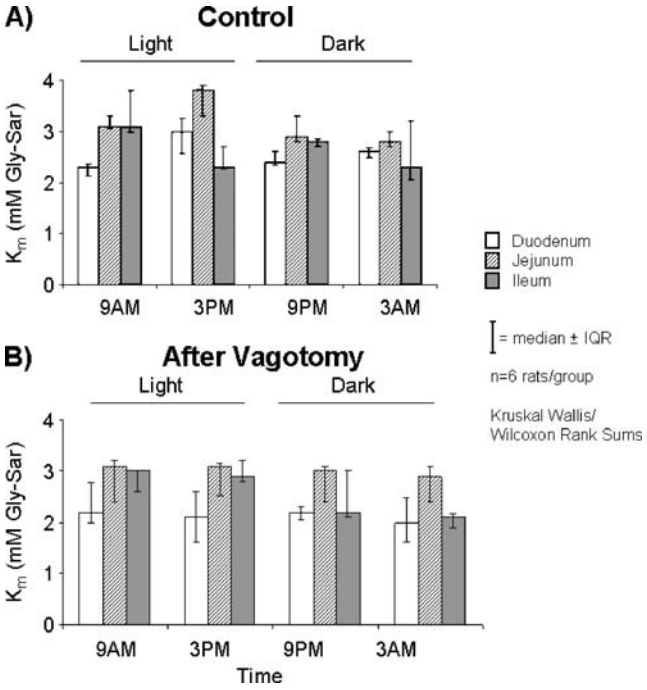

Figure 8 Values of K_m in each intestinal segment at four time points in **a** control rats and **b** vagotomized rats. K_m remained constant in both groups in each segment.

and 0.48 vs. 0.29 mm, respectively in controls; 0.51 and 0.49 vs. 0.32 mm, respectively in vagotomy; $p<0.0001$). There were no differences in villous heights between controls and vagotomized rats for each site-specific segment ($p>0.05$).

Discussion

Diurnal rhythmicity of absorptive function in the small intestine in rodents is a fascinating but poorly understood phenomenon. Diurnal variations in expression and function of mucosal transporters serve, presumably, to match the expected amounts of nutrients being delivered "diurnally" to the gut lumen; the mechanisms entraining these "anticipatory" rhythms are not known.[16,27] Recent studies from our laboratory and others suggested that extrinsic nervous innervation, primarily the vagal innervation, modulates in part this diurnal expression of mucosal hexose transporters;[8,23,28,29] however, to the best of our knowledge, no groups have investigated the role of vagal innervation in control of gene expression, transport function, and diurnal rhythm of the mucosal transporter "PEPT1," whose physiologic and clinical importance has been well recognized recently.

In this study, we delineated the impact of total abdominal vagotomy on baseline levels and diurnal variations in gene expression and absorptive function of PEPT1 throughout the rat small intestine. In order to assess the effect of vagal ablation on PEPT1 expression and function, we needed to show that the amount of food intake, pattern of food intake (light vs. dark cycle), and body weight were unchanged after vagotomy at the time of harvest. In fact, there was an initial postoperative lag in food intake and weight gain in vagotomized rats in the first week post-op. Our previous studies addressing hexose transporters showed a similar initial lag in vagotomized rats compared to sham laparotomy and normal controls,[7,8] most likely related to the vagal ablation; however, at the time of harvest 4 weeks later, there were no differences between controls and vagotomized rats in terms of feeding pattern (nocturnal feeding), amounts of food consumed daily, or overall weight gain, consistent with our past work. This sustained rhythmicity of nocturnal feeding after vagotomy correlated with the persistence of a diurnal variation in mRNA transcription in the duodenum and jejunum of vagotomized rats (as in controls); however, despite rhythmicity in feeding and mRNA expression, the diurnal variations of protein expression and transport function that occurred in controls were abolished when measured 4 weeks after vagotomy. These data suggest that neither the feeding pattern of rats nor the diurnal rhythm of mRNA expression are modulated by vagal innervation; however, diurnal variations of protein expression and transport function appear to be mediated in part by the vagal input to and/or from the small bowel.

Diurnal variations of mRNA expression in the proximal intestine of control and vagotomized rats had very similar patterns; mRNA levels peaked in anticipatory fashion 3 h before the dark cycle when most of the feeding occurred and declined to minimal levels 3 h before the light cycle when feeding had decreased. Preservation of this same diurnal pattern of mRNA expression after vagotomy (i.e., matched peak and trough levels with those of controls) reinforces the concept that these anticipatory rhythms of mRNA expression are not controlled or modulated by vagal innervation but rather entrained by other regulatory mechanisms, related probably to the role of peripheral clock genes in the gastrointestinal tract, which in turn, may be subject to control or modulation by other luminal, hormonal, and/or neural factors.[30–32]

Although an overall diurnal rhythmicity of mRNA levels was retained after vagotomy, several changes were noted in the absolute levels of mRNA for PEPT1 in the duodenum and jejunum in the two groups. Vagotomized rats had greater levels of total cellular mRNA of PEPT1 in duodenum (at 9PM) and jejunum (at 3PM) compared to the innervated control of rats; there were no differences between ileal segments from both groups. These differences might be related to loss of vagal input (vagal modulation) on cellular mRNA expression at the times when expression of mRNA for PEPT1 is maximal (late light phase, early

dark phase); no differences were noted at other times, raising the possibility that vagal innervation modulates signals controlling times of increased mRNA expression (transcription and/or stability). Indeed, in the vagally innervated control rats, the total cellular mRNA levels were similar across all three segments (duodenum, jejunum, and ileum), while after vagotomy, greater mRNA levels occurred in the more distal small intestine (ileum > jejunum > duodenum) at several time points in the diurnal rhythmicity, again suggesting a vagal modulation.

From a functional standpoint (peptide absorption), the changes in total cellular protein of PEPT1 and peptide uptake were equally interesting. Ultimately, protein absorption requires a functional peptide transporter in the apical membrane. Vagotomy led to a loss of the diurnal variation in total cellular levels of PEPT1 (as measured by the semiquantitative Western blots) despite the ongoing diurnal variations in mRNA, suggesting a potential vagal modulation of protein expression as has been postulated for hexose transporters.[8,23,29] But, measurement of total cellular protein by Western blots may not differentiate between functional and nonfunctional PEPT1 transporters or between intracellular stores of PEPT1 and apical, membrane-bound PEPT1 where the transport occurs. Therefore, it was necessary to evaluate actual transport function of PEPT1 to determine functional capacity for protein absorption. Consistent with the loss of diurnal expression of PEPT1 protein after vagotomy, diurnal variations in transport of Gly–Sar by PEPT1 were also absent after vagotomy, no longer peaking at 3PM and 9PM as in control rats. When the kinetics of uptake was evaluated, there was a loss of the diurnal variation in V_{max}, a measure of the number of "functional" PEPT1 transporters. In addition, the calculated K_m, a measure of transporter affinity for the substrate, remained unchanged, reinforcing the concept that the loss of the diurnal pattern of uptake after vagotomy is not a result of changes in type of transporter or protein conformation but rather a result of a loss of diurnal variations in the number of transporters expressed in the apical membrane of enterocytes after vagotomy.

A possible interpretation of these overall findings in PEPT1 expression (i.e., loss of rhythmicity of both protein expression and transport function after vagotomy) is that the mechanisms entraining these rhythms might have been impacted directly by loss of vagal input; thus, vagal input may modulate some aspect(s) of posttranscriptional and/or posttranslational processing; intracellular PEPT1 transporter proteins could be targeted for immediate breakdown and/or not be recruited to the apical membrane to participate in peptide uptake. As stated above, vagal modulation of protein translation for hexose transport has also been noted both by us[8] and by others.[23,29] In addition, there is good experimental evidence for recruitment of the hexose transporter GLUT2 to the apical membrane of the enterocyte by translocation from intracellular pools of transporter in response to greater concentrations of luminal substrate;[33–36] furthermore, some evidence suggests that PEPT1 may also be regulated in part by similar mechanisms of apical translocation.[22,37,38] Our study suggests strongly that vagal innervation modulates some aspect of the cellular regulation of PEPT1; however, under our experimental design, we cannot determine further the actual mechanism(s).

In order to assess the impact of vagotomy on mucosal histomorphometry, we measured villous height in three anatomic segments in controls and vagotomized rats. Changes in villous height (and thus the number of enterocytes per centimeter) could affect the results of dipeptide uptake (per 1-cm segment), although our measurements of expression of mRNA and protein would not be affected because these values were measured relative to the stably expressed housekeeper gene GAPDH and thus reflect indirectly the levels of mRNA and protein per enterocyte. Indeed, our measurements showed that there were no differences between the two groups in the villous height (for each corresponding segment), suggesting that vagotomy did not appear to cause a change in number of enterocytes. We cannot exclude the possibility of a change in enterocyte function, however, at least by this method of histomorphometry.

Our study, however, has several limitations that must be acknowledged that could affect the interpretation of our results. Vagotomy did lead to an obvious gastric distention at the 4-week time postoperatively. This gastric distention, although not affecting the diurnal pattern of food intake or weight gain of the animals, may have altered the timing of delivery of nutrients to the small intestine via a change in gastric emptying, a known effect of vagotomy. Similarly, vagotomy may alter transit through the small intestine. Different patterns of food delivery to the proximal and distal small intestine could affect the diurnal rhythms of protein expression and transport function via non-vagal mechanisms entraining these diurnal rhythms. Interestingly, the diurnal "anticipatory" patterns of gene expression were not affected by vagotomy, although the absolute levels were increased after vagotomy. Another potential limitation of our study is that we had no control group undergoing "sham laparotomy" to control for the anesthesia and celiotomy; however, previous studies from our laboratory addressing hexose transporters[8] and PEPT1[12] found no effects of sham laparotomy on gene expression, transport function, or on feeding patterns of rats.

Conclusion

While vagal innervation to the small bowel does not appear to regulate or modulate the diurnal rhythms of mRNA

expression for PEPT1, vagotomy does appear to mediate, in part, the diurnal variations of protein expression and transport function. This vagal control may have important implications in gut function after vagotomy, small bowel transplantation, bowel resection, or even in patients with short gut.

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2009 SSAT QUICK SHOT PRESENTATION

Predicting Organ Space Surgical Site Infection with a Nomogram

Luiz F. Campos-Lobato · Brian Wells · Elizabeth Wick · Kevin Pronty · Ravi Kiran · Feza Remzi · Jon D. Vogel

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Abstract
Purpose We hypothesized that organ space surgical site infections (organ space SSI) are a unique type of surgical site infection and therefore are associated with a unique set of risk factors. The aim of this study was to create a predictive model for organ space SSI after small bowel, colon, or rectal operations.
Methods The 2006 American College of Surgeons—National Surgical Quality Improvement Program (ACS-NSQIP) sample (N=12,373) was used to identify current procedural terminology codes for small bowel, colon, and rectal laparoscopic or open surgical procedures. The following variables were used to build a predictive model of organ space SSI within 30 days post-op: age, gender, body mass index, American Society of Anesthesiologists class, smoking, diabetes, steroid use, 30 days previous radiotherapy or surgery, preoperative serum creatinine and albumin, laparoscopic surgery, wound class, perioperative transfusion, operative time, and surgical site. Patients on chronic mechanical ventilation, dialysis, wound infection, or sepsis preoperatively were excluded.
Results Our organ space SSI model achieved a concordance index of 0.65 when validated in 2007 ACS-NSQIP patients (N= 9,521). A risk calculator designed based upon our model is available at www.clinicriskcalculators.com.
Conclusion This novel and validated nomogram is useful to predict organ space SSI associated with small bowel, colon, and rectal surgical procedures. It may also be useful for risk stratification and risk modification.

Keywords Risk factors · SSI · Abscess · Leak · Colectomy

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Introduction

The concept of surgical site infections (SSI) was introduced in 1992 by the National Nosocomial Infection Surveillance System of the Center for Disease Control. It is divided into three categories in accordance with the depth of the infection: superficial, deep, and organ space. In superficial SSI, the infection is restricted to the skin or subcutaneous tissue. Deep SSI is an infection that involves fascia and muscle layers of the incision. Organ space SSI (Org SSI) are best defined as infections that are related to the operation and involve any part of the anatomy opened or manipulated during an operation.[1] Abdominal or pelvic abscess after small bowel or colorectal surgery fits the definition of org SSI.

SSI has been reported as the second most common variety of hospital-acquired infections[2] and is the most common infection in surgical patients.[3] A recent study that evaluated the incidence of SSI after major colorectal procedures reported an incidence of superficial, deep, and organ space

SSI of 7.5%, 3.2%, and 3.6%, respectively.[4] Some additional ramifications of SSI, apart from their immediate effect upon the patient, are that they lengthen postoperative stay and significantly increase the cost of surgical practice.[5]

The first step in SSI prevention is identifying the risk factors related to it.[6] In this way, development of predictive models have become popular.[7,8] However, small sample sizes,[9] absence of external validation,[10] the use of variable definitions,[11] exclusion of small bowel resections, and grouping all subtypes of SSI as unique outcomes[12] are biases present in several of these models.

Therefore, the aim of this study was to develop a novel and validated National Surgical Quality Improvement Program (NSQIP)-based nomogram to predict organ space SSI after small bowel, colon and rectal operations.

Methods

Patient Cohort

The American College of Surgeons (ACS)-NSQIP database was queried for patients who underwent major colorectal surgeries from January 1st 2006 to December 31st 2006. Major colorectal surgeries were limited to the current procedural terminology (CPT) codes included in Table 1. Excluded from the study were any eligible patients with a preoperative diagnosis of sepsis, SSI (from a previous operation), ventilator-dependent patients, and concurrent dialysis.

Select variables known to be associated with SSI were chosen for inclusion in the nomogram.[6,8,13,14] These variables were used to create a linear regression model that predicts organ space SSI within 30 days of surgery and include smoking status, American Society of Anesthesiologist physical status classification (ASA class), wound classification, diabetes, steroid use in the past 30 days for a chronic condition, prior surgery in the past 30 days, radiotherapy for malignancy in the past 90 days, open or laparoscopic surgical technique, age, body mass index, most recent preoperative serum laboratory values (creatinine, albumin), gender, perioperative blood transfusion [>4 U of packed red blood cells (PRBC) prior to surgery, any transfusion during surgery, or >4 U of PRBC post-surgery], duration of surgery, and surgical site.

Table 1 CPT Codes

CPT	Definition
44120	Enterectomy with anastomosis
44140	Partial colectomy with anastomosis
44141	Colectomy, partial; with skin level cecostomy or colostomy
44143	Colectomy, partial; with end colostomy and closure of distal segment (Hartmann type procedure)
44145	Partial colectomy with low pelvic anastomosis
44146	Partial colectomy partial low pelvic anastomosis and colostomy
44150	Total colectomy with end ileostomys or ileoproctostomy
44153	Total colectomy (old code)
44155	Total proctocolectomy with end ileostomy
44156	Totalproctocolectomy with continent ileostomy
44157	Total proctocolectomy with ileoanal anastomosis,
44158	Total proctocolectomy with ileo-pouch anal anastomosis and loop ileostomy
44160	Partial ileocolectomy with ileocolostomy
44202	Laparoscopy enterectomy with anastomosis
44204	Partial laparoscopic colectomy with anastomosis
44205	Partial laparoscopic ileocolectomy with ileocolostomy
44207	Partial laparoscopic colectomy with low pelvic anastomosis
44210	Laparoscopy total colectomy, with ileostomy or ileoproctostomy
44211	Total laparoscopic proctocolectomy with ileo-pouch anal anastomosis and loop ileostomy
44212	Laparoscopy total colectomy with proctectomy, and ileostomy
44310	Ileostomy or jejunostomy, non-tube
44615	Intestinal stricturoplasty (enterotomy and enterorrhaphy) with or without dilation, for intestinal obstruction
44620	Closure of enterostomy
44661	Closure of enterovesical fistula; with intestine and/or bladder resection
45110	Abdomino-perineal resection
45113	Partial proctectomy,, with rectal mucosectomy and ileo-pouch anal anastomosis

Table 2 Surgical Site and Technique by CPT Code

Surgical site	Laparoscopic procedures	Open procedures
Small bowel	44202	44120
		44310
		44615
		44620
		44661
Colon	44204	44140
	44205	44141
		44143
	44210	44150
		44160
Rectum		44145
	44207	44146
	44211	44153
	44212	44155
		44156
		45110
		45113

Creation of Model

Values for all of the variables except for surgical site and surgical technique were obtained directly from the NSQIP dataset. Details about the NSQIP dataset have been published elsewhere.[12] Surgical technique (laparoscopic vs. open) and surgical site (small bowel, colon, or rectum) were determined from the CPT code documented for the principal operative procedure (Table 2).

Missing values for the predictor variables were imputed using the multiple imputations with chained equations (mice) package for R version 1.16. The imputation was performed without regard to the outcome. The linear regression model was fit using the 2006 NSQIP as the development dataset. Linearity assumptions of the continuous predictor variables were relaxed using restricted cubic splines.[15]

Model Validation

The model was validated on its ability to predict outcomes in the 2007 NSQIP dataset. Model validation was limited to those patients with complete data (i.e., no missing values for any of the predictor variables) since no imputation was performed on the validation dataset. Model discrimination was evaluated

Table 3 Patients Characteristics

Variable		Distribution (%)
Surgical Site	Colon	56%
	Rectum	25%
	Small bowel	19%
Smoking		18%
ASA class	1	4%
	2	53%
	3	39%
	4	4.0%
Wound class	1–2	86%
	3	9%
	4	5%
Diabetes		12%
Steroid in the past 30 days		7%
Prior surgery in the past 30 days		2%
Radiotherapy in the past 30 days		3%
Technique	Laparoscopic	27%
	Open	73%
Gender	Male	49%
	Female	51%
Transfusion		8%
Age (years)[a]		61 (49–73)
BMI (kg/m^2)[a]		26.9 (23.4–30.9)
Creatinine (mg/dL)[a]		0.9 (0.8–1.1)
Albumin (g/dL)[a]		4.0 (3.5–4.3)
Time of Surgery (min)[a]		147 (100–206)

[a] Values expressed in median and interquartile range

Table 4 Surgical Site Vs. Organ Space SSI

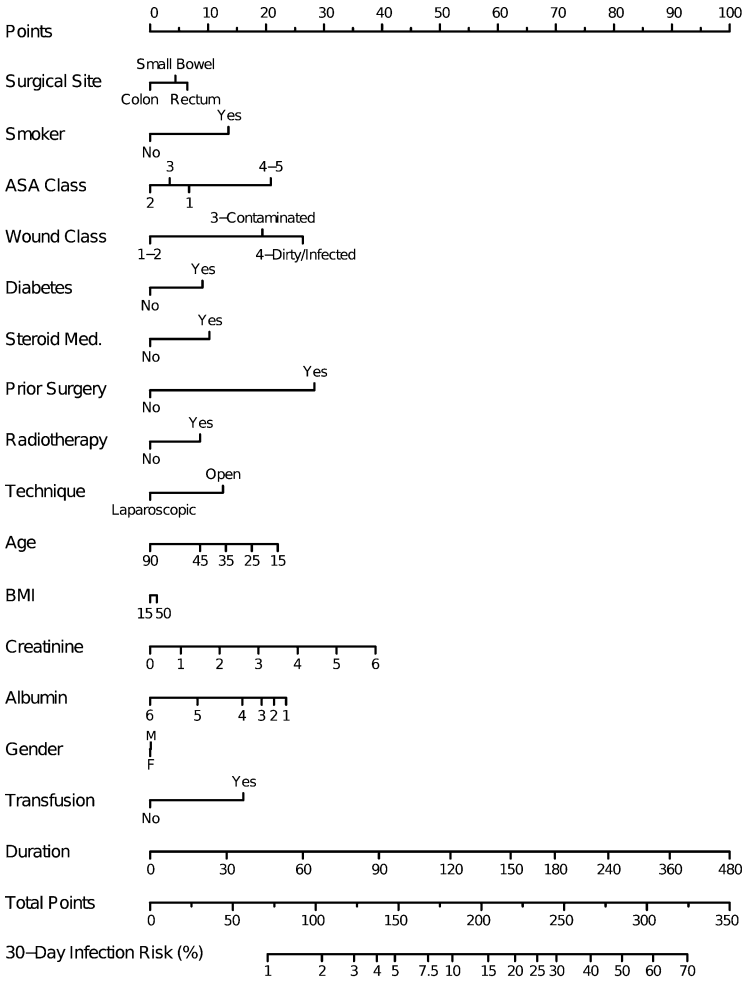
Site	Total		Organ space SSI	
	N	%	*N*	%
Small bowel	2,378	19	71	3.0
Colon	6,910	56	189	2.7
Rectum	3,085	25	125	4.1
Total	12,373	100.0	385	3.1

with the use of the concordance index (C index). The concordance index is defined as the probability that given two randomly selected patients, the patient with the worse outcome was, in fact, predicted to have a worse outcome. This measure, similar to an area under the receiver operating characteristic curve,[16] ranges from 0.5 (i.e., chance or a coin flip) to 1.0 (perfect ability to rank patients). Model calibration was assessed by plotting the proportion of patients predicted to develop organ space SSI versus the actual who developed organ space SSI in each quintile of predicted risk.

Results

Query returned 12,373 patients who underwent small bowel, colon, and rectal resections. Table 3 provides patient characteristics of the development dataset after imputation.

Of the patients, 56% underwent surgery of the colon, and 27% of the surgical procedures were performed



Instructions: Identify the patient's surgical site and draw a vertical line to the "points" axis on the top of the page. Repeat this process for the remaining variables. Sum the points for each individual variable and locate this on the "Total Points" axis at the bottom of the page. Draw a vertical line from this spot on the Total Points axis straight down to calculate the risk of OrgSSI within 30 days.

Variables	Low Risk	High Risk
Surgical Site	Colon	Small Bowel
Smoker	No	Yes
ASA Class	1	3
Wound Class	2	4
Diabetes	No	Yes
Steroids	No	Yes
Prior Surgery	No	Yes
Radiotherapy	No	No
Technique	Laparoscopic	Open
Age (years)	50	30
BMI (Kg/m2)	20	15
Creatinine (mg/dL)	1.0	2.5
Albumin (g/dL)	4.0	2.0
Gender	Male	Female
Transfusion	No	Yes
Duration	90 min	300
Risk of OrgSSI	1%	39%

Figure 1 Nomogram and example of a low-risk and a high-risk case. The low and high risk cases *depicted in the columns to the right are examples of nomogram use and do not represent aspects of the nomogram itself.*

laparoscopically. Three hundred eighty-five patients experienced organ space SSI within 30 days of surgery (Table 4).

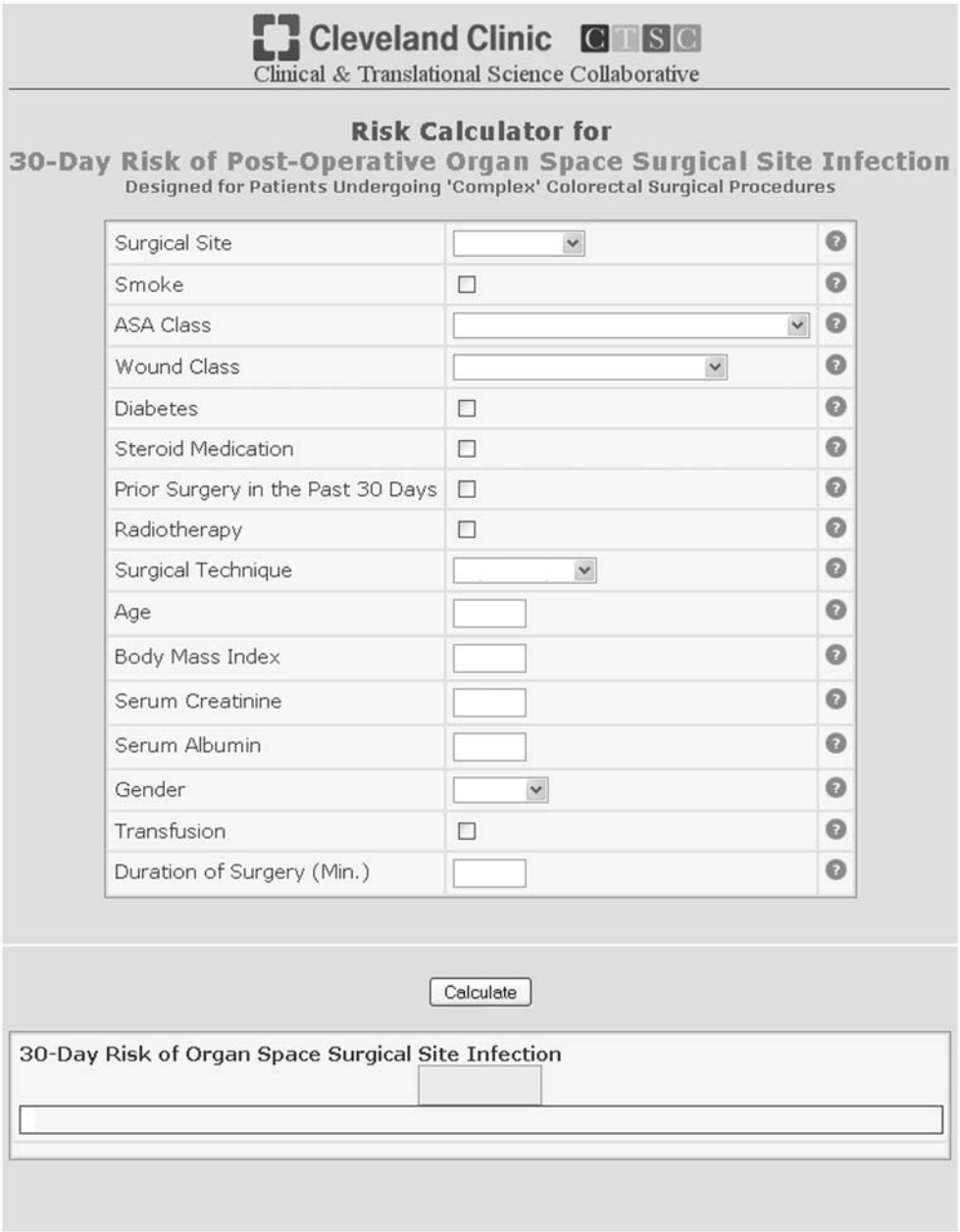
The nomogram for predicting the risk of organ space SSI within 30 days is illustrated in Fig. 1.

The duration of surgery appears to have the highest potential for increasing organ space SSI. Elevated creatinine levels and previous surgery were also stronger organ space SSI predictors. Age was inversely associated with infection risk, which might be a result of higher incidence of inflammatory bowel disease in younger patients.

A risk calculator was designed based on the nomogram and it available for free access at www.clinicriskcalculators.org (Fig. 2).

The validation dataset based on the 2007 NSQIP contained 9,521 patients and 343 events. The results of the validation are shown in Fig. 3. Overall, the model shows very good calibration. The model appears to slightly underestimate the risk of infection in low-risk patients while slightly overestimating risk in patients at the highest level of risk. The model accurately identified the highest risk patient 65% of the time among all pairs of patients with discordant outcomes (C index=0.65).

Figure 2 Risk calculator.



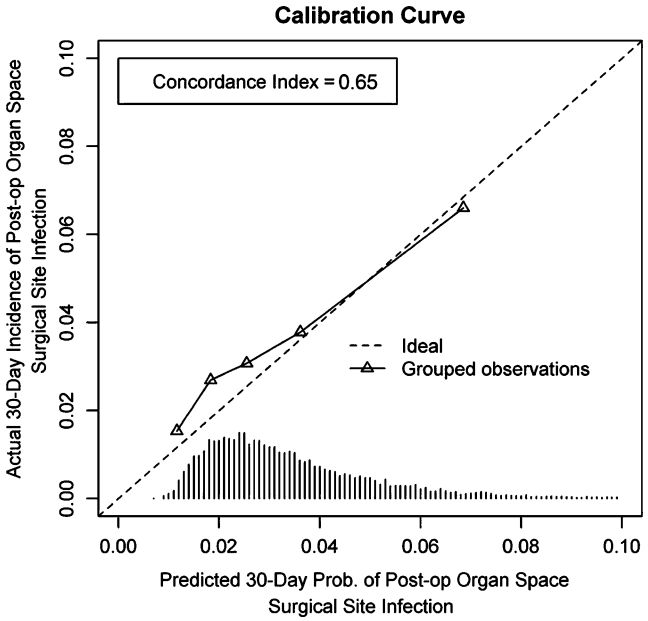


Figure 3 Calibration curve.

Discussion

The purpose of our study was to develop and validate a NSQIP-based nomogram to predict org SSI in patients that undergo small bowel, colon, or rectal surgery. Several prognostic factors were incorporated into the nomogram, including nonlinear and more complex relationships. Unlike systems that assign prognosis based on risk groups, nomograms such as ours can be used to estimate risk based on a combination of variables. As a result, the outcome prediction can be more individualized.[17]

Previous tools for predicting postsurgical infections typically combined potentially minor superficial wound infections with deeper, more serious infections.[18,19] Not only are superficial infections less clinically relevant, but they may also not share the same risk factors with org SSI. Other predictor models were designed for specific diagnosis or surgical procedures alone,[9,19–23] but our model has the advantage of being useful for a variety of diagnoses and surgical procedures. The current ACS-NSQIP model for SSI has a concordance index of 0.61 and is designed for use in predicting all types of SSI after colon and rectal surgery. The concordance index of our model compares favorably to the ACS-NSQIP model and has the advantage of being specifically designed to predict Org SSI after small bowel, colon, or rectal operations.[24]

The emphasis on a more accurate outcome estimate may not seem attractive to all surgeons and patients. Undoubtedly, the majority of surgeons currently use the prognostic factors included in our nomogram when discussing with their patients the potential benefits and complications of an eventual surgical procedure. However, the nomogram provides a sound mechanism for conveying the impact of multiple clinical and surgical factors. In this way, it might be most functional for those patients for whom the potential surgical procedure benefit is marginal.

The main strength of our study is that it is based on the ACS-NSQIP dataset which includes a large and diverse patient sample. Additional strengths are that our nomogram was developed and validated in two different and equally large samples and the online risk calculator is simple, free, and easy to use. A limitation of our study is that the diagnostic indication for small bowel, colon, or rectal surgery was excluded from the nomogram. In the ACS-NSQIP dataset, nonspecific diagnoses such as "unspecified small bowel obstruction," "hemorrhage," and "obstruction/perforation" may be used. In these cases, the actual diagnosis may be cancer or a broad range of benign diseases. Rather than exclude any case with an uncertain diagnosis, we chose to eliminate this variable and group cases by procedure type rather than by diagnosis. Another limitation of our study is that we did not asses the impact of other relevant unique occurrences, including "sepsis," on the risk for organ space SSI.

Conclusion

This novel and validated nomogram can be used to predict organ space surgical site infections associated with common, major small bowel, and colorectal procedures. Risk stratification and risk modification are potential uses of this nomogram.

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2009 SSAT PLENARY PRESENTATION

The Impact of Socioeconomic Status on Presentation and Treatment of Diverticular Disease

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Abstract
Introduction Diverticular disease is a common medical problem, but it is unknown if lower socioeconomic status (SES) affects patient outcomes in diverticular disease.
Material and methods The New York (NY) State Inpatient Database was used to query 8,117 cases of diverticular disease occurring in patients aged 65–85 in 2006. Race and SES were assessed by creating a composite score based on race, primary insurance payer, and median income bracket.
Results Primary outcomes were differences in disease presentation, use of elective surgery, complication rates when surgery was performed, and overall mortality and length of stay. Patients of lower SES were younger, more likely to be female, to have multiple co-morbid conditions, to present as emergent/urgent admissions, and to present with diverticulitis complicated by hemorrhage ($p<0.0001$).
Discussion Overall, patients of low SES were less likely to receive surgical intervention, while rates of surgery were similar in elective cases. When surgery was performed, patients of lower SES had similar complication rates (25.4% vs. 20.2%, $p=0.06$) and higher overall mortality (9.0% vs. 4.4%, $p=0.003$).
Conclusion Patients of low SES who are admitted with diverticular disease have an increased likelihood to present emergently, have worse disease on admission, and are less likely to receive surgery.

Keywords Diverticulitis · Socioeconomic status · Race · Surgery · Propensity scores · Access · Disparities · NIS

Introduction

Disparities in health care attributed to race and socioeconomic status (SES) are well documented.[1–3] This has been attributed to a number of factors, including access to care,[4,5] patient education and attitudes toward care,[6,7] and differences in provider/hospital quality.[8,9] Differences in care attributed to race or SES have not previously been described for most gastrointestinal disorders.

The development of colonic diverticula is extremely common in the westernized, industrialized world. It is estimated that 60% of people in these countries will develop diverticula at some point in their lives.[10] Many of these patients will experience complications of this disease, most commonly manifested as diverticulosis with bleeding or diverticulitis. The treatment of acute diverticular disease requires a combination of medical and surgical therapy, as well as long-term care consisting of diet modification and prompt follow-up for any recurrences. These factors contribute to make this an ideal medical–surgical condition to use in investigating for differences in disease presentation, hospital management, and overall outcomes based on race and SES.

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Methods

A retrospective analysis was performed using discharge records from the State Inpatient Databases (SID) from New York State (NY) from 2006. The Healthcare Cost and Utilization Project (HCUP) supports these databases, which contain all patient discharge records from participating hospitals. All acute care nonfederal hospitals including both academic and specialty hospitals reporting data are included in the databases. In NY, this constituted 206 out of a possible 207 hospitals.

This study was reviewed by and received exemption from the University of Massachusetts Institutional Review Board, as no personal identifiers are listed in the SID data.

Study Population

The Clinical Modification of the International Classification of Diseases, 9th Revision (ICD-9-CM) diagnostic and procedural codes was used to identify diagnoses and procedures. All patients aged 65–85 with a primary diagnosis code for colonic diverticular disease (ICD-9 diagnosis codes 562.10, 562.11, 562.12, and 562.13) were identified. From an initial cohort of 8,537 cases, 420 cases (4.9%) were eliminated due to missing race or income bracket variables, leaving a final cohort of 8,117 cases.

Race/Socioeconomic Score

Patient demographic characteristics compiled in the SID were used to create a composite score based on race and SES. Race was grouped into white (2 points) and non-white (0 points). Income bracket is a categorical variable based on the estimated median household income in a patient's ZIP code, divided into quartiles. In 2006, the poorest quartile was defined as those making less than $38,000 per year (0 points), and the highest income bracket was defined as those making more than $63,000 per year (2 points), with the middle income brackets (1 point) falling between these two values. Insurance status was divided into three groups: those with primary private insurance or primary Medicare and secondary private insurance (2 points); those with Medicare alone (1 point); and all others (0 points). These points were summed, and patients were grouped into tertiles based on total score (Table 1). Due to a dichotomous comparison, groups were divided into Low (lowest third) and High (middle and upper thirds) SES groups.

Study Variables

Age was incorporated as a continuous variable. Admission type was divided into two groups: emergent/urgent (emergent) and elective cases. Admission source was similarly divided into those patients coming through the emergency room (ER) and those admitted through other channels (i.e., outpatient clinic, scheduled admit, etc.). Additional characteristics of disease presentation were identified by determining all patients with any diagnosis present at admission of peritonitis (ICD-9 diagnosis code 567), intestinal obstruction (ICD-9 code 560), or intestinal abscess (ICD-9 code 569.5).

Patients undergoing a primary procedure of colectomy (ICD-9 procedure codes 45.73–45.79) were identified. Laparoscopic colectomy was identified as those patients with a primary procedure code for colectomy along with a secondary procedure code of laparoscopy (ICD-9 procedure code 54.21) as previously described.[11] Major postoperative complications were identified using a validated set of ICD-9 diagnosis and procedure codes. These complications consisted of postoperative infection, myocardial infarction, aspiration pneumonia, deep vein thrombosis/pulmonary embolus, pulmonary compromise, gastrointestinal hemorrhage, reopening of surgical site, and procedure-related perforation or laceration were identified as previously described (Simons, in press).

To evaluate comorbidity, the Elixhauser comorbidity index was used. This previously validated index identifies 29 specific disease entities that are considered true preoperative comorbidities rather than complications of care.[12] Scores between zero and three were created based on how many comorbid diseases each patients had.

A unique feature of the SID is its inclusion of detailed, itemized charges for each admission. HCUP provides software that can be used to combine information from these charges codes as well as ICD-9 codes to identify a series of "utilization flags" that cover a wide range of hospital resources, including ICU stay and administration of blood products. The NY SID also provides the total number of units of blood used in each admission, allowing for further quantification of this important resource.

Outcomes

The goal of this study was to identify any differences in outcome for patients suffering from a medical–surgical

Table 1 Racial/Socioeconomic Score System

Variable	Low SES (n=2,978, 32% of cases)				High SES (n=5,536, 68% of cases)		
SES score	0	1	2	3	4	5	6
Number of cases	77	826	703	975	1,739	2,292	1,505

SES socioeconomic status

condition based on racial and socioeconomic factors. Furthermore, efforts were made to identify any precipitating factors that could explain these differences. Primary outcomes included differences in disease presentation (disease type, comorbid conditions, and admission type/source), disease treatment (use and timing of surgical intervention), and overall outcomes (in-hospital mortality, surgical morbidity, length of stay, use of hospital resources, and total hospital charges).

Volume

Volume is an inexact method of assessing hospital and provider ability but does provide some indication of the experience individual institutions and physicians have in treating this disease, and higher volume providers and hospitals have been shown to be associated with improved outcomes in some disease conditions.[13] Physician and hospital identifiers were used to determine the total number of cases of diverticular disease treated by individual attending physicians and hospitals, as well as the number of colectomies performed by individual surgeons and hospitals.

Case-Controlled Analysis

Propensity scores were used to further investigate whether differences in outcomes were dependent on disparities in patient population, hospital characteristics, and patient comorbidities.[14] An advantage to the use of propensity scores is that the model is not constrained with overfitting, multiple testing, and the conventional $p<0.05$ criteria for variable inclusion. Candidate factors for the propensity model were important demographic and disease factors, including age, gender, and Elixhauser comorbidity score. The propensity groups reduce all of these differences between patients. We used a Greedy 5→1 digit matching algorithm for matching.[15] This algorithm first matches on five digits of propensity score and then subsequently on four, etc. A matched cohort was created in which all demographic differences between low and high SES groups (n=2,581 in each group) were eliminated, allowing us to evaluate the effect of race and SES status in a case–control fashion. Within each group, the association between each demographic or disease characteristic was determined by the χ^2 or Student's t test.

Statistical Analysis

SAS 9.1 software (SAS Institute, Cary, NC) was used to analyze data. A Student's t test was used to determine statistical significance for continuous variables. χ^2 analysis tested categorical variables. Statistical significance was defined as $p<0.05$.

Logistic regression was used to generate propensity scores to minimize bias from non-random assignment. Covariates included age, gender, race/SES, comorbidities, and hospital characteristics. A Hosmer–Lemeshow goodness-of-fit test was performed to confirm the final model. All results in the regression model were represented by an odds ratio and 95% confidence interval (CI). All regression models were performed separately.

Results

Patient Population and Disease Presentation

There were 8,117 patients with a primary diagnosis of diverticular disease in NY in 2006 that were included in this study. The average age of all patients was 75, and 64% of all patients were female (Table 2). Forty-one percent of patients presented with three or more co-morbid conditions. Low SES patients were younger (75 vs. 76, $p<0.0001$), more commonly female (70% vs. 62%, $p<0.0001$), and more likely to have multiple co-morbid conditions than high SES patients (44% vs. 39%, p<0.0001). High SES patients were more likely to be white race (97.7% vs. 22.2% in the low SES). Non-white race therefore comprised 77.8% of the low SES group compared to 2.3% of the high SES group.

Sixty-one percent of patients presented with diverticulitis, while the remainder presented with diverticulosis (39%). In cases of diverticulosis, the majority of patients (82%) presented with hemorrhage. Hemorrhage was rarely seen in cases of diverticulitis (8% of cases). Low SES patients more commonly presented with diverticulosis than high SES patients (47% vs. 36%, $p<0.0001$), and cases of diverticulitis were more likely to present with hemorrhage (11% vs. 7%, $p<0.0001$).

As one would expect, most patients were admitted emergently (91%), and most commonly came through the ER (82%). Low SES patients were more likely to be admitted emergently (95% vs. 89%, $p<0.0001$) and more likely to enter the hospital through the ER (86% vs. 80%, $p<0.0001$).

Hospital Management

Fourteen percent of all patients were treated surgically during their hospital stay with a colectomy. Surgery was less commonly performed in low SES patients (11% vs. 15%, $p<0.0001$), although this difference was not statistically significant when looked at by admission type. Similarly, low SES patients were less likely to receive laparoscopic surgery (1.7% of colectomies vs. 5.3%, $p=0.01$), which, again, was due to differences in admission type.

Table 2 Patient and Admission Characteristics of 8,117 Patients Admitted with Diverticular Disease in 2006 in New York

Variable	Total (n=8,117)	Low SES (n=2,581)	High SES (n=5,536)	P value
Percent of total cases	100%	31.8%	68.2%	
Patient characteristics				
Mean age (median)	75.3 (76)	74.6 (75)	75.6 (76)	<0.0001
Female gender	64.1%	69.6%	61.6%	<0.0001
Race				<0.0001
White	73.7%	22.2%	97.7%	
Non-white	26.3%	77.8%	2.3%	
Elixhauser comorbidity				<0.0001
0	8.8%	6.9%	9.7%	
1	22.8%	21.3%	23.6%	
2	27.7%	28.0%	27.6%	
≥ 3	40.7%	43.9%	39.3%	
Disease presentation				
Primary diagnosis				<0.0001
Diverticulosis	39.3%	46.8%	35.8%	
% with bleed	82.3%	80.2%	83.5%	
Diverticulitis	60.7%	53.2%	64.2%	
% with bleed	7.9%	11.4%	6.6%	
Admission type				<0.0001
Emergent/urgent	90.9%	94.8%	89.0%	
Elective	9.1%	5.2%	11.0%	
Admission source				<0.0001
ER	81.9%	85.7%	80.1%	
Routine	16.7%	12.8%	18.6%	
Surgical intervention				
Colectomy performed	13.9%	11.2%	15.2%	<0.0001
In emergent admissions	8.8%	8.5%	9.0%	0.48
In elective admissions	64.7%	60.2%	65.7%	0.23
Laparoscopic colectomy	4.4%	<4%	5.3%	0.01
In elective admissions	9.4%	<13%	10.0%	0.29
Volume—all cases				
Hospital volume mean (Median)	66.8 (57)	60.5 (55)	69.8 (61)	<0.0001
Attending volume mean (median)	3.3 (2)	3.1 (2)	3.3 (2)	<0.0001
Volume—colectomy				
Hospital volume mean (median)	12.3 (10)	9.4 (8)	13.3 (10)	<0.0001
Surgeon volume mean (median)	3.4 (2)	2.5 (2)	3.7 (3)	<0.0001

SES socioeconomic status, *ER* emergency room

Provider and Hospital Volume

The mean number of cases of diverticular disease treated by an individual hospital was 67 (median, 57; range, 1–208). Low SES patients were treated at hospitals with less experience with the disease than high SES patients (mean 61 vs. 70, $p<0.0001$). The mean number of cases of diverticular disease treated by an individual attending physician was 3.3 (median, 2; range, 1–20). Low SES patients were also treated by attending physicians with less experience than high SES patients (mean, 3.1 vs. 3.3, $p=0.003$). The mean number of surgical cases performed by an individual surgeon was 3.4 cases (median, 2; range, 1–24 cases). In surgically managed cases, low SES patients were treated at lower volume hospitals (9.4 vs. 13.3 cases, $p<0.0001$) by lower volume surgeons (2.5 vs. 3.7 cases, $p<0.0001$). In cases that were performed electively, there was also a volume effect favoring high SES. Patients with High SES were operated on at higher volume hospitals (mean, 14.6 vs. 11.2; range, 1–37; $p=0.002$) and by higher volume surgeons (mean, 4 vs. 3 cases; range, 1–24; $p=0.007$)

Unadjusted Outcomes

Seventy-one percent of patients were discharged directly to home (Table 3). Overall mortality was 1.6%, which did not differ significantly between low and high SES (1.8% vs. 1.5%, $p=0.34$). Low SES patients were less likely to be discharged directly to home (68% vs. 72%, $p=0.002$) and almost three times more likely to leave against medical advice (1.1% vs. 0.4%, $p=0.0002$). Low SES patients had longer hospital stays (mean 6.7 days vs. 6.3, $p=0.03$) and higher hospital charges (mean, $30,000 vs. $27,500, $p=0.02$) than high SES patients. Sixteen percent of patients in both groups required care in the intensive care unit. Low SES patients were more likely to require a blood transfusion (35% vs. 28%, $p<0.0001$).

Outcomes in Surgical Cases

In cases treated with colectomy, only 40% of patients were discharged directly to home without services, and mortality was 6%. Low SES patients were less likely to be discharged directly to home (33% vs. 42%, $p=0.007$) and had higher mortality than high SES patients (9.0% vs. 4.4%, $p=0.003$). Low SES patients had longer hospital stays (15 days vs. 12, $p=0.002$) and higher hospital charges ($70,500 vs. $58,700, $p=0.007$). Rates of surgical complications were not significantly different between the two groups (25% in low SES, 20% in high SES, $p=0.06$).

Logistic Regression Models

In order to further investigate differences in treatment and outcomes, logistic regression models were created to identify factors that were independently predictive of outcomes. Factors included in these models were age, gender, patient comorbidities, admission type, and low vs. high SES. Age, male gender, and increasing comorbidity were all independently predictive of increased likelihood to enter the hospital emergently, as was low SES, which was 2.5 times more likely than high SES to present emergently (OR, 2.49; 95% CI, 2.04–3.00, $p<0.0001$; Table 4). Increased use of surgery was independently linked to younger age, increased comorbidity, and elective admissions. Low SES patients were slightly less likely to receive

Table 3 Outcomes of 8,117 Patients Admitted with Diverticular Disease in 2006 in New York

Variable	Total		Low SES		Middle/high SES ($n=843$)	High SES ($n=5,536$)	*P* value
	$n=8,117$	$n=1,131$	$n=2,581$	$n=288$			
Disposition							<0.0001
Home	70.7%		68.4%			71.8%	0.0002
Transfer	11.7%		11.6%			11.8%	
Home health care	15.4%		17.1%			14.5%	
AMA	0.6%		1.1%			0.4%	0.0002
Died	1.6%		1.8%			1.5%	0.34
Mean LOS (median)	6.5 (5)		6.7 (5)			6.3 (4)	0.03
ICU Stay	16.0%		16.2%			16.0%	0.83
Mean total charges (median)	$28.3K (16.9K)		$30K (18K)			$27.5K (16.4K)	0.02
Surgical cases							
Disposition							0.008
Home		40.1%		33.3%	42.4%		
Transfer		23.7%		26.0%	22.9%		
Home health care		30.7%		31.6%	30.4%		
Died		5.6%		9.0%	4.4%		
LOS—mean (median)		12.8 (10)		14.7 (12)	12.2 (9)		0.002
Blood transfusion		45.0%		58.3%	40.4%		<0.0001
Mean pints of blood (median)		1.5 (0)		2.8 (0)	1.1 (0)		0.2
ICU stay		44.5%		47.2%	43.5%		0.28
Mean total Charges (median)		$61.7K (42.9K)		$70.5K (51.8K)	$58.7K (40.9K)		0.007
Any complication		21.5%		25.4%	20.2%		0.06

SES: Socioeconomic status; LOS: Length of stay; ICU: Intensive care unit

Table 4 Logistic Regression Models: Likelihood of Entering Hospital Emergently and Receiving Surgery

Variable	Emergent Admit			Receive Surgery		
	Odds Ratio	95% CI	*P* value	Odds Ratio	95% CI	*P* value
Age	1.08	1.07–1.10	<0.0001	0.96	0.94–0.97	<0.0001
Female	0.71	0.61–0.84	<0.0001	1.11	0.95–1.29	0.18
Comorbidity	1.16	1.10–1.22	<0.0001	1.09	1.04–1.14	0.0007
Emergent				0.06	0.05–0.07	<0.0001
Low SES	2.49	2.04–3.00	<0.0001	0.86	0.73–1.00	0.055

surgery, although this difference was not statistically significant (OR, 0.86; 95% CI, 0.73–1.00, $p=0.055$).

Overall mortality was significantly related to increased age and comorbidity (Table 5). In cases where surgery was performed, risk factors for mortality were increasing age and comorbidity, emergent admission, and low SES (hazards ratio, 1.8; 95% CI, 1.0–3.1, $p=0.039$).

Propensity Matching

Because significant differences in age, gender, and pre-existing comorbidities existed between the low and high SES groups, propensity scores were used to create a matched cohort in which the differences in these variables were eliminated.

Differences in disease presentation remained in the propensity-matched groups (Table 6). Patients of low SES were more likely to present emergently, enter the hospital through the ER, and be treated by higher volume physicians at higher volume hospitals. Differences in utilization of surgery and of laparoscopic surgery were again insignificant after accounting for differences in admission type.

Outcomes in the propensity matched groups were significantly different (Table 7). As in the unmatched cohort, patients of low SES were less likely to be discharged directly home, more likely to leave AMA, and had higher rates of blood transfusion. Mortality, length of stay, and total charges were not significantly different between the two groups. In surgical cases, low SES patients were less likely to be discharged directly to home and had higher rates of blood product usage, longer lengths of stay, higher total charges, higher complication rates, and higher mortality.

Discussion

The results of this population-based study of inpatient NY data from 2006 suggest that SES accounts for significant differences in patient presentation, management and outcome in patients admitted with diverticular disease. Patients of low SES were more likely to present with emergent or urgent visits, diverticulitis with bleeding, and admission through the ER. There were also differences observed in medical and surgical management with patients of low SES less likely to undergo colectomy. Finally, there were dramatic differences in outcome after surgery, including increased use of blood products, complications, and in-hospital mortality. We chose diverticulitis as the model of our analysis, since it has a medically and surgically based management with varying degrees of severity. Its management is complex; yet, it represents a common disease entity that can be studied at a regional level with sufficient power.

This study centered on the creation of a novel race/SES score. This score was devised to incorporate available variables from the NY HCUP database. The demographics included were race, income bracket, and insurance type. Race was categorized as white or non-white, since race alone is not a predictor of low SES class. Income bracket was categorized into the three possible scores (lowest quartile, second and third quartiles, and highest quartile), and insurance type was based on government or private insurance. Due to the incorporation and reliance of Medicare and Medicaid, we felt that it was necessary to include only patients aged 65–85 years in this study.

Multiple studies have shown that patients of non-white race and low SES are more likely to present emergently,

Table 5 Logistic Regression Models: Mortality for Patients Admitted with Diverticular Disease

Variable	Overall mortality			Mortality in colectomy		
	Hazards ratio	95% CI	*P* value	Hazards Ratio	95% CI	*P* value
Age	1.08	1.05–1.12	<0.0001	1.09	1.04–1.14	0.0002
Female	0.92	0.64–1.32	0.64	1.18	0.66–2.1	0.59
Comorbidity	1.44	1.30–1.59	<0.0001	1.39	1.18–1.64	<0.0001
Emergent	2.13	0.78–5.84	0.14	9.29	2.84–30.4	0.0002
Low SES	1.22	0.84–1.76	0.29	1.79	1.03–3.11	0.039

SES socioeconomic status

Table 6 Admission and Treatment Demographics in Propensity Matched Groups of 5,162 Patients

Variable	Low SES (n=2,581)	High SES (n=2,581)	P value
Admission diagnosis			<0.0001
Diverticulosis	46.8%	32.6%	
Percent with bleed	80.2%	82.9%	0.13
Diverticulitis	53.2%	67.4%	
Percent with bleed	11.4%	5.6%	<0.0001
Emergent/urgent admission	94.8%	87.9%	<0.0001
Admitted through ER	85.7%	78.9%	<0.0001
Treated with colectomy	15.7%	11.2%	<0.0001
In elective admissions	60.2%	65.4%	0.29
Laparoscopic colectomy	–	5.2%	0.02
In elective admits	–	9.3%	0.40
Hospital volume (median)	60.5 (55)	69.2 (58)	<0.0001
Surgeon volume (median)	3.1 (2)	3.4 (2)	0.005

SES socioeconomic status, *%* percent, *ER* emergency room, (–) actual numbers not reported due to data use confidentiality agreement

perhaps indicating that they are less likely to seek care when a disease is still in its early stages.[16] This increase in emergent presentations is undesirable for both the patient and the health care system, as it may contribute to higher overall health care cost and resource utilization. Becker[17] showed in a 10-year analysis of patients with uterine fibroids that patients of non-white race/ethnicity had higher total cost and length of stay compared to white patients. In this study, it is unclear whether disease stage and presentation were more severe in the low SES group. Patients in this group did present more emergently and had less elective surgery, but this could be due to a variety of reasons not related to race or SES.

Poor attitudes toward health care and lack of patient motivation are often cited as explanations for adverse outcomes in patients of non-white race and low SES. While this may sometimes contribute, there are multiple indications that the health care system bears a significant portion of this burden as well. Reporting on the SHARE trial, Siciliani and Verzulli[18] found that patients of lower education level and lower income had longer waiting times for specialty care and non-emergent surgery. In an assessment

Table 7 Outcomes in Propensity Matched Groups of 5,162 Patients

Variable	Low SES		High SES		P value
	(n=2,581)	(n=288)	(n=2,581)	(n=405)	
Disposition					0.04
Home	68.4%		71.4%		0.02
AMA	1.1%		0.5%		0.02
Died	1.8%		1.3%		0.18
ICU stay	16.2%		14.8%		0.18
Blood transfusion	35.3%		26.7%		<0.0001
LOS—mean (median)	6.7 (5)		6.5 (5)		0.31
Total charges—mean (median)	$30K (18K)		$28K (16.7K)		0.11
Surgical cases					
Disposition					0.005
Home		33.3%		42.5%	0.01
Died		9.0%		3.7%	0.003
ICU stay		47.2%		40.7%	0.09
Blood transfusion		58.3%		38.8%	<0.0001
LOS—mean (median)		14.7 (12)		12.5 (9)	0.01
Total charges—mean (median)		$70.5K (51.8K)		$61.4K (40.8K)	0.08
Any complication		25.4%		18.5%	0.03

SES socioeconomic status, *AMA* against medical advice, *U* units, *ICU* intensive care unit, *LOS* length of stay

of the treatment of non-small cell lung cancer, Hardy et al.[19] found that blacks were less likely to receive surgery or chemotherapy for their disease. Provider characteristics also represent an important factor in determining health outcomes. Birkmeyer and others have shown that higher case volume for both physicians and hospitals may significantly improve outcomes in a variety of surgically managed conditions. This group and others have also shown that black patients, and other disadvantaged groups, may be less likely to receive care from high volume physicians and centers.[2,20,21] Further studies to identify the scope of this discrepancy and to develop plans to improve on it are necessary.

Due to differences in the unadjusted cohort, a matched analysis is critical to make sure that observed differences in outcome are not due to inherent patient factors such as age or comorbidity. The use of propensity scores to create a risk-adjusted, demographically matched cohort is an advantage of our study. Propensity scores reduce the entire collection of observed background characteristics to a single variable that appropriately summarizes those characteristics.[22] In the matched cohort, we still observed that patients of low SES had a higher percentage of emergent admissions and admissions through the ER. In addition, they were less likely to be treated with colectomy (11.2% vs. 15.7%). There was a mortality benefit in the high SES group, which may be attributed to use of less blood products. Use of surgery only attenuated these findings, resulting in patients in the high SES group received less blood products, lower total hospital charges, and surgical complications.

Receipt of care and access to experienced resources have been an ongoing area of investigation in field of disparities and outcomes research. We were surprised to see stark differences in provider (hospital or physician) volume in the care of patients in the two groups. Although it is difficult to make judgments about access to care in this retrospective analysis because of the emergent nature of diverticular disease, given that a large proportion of the surgical cases were treated with elective admissions, one could surmise that access to surgery may be hindered in the low SES group. This is an area that warrants further investigation.

Several limitations to this study must be considered. This was a retrospective study and has the associated constraints due to the level of the NY SID data. For example, we were unable to confirm the validity and accuracy of the diagnostic and procedure coding.[23] The main outcome measure of this study was in-hospital mortality. This may reflect a lower mortality rate compared with studies using 30-day mortality, as most patients were likely discharged from the hospital prior to the potential death (if applicable). Our study used population-based data with only limited information on patient and treatment factors, thereby limiting our evaluation of medical factors such as presence of cancer, cirrhosis, antibiotic use, mechanical ventilation, and prior surgery. Use of colectomy and type of admission can lead one to decipher when surgery was performed in an elective vs. emergent setting, but this may also lead to inaccuracies based on coding. In addition, if patients were treated for diverticular disease in NY, but then underwent surgery outside of the state, then those cases were obviously not captured.

In summary, significant differences in patient presentation, hospital management, and surgical outcomes were observed based on SES and race in this retrospective analysis of inpatient NY state data. Access to care remains important both for individual patient outcomes and for health care utilization. Further studies, especially those incorporating longitudinal data from both inpatient and outpatient facilities, are necessary to validate our findings.

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Conflicts of interest Authors have no disclosures to report. No conflicts of interest exist.

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Discussant

Dr. Timothy M. Pawlik (John's Hopkins, Baltimore, MD): Nick, that was a really fantastic presentation and another great paper from your group at University of Massachusetts. I appreciate being provided the manuscript beforehand.

Most of my comments concern the creation of the composite scoring system because everything flows from the scoring system. My questions are as follows.

First, I think that most of us would agree that there are disparities. The challenge is in trying to understand what are the root causes of these disparities. So, why did your group choose to create a composite score that combined race, income, and insurance status into one score? Why not analyze each of these factors separately and then look for interactions, etc.? It seems that you lost some of your power in the study by combining all the factors into an aggregate score right from the start.

My second question is how exactly was the composite score derived? It does not seem like that the score was weighted according to the potential impact of each factor. For example, if you were white and you were in the poorest SES bracket, you got 2 points. Similarly, if you were non-white but you were high in the highest SES bracket, then you also got 2 points. I do not know that it makes sense empirically to me that you are mixing and equating pointwise race with income. So, how exactly was the composite score derived and has it been validated with a test set of data?

Third, you divided your data into tertiles but then you analyze it dichotomously? Why did you do that? Why not just analyze the data in tertiles and use the statistical methodology for doing that?

My final question surrounds the disease that you chose to study—diverticular disease. Diverticular disease is a longitudinal disease where we frequently see patients multiple times in the clinic or they have multiple hospitalizations. One of the limitations of the NIS data set is that it does not allow for longitudinal tracking of the patients. Do you have any insight if patients had multiple hospital admissions prior to the one you measured? Perhaps, low SES patients were presenting with more extensive disease? Can you just shed some additional light on some of the limitations of the NIS data set for looking specifically at diverticular disease?

Again, a great presentation. I enjoyed reading your paper, and I look forward to your responses.

Closing discussant

Dr. Nicholas Csikesz (Worcester, MA): First, to talk about how we created the score and why we created the score. We wanted to look at SES primarily, but doing that in an administrative hospital database is challenging.

How do you really define SES using the variables that you have there? For instance, patient education level, as I mentioned, is not available at all.

The income bracket, the variable that we have, is based on ZIP code. As we all know, ZIP codes can contain a wide heterogeneity of income levels within them. So, we felt that there were not any individual variables that would give us a sense of SES within this database. Therefore, our plan was that, by combining them, we would, hopefully, get something that would give a little better sense of SES.

In terms of how we derived at it, to some extent, it was a little arbitrary in choosing the scoring system, and it has not been validated. However, I am not sure how we would validate it in this database. There is not something that you can really test for to say that you have accurately identified low SES.

I think the take home is, as you mentioned, that there are disparities in care. What we found were these disparities were at least partially due to, it seems like, a decrease in likelihood to present electively and perhaps some differences in surgical management and the volume of providers and hospitals that are treating these patients.

I think further studies are going to need to hone in on identifying who these patients are and how we can get them better care, both for them and to decrease the burden to the health care system.

Therefore, we divided the SES score into tertiles as you mentioned. In some other work that we have done with creating groups like this, we found tertiles worked well for being able to focus either on the low end of the spectrum or the high end of the spectrum and still have two large groups to compare. I think anytime you take a continuous variable and divide it into groups, to some degree, it is arbitrary how you do it.

We did look at differences with different groupings or using as a continuous variable. Actually, I thought I might get a question like this, so I made a slide looking at just with the raw SES score; this is the percentage of elective admits. We see that there was an increase in elective admits with SES score and this is where we had drawn our cut-off.

You also mentioned that diverticular disease is a longitudinal disease. We are not going to be able to capture all of the intricacies of its care with an administrative database that does not have information about readmissions or anything like that.

We have a snapshot of one year with how many admissions there were. We do not know whether in our group of 2,000 low SES patients, maybe it was 1,500 patients and maybe 500 were readmits. We do not know things like that. So that is something where getting some data that had longitudinal and especially outpatient care, to figure out what kind of primary care these patients were getting. That would be really valuable.

Discussant

Dr. Neil Hyman (Burlington, VT): An excellent presentation. It is always a big problem trying to draw conclusions from administrative databases because you have no idea what the data really means. For example, did the higher socioeconomic patients have completely different presentations to the emergency department since they had access to primary care for "milder" episodes?

I also have a question about your three categories. It looked like you had a large group of patients coded as diverticulitis with hemorrhage, if that really existed in any of the patients. And then you had another category called diverticulitis and then almost half being admitted with "diverticulosis." Can you explain to me what it means if you were admitted to the hospital and you were not bleeding and you did not have diverticulitis but you had "diverticulosis" as the reason for admission to the hospital?

Closing discussant

Dr. Nicholas Csikesz (Worcester. Massachusetts): Sorry, I should have probably expanded on that slide. The diverticulosis patients, for the vast majority, did present with hemorrhage, and there were not really significant differences between the two groups, so I did not report that. I should have mentioned it.

And you are right, the diverticulitis with hemorrhage was a little confusing to us, but that is what the coding showed us.

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2009 SSAT PLENARY PRESENTATION

Impact of Neoadjuvant Chemotherapy with FOLFOX/FOLFIRI on Disease-Free and Overall Survival of Patients with Colorectal Metastases

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Abstract
Study Aims To determine if neoadjuvant FOLFOX/FOLFIRI is associated with improved disease-free survival (DFS) or overall survival (OS) in patients with colorectal metastases (CRM) to the liver.
Methods Ninety-nine patients (from 457 eligible) with CRM that underwent hepatic resection during 2000 to 2005 were included. Group 1 (n=44) patients received neoadjuvant FOLFOX/FOLFIRI, and Group 2 (n=55) did not receive neoadjuvant therapy.
Results There were 58% men. The median age for Group 1 was 58 and Group 2, 64 (p=0.03). OS for Group 1 at 1, 3, and 5 years was 93%, 62%, and 51%, respectively, with a median OS of 5.8 years. In Group 2 survival at 1l, 3, and 5 years was 90%, 63%, and 45%, respectively, with a median OS of 3.7 years (HR 1.06, p=0.87). The DFS for Group 1 at 1, 3, and 5 years was 51%, 20%, and 20%, with a median DFS of 1.1 years and Group 2 at 1, 3, and 5 years was 58%, 32%, and 32% (median DFS—1.2 years; HR=1.24, p=0.45).
Conclusions Neoadjuvant FOLFOX/FOLFIRI was employed more frequently in younger patients with CRM; however, neoadjuvant chemotherapy for CRM was not significantly associated with an increase in OS or DFS, despite additional adjuvant therapy.

Keywords Neoadjuvant therapy · FOLFOX · Hepatectomy · Colorectal cancer metastases · Survival

Introduction

Colorectal cancer is the third most common malignancy and is the second leading cause of cancer mortality, attributing to approximately 50,000 deaths in the USA in 2005.[1] Metastatic colorectal cancer is associated with an even poorer prognosis with a 5-year survival rate of 5–37%.[2–4] Approximately 25–30% of patients with colorectal carcinoma will initially present with metastatic disease, and the liver is the most common site of involvement.[3,5,6] Resection of isolated hepatic metastases is currently the most effective form of curative treatment offering a 5-year survival rate ranging from 25–58%.[7–11] While 5-fluorouracil (5-FU)-based chemotherapy was used in most patients with metastatic colorectal cancer, during the past decade, combinations of 5-FU, leucovorin (LV), and the topoisomerase I inhibitor irinotecan (FOLFIRI) or the platinum analog oxaliplatin (FOLFOX) have proven more effective.[2] Moreover, the novel use of neoadjuvant FOLFOX initially reported in 1996 changed the surgical paradigm for patients with colorectal metastases to the liver converting 16% of patients with unresectable to resectable disease.[5] Expansion of the use of neoadjuvant therapy to include patients with resectable metastatic disease has occurred, but data for such patients is limited and whether outcomes are improved remains debatable. The objective of our study was to

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determine if neoadjuvant FOLFOX/FOLFIRI is associated with increased disease-free survival (DFS) or improved overall survival (OS) in patients with surgically resectable colorectal metastases (CRM) to the liver.

Material and Methods

A retrospective, institutional review board approved, review was performed on 457 consecutive patients who underwent hepatic resection for metastatic colorectal disease between January 1, 2000, and December 31, 2005, at Mayo Clinic, Rochester, Minnesota. Inclusion criteria included age greater than 18 years, pathologically documented metastatic colorectal cancer to the liver, resectability of hepatic metastases by a hepatobiliary surgeon prior to initiation of neoadjuvant therapy with FOLFOX/ FOLFIRI, and patients who were not exposed to any prior neoadjuvant chemotherapy.

However, patients were also eligible for inclusion into Group 1 if they received adjuvant chemotherapy for their primary malignancy within 6 months of the hepatic resection, which was considered neoadjuvant chemotherapy in the relationship to the hepatic resection.

Exclusion criteria included a history of hepatic arterial infusion chemotherapy (n=82) or other any other neo-adjuvant or adjuvant chemotherapeutic agents (n=126), documented presence of extra-hepatic metastases (n=60), upper abdominal/intraoperative radiation (n=25), history of hepatitis/cirrhosis (n=2), concomitant cancers (n=5), or a combination of the above (n=58). Data elements abstracted from the patient chart included demographics, symptomatic presentation, preoperative imaging, operative and pathologic findings, chemotherapy regimen, postoperative complications, date of death, and date of disease recurrence. Eligible patients were divided into two groups. Group 1 (n=44) consisted of patients who received neoadjuvant FOLFOX/ FOLFIRI, and Group 2 (n=55) consisted of patients who did not receive neoadjuvant therapy.

We have reported descriptive statistics as number (percent) and as mean (SD) or median [range] where appropriate. Time of hepatic resection to death or last follow-up was used to calculate OS, while DFS assessment was from time of hepatic resection to either recurrence or last follow-up, censoring at patient death when not due to disease progression. Kaplan–Meier survival was used to calculate OS and DFS estimates 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 treatment group and overall, as well as DFS; the hazard ratio (HR) and 95% confidence interval are reported. Models included age and gender in addition to treatment group. The association between recurrence and patient death was assessed considering the date of recurrence as a time-dependent covariate in the Cox model. The study had 80% power to detect associations between neoadjuvant use and overall survival of HR≥2.44, and DFS of HR≥2. All missing data was excluded from analysis. The alpha-level was set at $p<0.05$ for statistical significance.

Results

Demographics and Presentation

Of the 457 eligible patients, 358 patients did not meet inclusion criteria, leaving a study group of 99 patients (22% of the hepatic resection patients), 58 males and 41 females. The median age of the overall study group at hepatic resection was 63 years (range 33–90). The median age of Group 1 and Group 2 was 58 and 64 years, respectively (p=0.03; Table 1). Overall, patients were followed a median (range) of 3.9 years (0–6.6), to either death or last contact. There were 44 patients (20% of total) in Group 1 and 55 patients in Group 2 (12% of total). Hepatic metastases were evident at the initial colorectal operation in 48 patients of Groups 1 and 2 (49%). Twenty-one patients (21%) underwent simultaneous resection of the primary colorectal cancer and the hepatic metastases while 78 patients (79%) underwent staged resections of the primary tumor and hepatic metastases. Among the 61 patients for whom preoperative carcinoembryonic antigen (CEA) serum levels were available, only five patients had levels >200 ng/mL and all of these patients were in Group 2.

Surgical Management

The average interval from initiation of chemotherapy to hepatic resection in Group 1 was 7 months (3–26 months). The median interval from diagnosis of hepatic metastases to hepatic resection in Group 1 was 192 days [2–906 days] and in Group 2 was 38 days [0–804]. The average smallest resection margin overall was 0.91 cm. The average closest resection margin in Group 1 and Group 2 was 0.81 and 0.97 cm, respectively (p=0.08; Table 1). Metastases were described pathologically as poorly differentiated in 70 patients overall (81.4%), moderately differentiated in 15 patients (17.4%), dedifferentiated in one patient (1.2%), and revealed post-chemotherapy fibrotic changes in six patients (6%; Table 1).

There were no differences in the extent of resections between the two groups. Most patients underwent a major hepatic resection: right hepatectomy in 48% and left hepatectomy in 6%. The remaining patients underwent: left lateral sectorectomy in 4%, bisegmentectomy in 3%, segmentectomy in 19%, nonanatomic subsegmental resec-

Table 1 Group Characteristics

	Overall n=99 N (%)	Group 1* n=44 N (%)	Group 2* n=55 N (%)	P value
Gender				0.41
Male	58 (59)	28 (64)	30 (55)	
Female	41 (41)	16 (36)	25 (50)	
Median age at liver surgery (years)	63	64	57.5	0.03
Primary disease				
Synchronous diagnosis	48 (48)	24 (55)	24 (44)	0.28
Positive primary LN	57 (61)	28 (67)	29 (56)	0.28
Total # LN, median [min–max]	14 [3,77]	14 [3,77]	13.0 [5,36]	0.93
Total # LN positive, median [min–max]	1.0 [0,19]	1.0 [0,19]	2.0 [0,19]	0.13
Metastatic disease				
Synchronous resection	21 (21)	7 (16)	14 (25)	0.25
Time CRM diagnosis to hepatic surgery, median days [min–max]	75 [0–903]	192 [2,903]	38 [0,804]	<0.001
Final pathology, differentiation				0.38
Well	0	0	0	
Moderate	15 (17.4)	5 (13.5)	10 (20.4)	
Poor	70 (81.4)	31 (83.8)	39 (79.6)	
Dedifferentiated	1 (1.2)	1 (2.7)	0	
Largest tumor size (cm)	3.9	3	4.7	0.001
Positive margin	3	1	2	1.00
Mean resection margin	0.91 cm	0.81 cm	0.97 cm	0.08

Missing data excluded from analysis. Final pathology missing for n=6 in Group 1 and n=7 in Group 2

Group 1 neoadjuvant chemotherapy, *Group 2* no neoadjuvant chemotherapy

tion in 39% (Table 2). Of the patients who underwent a segmentectomy, the median number of segments removed was one, while the median number of nonanatomic subsegmental resections was two. The lymph node status of the primary colorectal cancer was pathologically positive in 57 patients, negative in 37 patients, and unknown in five patients. Of the patients with lymph nodes excised during the colorectal procedure, the average number of lymph nodes excised during resection of the primary colorectal cancer was 14 with an average number of one node positive in Group 1 and an average number of two nodes positive in Group 2 for metastatic cancer (p=0.12).

Table 2 Operative Procedures

Type of procedure	Overall %	Group 1 (n=44) N (%)	Group 2 (n=55) N (%)
Right hepatectomy	47 (47.5)	22	25
Left hepatectomy	6 (6.1)	2	4
Left lateral sectorectomy	4 (4.0)	4	0
Bisegmentectomy	3 (3.0)	1	2
Segmentectomy	19 (19.2)	9	10
Non-anatomic wedge	39 (39.4)	23	16

Group1=neoadjuvant chemotherapy, Group 2=no neoadjuvant chemotherapy

Chemotherapy Treatment

Neoadjuvant Chemotherapy Prior to Hepatic Resection

Forty-four patients received neoadjuvant chemotherapy (Group 1) consisting of FOLFOX, FOLFIRI, or both. Fifty-five patients did not receive neoadjuvant chemotherapy (Group 2). All patients were deemed resectable by a hepatobiliary surgeon based on computed topography (CT) prior to the initiation of chemotherapy. Neoadjuvant FOLFOX/FOLFIRI was employed more frequently in younger patients: median age of 58 versus 64 years for Group1 and Group 2 respectively (p=0.03).

Adjuvant Chemotherapy After Hepatic Resection

Forty-nine patients (58%) received adjuvant chemotherapy after hepatic resection. Of these patients, 16 (33%) received FOLFOX, 13 (27%) received FOLFIRI, 12 (24%) received both, three (6%) received another form of chemotherapy, and the regimen was unknown in five (10%) patients. Adjuvant chemotherapy was used in 68% of patients in Group 1 and 49% of the patients in Group 2 (p=0.08).

Eighteen patients in Group 1 and 23 patients in Group 2 received adjuvant FOLFOX, FOLFIRI or both.

Among the 85 patients where adjuvant therapy use was recorded, adjusting for gender and age at surgery, adjuvant therapy use was not significantly associated with patient improved survival, s=0.25 (HR=0.63, 95% CI 0.29–1.39).

Adjuvant therapy use was significantly associated with an increased risk of recurrence, p=0.046 (HR=2.00, 95% CI 1.01, 3.96).

Disease-Free Survival Group 1 Versus Group 2

Disease-free survival at 1, 3, and 5 years was 51%, 20%, and 20% in Group 1, with a median DFS of 1.1 years. The DFS for Group 2 at 1, 3, and 5 years was 58%, 32%, and 32% with a median DFS of 1.2 years (Fig. 1). Adjusting for age and gender, use of neoadjuvant therapy was not significantly associated with DFS (p=0.45), a patient receiving neoadjuvant therapy relative to a patient who did not receive neoadjuvant chemotherapy had a slightly increased risk of recurrent disease, HR=1.24 (95% CI 0.71–2.14).

In Group 1, 39 % of patients did not have any disease recurrence compared to 51% in Group 2. Group 1 had a recurrence pattern of primarily intrahepatic recurrence (36%), with 11% extrahepatic alone and 14% both intra- and extrahepatic. In Group 2, the pattern of recurrence was similar with 50% intrahepatic alone, 15% extrahepatic alone, and 18% with both intra- and extrahepatic disease.

Overall Survival Group 1 Versus Group 2

There were three in-hospital deaths, two in Group 1 (post operative myocardial infarction and multiorgan system failure from undetermined etiology) and one in Group 2 (hepatic failure). Overall survival for Group 1 at 1, 3, and 5 years was 93%, 62%, and 51%, respectively, with a median survival of 5.8 years. Overall survival at 1, 3, and 5 years for Group 2 was 90%, 63%, and 45%, respectively, with a median survival of 3.7 years (Fig. 2). Adjusting for age and gender, use of neoadjuvant therapy was not associated with improved overall patient survival (p=0.87), in fact a patient receiving neoadjuvant therapy relative to a patient who did not had a slightly higher risk of death, HR=1.06 (95% CI 0.54–2.07).

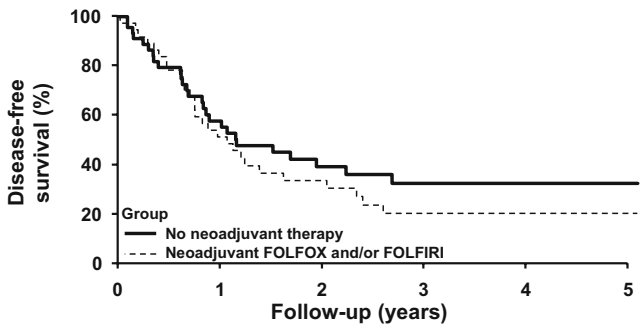

Figure 1 Group 1 vs Group 2—disease-free survival.

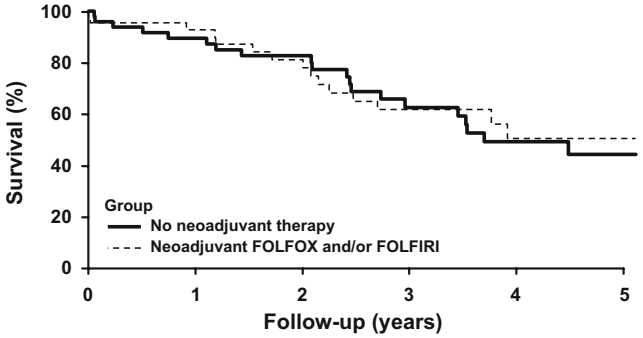

Figure 2 Group 1 vs Group 2—overall survival.

Disease-Free Survival Synchronous Versus Metachronous

Of the total population of patients, DFS at 1, 3, and 5 years was 38%, 30%, and 30% in the patients undergoing synchronous resection. Disease-free survival at 1, 3, and 5 years was 58%, 26%, and 26% (Fig. 3).

Overall Survival Synchronous Versus Metachronous

Overall survival rates at 1, 3, and 5 years were 94%, 64%, and 55% in those undergoing a synchronous resection and were 90%, 61%, and 46% in those undergoing a metachronous resection (Fig. 4).

Discussion

The results of our study revealed that the use of neoadjuvant chemotherapy in patients with resectable metastatic disease

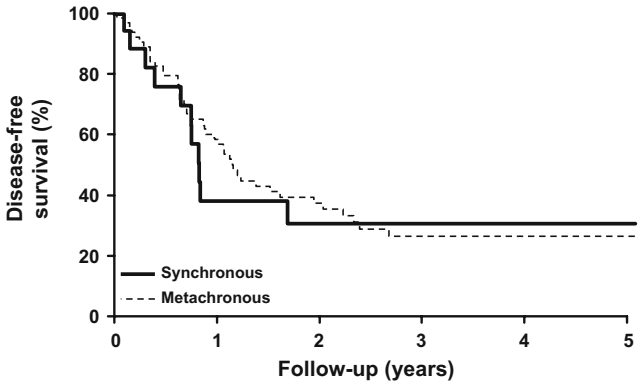

Figure 3 Synchronous vs metachronous disease-free survival.

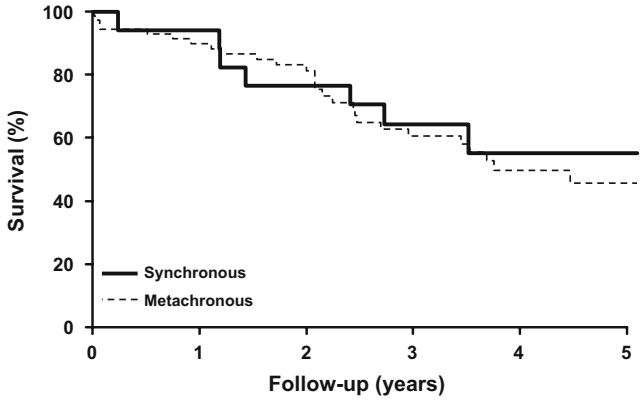

Figure 4 Synchronous vs metachronous—overall survival.

does not offer a definite OS or DFS advantage over surgical resection alone with or without adjuvant chemotherapy. Our data also demonstrated no survival advantage of neoadjuvant chemotherapy with synchronous versus metachronous resection. These findings were in the setting of strict exclusion criteria, similar tumor burden, and types of surgical resections performed. The only apparent significant difference among treatment groups was that the mean age of those treated with neoadjuvant chemotherapy was younger than those that went straight to operative resection.

The mean 1-year OS was 93% in Group 1 and 90% in Group 2. At 5 years, the OS had decreased to 48% in Group 1 and 45% in Group 2. Lubezky et al.[12] noted a similar 1-year OS rate (91%) for patients receiving adjuvant chemotherapy when compared to patients receiving neoadjuvant therapy (95%). Lubezky also reported similar 3-year OS of 84% for patients with adjuvant therapy compared to 70% OS for patients receiving neoadjuvant treatment (the OS was similar in both groups). Both Lubezky and we concluded that there is no OS advantage for neoadjuvant chemotherapy compared to adjuvant therapy expect for those truly made resectable with neoadjuvant chemotherapy.

Our study demonstrated no difference in DFS at 1-year (51% in Group 1 and 58% in Group 2). At 5 years, however, the DFS was only 20% in Group 1 and 32% in Group 2. Lubezky et al.[12] described a 1-year DFS of 63% for patients having adjuvant therapy compared to a 94% 1-year DFS in patients receiving neoadjuvant therapy. Although the neoadjuvant group appeared to have a DFS benefit in the first year (94% vs. 63%), the benefit disappeared by 3 years (50% vs 49%).

Another study by Adam et al.[13] found similar results with respect to DFS in a series of 1,104 unresectable patients who received neoadjuvant FOLFOX/FOLFIRI, of those patients 12.5% became eligible for curative resection. The DFS in the surgically resected patients was 30%, 22%, and 17% at 3, 5, and 10 years, respectively. The OS for this group was 52%, 33%, and 23% at 3, 5, and 10 years, respectively. By comparison, a control group that was primarily resected had a superior OS of 66%, 48%, and 30% at 3, 5, and 10 years, respectively. Unlike our study, Adam et al. included patients with extrahepatic disease, preoperative portal vein embolization (PVE) and two-stage hepatectomy. In our study, we followed the methodology of the phase II North Central Cancer Treatment Group Study[14] that excluded patients with extrahepatic disease and those who underwent PVE.

Allen et al.[15] showed neoadjuvant treatment was an important prognostic factor for response to chemotherapy; however, neoadjuvant therapy failed to improve patient survival. Our results are commensurate with prior published survival rates for patients treated with neoadjuvant chemotherapy and surgical treatment alone.

Neoadjuvant chemotherapy does allow for resection of metastases initially considered unresectable, but the role of neoadjuvant chemotherapy in patients with resectable metastases is debatable. Disease-free survival and OS after resection of hepatic metastases following neoadjuvant chemotherapy does not appear to significantly differ after resection alone. The only trial to address this question in a controlled fashion was the recently published trial from the European Organization for Research and Treatment of Cancer (EORTC Intergroup 40983). This phase III trial and the data suggest that neoadjuvant chemotherapy followed by hepatic resection of metastases may improve OS, there was a trend toward improved OS following neoadjuvant chemotherapy, but statistical significance was not achieved.[16]

Firm guidelines for initiating neoadjuvant chemotherapy in patients with colorectal metastases to the liver have yet to be established. Instead, factors reflective of aggressive disease such as synchronous presentation have been used as surrogate indicators. Such patients are thought to have biologically less favorable disease and, have a worse overall and disease-free interval compared to patients with metachronous disease.[17,18] Although the interval for defining synchronous metastases vary in the literature, most studies have shown that outcome after resection of synchronous metastases is worse than that for metachronous metastases. Scheele et al. describes a 5-year survival decrease from 43% to 30% in patients with synchronous metastases compared to those with metachronous disease,[8] whereas Sugawara et al. found that the only factor associated with survival in patients with synchronous disease was the resection margin status.[19]

Simultaneous resection can be performed with minimal morbidity; however, the concern of survival after synchronous resection compared to delayed resection following neoadjuvant therapy has not been well-described.[20–22] In our series, patients undergoing synchronous resection, the

1, 3, and 5-year DFS rates were 38%, 30%, and 30%; and OS rates were 94%, 64%, and 55%. In patients undergoing a metachronous resection, the DFS rate was much improved at 1 year (58%) but the outcomes were similar to the patients with synchronous resection at three and 5 years (26% and 26%, respectively). The 1, 3, and 5-year OS rates of patients with metachronous resections were 90%, 61%, and 46%, respectively, not significantly different than to those with synchronous disease. Our data demonstrated the OS and DFS curves of the comparable groups were parallel, suggesting no survival advantage for neoadjuvant chemotherapy.

Today, hepatic resection is associated with morbidity and limited mortality. Most referral centers specializing in hepatobiliary procedures report mortality rates <5% after major liver surgery. However, with the advent of neoadjuvant chemotherapy, the morbidity of liver resection may be higher due to the hepatic parenchymal changes caused by the chemotherapy. Hepatic steatohepatitis has been associated with neoadjuvant therapy limiting the extent of liver resection and leading to increased operative morbidity and mortality.[23] The EORTC Intergroup Trial reported a 25% postoperative complication rate in the group receiving neoadjuvant chemotherapy.[16] Similarly, Aloia et al. reported that the only significant contributing factor with multivariate analysis of patients requiring intraoperative blood transfusion and neoadjuvant chemotherapy. Surgical evaluation and histopathologic analysis also revealed an increase in liver fragility and an increased incidence of vascular hepatic lesions in the neoadjuvant patient population.[24] These concerns are valid and must be taken into consideration when deciding on a treatment plan that includes neoadjuvant chemotherapy for CRM to the liver.

Despite attempts to limit confounding factors in our study there are several obvious limitations. Primarily this is a retrospective study and patients did not undergo randomization. The second limitation is the small sample sizes with only 44 of the 99 patients receiving neoadjuvant chemotherapy due to the variable use of FOLFOX and FOLFIRI neoadjuvant chemotherapy during the period of study. Lastly, postoperative therapy was not standardized in either group adding to patient heterogeneity.

Conclusion

Hepatic resection for colorectal metastases is potentially curative. Neoadjuvant chemotherapy may downsize unresectable metastases allowing resection and improving OS in patients who are unresectable at presentation. The utility of neoadjuvant chemotherapy with or without adjuvant therapy in patients with resectable metastatic disease does not offer a definite DFS or OS advantage over initial surgical resection; however, there may be a subset of patients, namely those younger than 60 years old that may benefit from neoadjuvant therapy. Further research is justified in this area in order to detect a true benefit.

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Dr. Sarah York Boostrom, Presenter (Mayo Clinic—Rochester, Rochester, MN)

Discussant

Dr. J. Nicholas Vauthey (MD Anderson, Houston, TX): The authors should be congratulated for reporting a subset of patients operated at their institution with or without preoperative chemotherapy. However, the study is limited by the small number of patients in each group, and an overoptimistic statistical expectation that chemotherapy would improve the survival by a hazard ratio of 2.0 or more, while the only randomized study evaluating perioperative FOLFOX, indicates a significant but modest 8% increase in disease-free survival in greater than 300 eligible patients.

I have two questions for you.

First, why did you choose systemic chemotherapy in some patients and not in others? What is, currently, the recommendation of your medical oncology group?

Second, the comparison of your preoperative variables did not include prechemotherapy variables such as carcinoembryonic antigen, number of tumors, or neutrophile lymphocyte ratio. I know your group has shown no value in the colorectal risk score. However, studies predating the era of chemotherapy have shown values in these criteria using prechemotherapy values.

Closing Discussant

Dr. Sarah York Boostrom: Thank you for your questions. I believe the first question was in regard to the intergroup trial by Nordlinger. Even though they did show improvement in disease-free survival in the first few years, it was not significant after approximately 3 to 5 years.

In addition, when you adjust for the time period between the neoadjuvant and the adjuvant group in receiving their surgical procedure, it was not significant for disease-free survival, or overall survival.

The second question is regarding how we choose our patients for chemotherapy. Unfortunately, that is not something that we have a lot of control over. Approximately half of our patients were found to have hepatic metastases at the time of their primary initial operation, but received their operation at another institution. Only 20% underwent a synchronous resection so, unfortunately, most of our patients who received neoadjuvant chemotherapy were given their regimen at another institution prior to referral for their hepatic resection.

In regard to CEA as well as number of tumors, we did abstract that data. However, the CEA postop levels were not followed as closely because most of our patients do return to their former institutions for follow-up.

The majority of our patients who have CEA levels that were elevated were in the non-neoadjuvant group. The number of tumors was recorded prior to surgery as well as at pathology after resection. The median number of segments removed was one and the median number of nonanatomic wedge resections was two.

I recently read the paper on the neutrophil to lymphocyte ratio. However, I did not abstract that data. That is something we could look at.

Discussant

Dr. Margo Shoup (Loyola University, Chicago, IL): I appreciate your talk. I think you have to be a little bit careful, though, about when you say neoadjuvant chemotherapy in these patients.

The medical oncology data is pretty clear that patients benefit from neoadjuvant chemotherapy followed by surgery followed by post-operative adjuvant chemotherapy for a total of 12 cycles of chemotherapy. This can be broken up into four cycles preoperatively then eight postoperatively, or six cycles then surgery, and then six more, as long as they get 12 cycles of chemotherapy and they are with targeted therapy as well.

So my question to you is, in your population of patients, how many of these actually received adjuvant chemotherapy in addition to neoadjuvant chemotherapy?

Closing Discussant

Dr. Sarah York Boostrom: Thank you for your question. Fifty-eight percent received adjuvant chemotherapy. Sixty-eight percent were in the neoadjuvant group and only 49% were in the non-neoadjuvant group. I do agree that studies show that the adjuvant therapy may be beneficial for the patient.

In some of the studies that are comparing neoadjuvant chemotherapy and survival, it is difficult to determine whether it is the neoadjuvant chemo that is beneficial or whether it is actually the adjuvant chemotherapy because the majority of the patients who do receive neoadjuvant are also receiving the adjuvant therapy.

Discussant

Dr. Timothy Pawlik (Johns Hopkins, Baltimore, MD): In one of your conclusions, you said neoadjuvant chemotherapy was associated with an increased risk of death. I can only fathom two ways that neoadjuvant chemotherapy could be causing increased death. Number 1, that the neoadjuvant chemotherapy is causing liver toxicity and therefore increased perioperative mortality from liver insufficiency or failure. Or, number 2, that those patients who received neoadjuvant chemotherapy have worse tumor biology and therefore eventually die from more aggressive disease.

In looking at your survival curves, the separation in survival comparing the two groups was late. To me, this seems to imply that the tumor biology in the two groups may be different, which may mean that the groups are not comparable. I fear that the study suffers from a significant selection bias pertaining to whom received neoadjuvant chemotherapy—thereby making conclusions biased and potentially misleading.

How would you explain the conclusion that neoadjuvant chemotherapy is associated with increased risk of death? Is it increased perioperative mortality or different tumor biology in the two groups leading to a potential selection bias? Thank you.

Closing Discussant

Dr. Sarah York Boostrom: I think it may be a combination of both in the neoadjuvant chemotherapy group.

However, I think what we noticed in our neoadjuvant group is that at pathology, we were unable to ascertain whether there were negative margins. Pathology revealed only post-chemotherapeutic fibrotic changes.

So in these surgically resectable patients who are receiving neoadjuvant chemotherapy, their tumors are not detected at surgery. Dr. Vauthey and colleagues wrote a paper on this topic and observed that after receiving neoadjuvant chemotherapy the tumor were disappearing and were not apparent during pathologic analysis of the specimen. They are recommending possible preop coiling in the location of the tumor in order to identify the tumor at surgery.

So in some of the neoadjuvant chemotherapeutic patients, we feel perhaps that there is disease persistence rather than recurrence, but we are calling their disease a recurrence. There may have been microscopic tumor left behind at the time of the initial operation. In addition, these patients may have aggressive tumor biology, thus they do not respond to chemotherapy and they have disease persistence which leads eventually to an earlier death.

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2009 SSAT PLENARY PRESENTATION

The Relationship Between the Local and Systemic Inflammatory Responses and Survival in Patients Undergoing Curative Surgery for Colon and Rectal Cancers

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Abstract
Introduction Both local (Klintrup criteria) and systemic (Glasgow Prognostic Score, mGPS) inflammatory responses have been reported to be independent predictors of cancer-specific survival in colorectal cancer. However, high-grade local inflammatory response appears more common in rectal and high mGPS more common in colonic tumors. Whether relationships with survival are similar in colon and rectal tumors is unclear. The present study assesses the prognostic value of local and systemic inflammation in colon and rectal cancers and defines 3-year survival according to inflammation-based criteria for stage II/III disease.
Methods Two hundred forty colon and 140 rectal cancer patients underwent potentially curative surgery between 1997 and 2007. C-reactive protein and albumin (mGPS) were measured preoperatively. Routine pathology specimens were scored according to Klintrup criteria for peritumoral infiltrate.
Results Patients with colon cancers were older ($P<0.05$) and had higher T stage ($P<0.001$) and mGPS ($P\leq0.001$) compared with rectal cancers. The proportions of patients with a high-grade tumor inflammatory cell infiltrate were similar in colon and rectal cancers. mGPS and Klintrup criteria were independent predictors of cancer survival. The mGPS hazard ratios were 1.56 and 1.76 for the mGPS, and the Klintrup hazard ratios were 2.12 and 5.74 for colon and rectum, respectively. For stages II and III colorectal cancer, 3-year survival was 91% and 73%, respectively. Three-year survival varied between 100% and 68% depending on Klintrup score/ mGPS in stage II disease and between 97% and 60% in stage III disease.
Conclusion Local and systemic inflammatory responses are important independent predictors of survival in colon and rectal cancers. These scores combined with tumor–node–metastases stage improve the prediction of survival in these patients.

Competing interests There are no sources of funding or competing interests to declare.

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Introduction

Colorectal cancer is the second most common cause of cancer death in Western Europe and North America. Each year in the UK, there are approximately 35,000 new cases and 16,000 deaths attributable to this disease.[1] Overall survival is poor; even in those who undergo resection with curative intent, only half survive 5 years.[2,3] It is increasingly recognized that disease progression in colorectal cancer patients is not solely determined by the intrinsic characteristics of the tumor but also by host local and systemic inflammatory responses.[4,5]

In terms of the local inflammatory response, there is now persuasive evidence that a pronounced lymphocytic infiltrate in and around the infiltrating tumor identified on routine pathology is associated with improved cancer-specific survival.[6–9] Furthermore, Galon and colleagues, using more sophisticated techniques, have reported that the type, density, and location of immune cells in colorectal tumors can provide prognostic information superior to that of existing tumor staging.[10]

Klintrup and colleagues[9] have simplified the assessment of inflammatory cell infiltrate at the tumor's invasive margin. The authors reported that, on routine hematoxylin and eosin stained sections, the tumor inflammatory infiltrate (scored high or low) was reproducible and that a high grade was associated with improved survival independent of tumor stage in patients undergoing potentially curative resection for colorectal cancer. These observations have recently been validated in a cohort from another center in another country.[11]

McMillan and colleagues[12] have simplified the assessment of the systemic inflammatory response. The authors reported that the presence of an elevated systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein and hypoalbuminemia termed the Glasgow Prognostic Score (GPS), was associated with poor survival, independent of tumor stage, in patients undergoing curative resection for colorectal cancer.[12] These observations have been validated in a cohort from another centre in another country.[13]

The relationships between the local and systemic inflammatory responses, using the above methods, were examined in a recent study in 287 colorectal cancer patients.[14] Both local and systemic inflammatory responses predicted cancer-specific survival independent of tumor stage, and these were linked through circulating white cells.

It is also of interest that a high-grade local inflammatory cell response was more commonly present in rectal tumors,[9] whereas an elevated systemic inflammatory response was more commonly present in colonic tumors.[12] In addition, the original description of the prognostic value of tumor margin inflammatory cell reaction was initially made in rectal cancer.[6] Therefore, in terms of local and systemic inflammatory responses and survival, it is not clear whether such relationships are similar in colon and rectal tumors.

To our knowledge, detailed analysis of local and systemic inflammatory responses in colon and rectal cancers has not previously been undertaken. Therefore, the aim of the present study was to assess local and systemic inflammatory responses and their relationship with survival in a large cohort of patients with colon and rectal cancers. The present study also aimed to define 3-year survival rates according to local and systemic inflammatory response in different tumor stages.

Materials and Methods

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and preoperative abdominal computed tomography, were considered to have undergone potentially curative resection for colon and rectal cancers (stages I–III) between January 1997 and February 2007 in a single surgical unit at Glasgow Royal Infirmary were included in the study. Patients were identified from a prospectively maintained database. Clinical conditions known to acutely or chronically evoke a systemic inflammatory response were excluded from the present study. These include (1) emergency presentation, (2) clinical evidence of infection such as pyrexia and elevated white cell count, and (3) presence of a chronic inflammatory condition such as active rheumatoid arthritis or inflammatory bowel disease. Patients receiving preoperative radiotherapy were excluded from the study since radiotherapy has been reported to evoke an inflammatory response.[15,16] Patients who died within 30 days of surgery were excluded from the analysis. The tumors were staged using the conventional Tumor, Node, and Metastases (TNM) classification (from the fifth edition and according to the Royal College of Pathologists Dataset 2007).[17] All rectal cancer resections were performed with a total mesorectal excision.[18] All other pathological data were taken from the pathology reports issued at the time of resection.

The routine hematoxylin and eosin slides were retrieved from the pathology archives. A minimum of three slides per specimen were selected from the deepest area of tumor invasion and scored according to Klintrup criteria.[9] The Klintrup method is based on the deepest point of invasion identified from the three slides, and this provides the overall score for the specimen. Klintrup scoring of slides was carried out as described previously.[9,11] Briefly, tumors were scored according to a four-point score. Scores were based on appearances at the deepest area of tumor invasion. A score of 0 indicated there was no increase in inflammatory cells at the deepest point of the tumor's invasive margin; score 1 denoted a mild and patchy increase in inflammatory cells; score 2 denoted a prominent inflammatory reaction forming a band at the invasive margin with some evidence of destruction of cancer cell islands; and score 3 denoted a florid cup-like inflammatory infiltrate at the invasive edge with frequent destruction of cancer cell islands. These scores were then subsequently classified as low-grade (scores 0 and 1) or high-grade (scores 2 and 3) (Figs. 1 and 2, respectively).

A total of 197 tumor specimens were scored independently by two observers (CSDR and JMS), who were blinded to patient outcome, to confirm consistency of scoring. Training was provided by a consultant pathologist

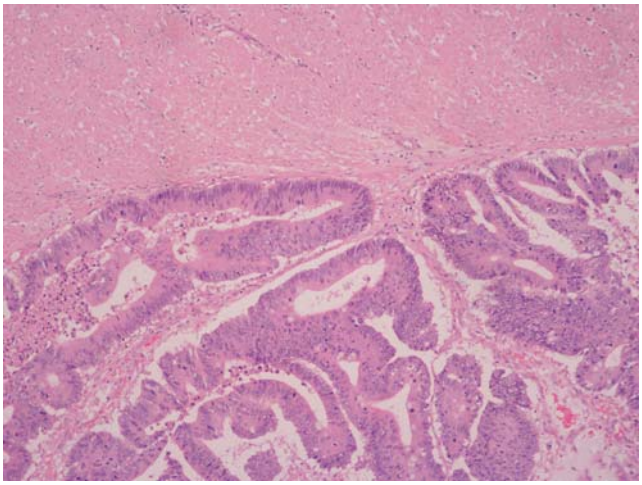

Figure 1 Low-grade or absent inflammatory cell infiltrate at the tumor's invasive margin.

(KO). The inter-observer intraclass correlation coefficient provides a measure of inter-observer agreement. The inter-observer intraclass coefficient for Klintrup's assessment of peritumoral inflammatory cell infiltrate was 0.81. (values of ≥0.6 are considered acceptable, and >0.7 is considered good). CSDR then scored all slides (n=385), and these data were used in the analysis.

Blood samples were taken for routine laboratory measurements of C-reactive protein and albumin prior to surgery. All colon and rectal cancer patients have C-reactive protein and albumin measured preoperatively as standard in our institution. The coefficient of variation for these methods, over the range of measurement, was less than 5% as established by routine quality control procedures.

The GPS was constructed as previously described.[19] Briefly, patients with both an elevated C-reactive protein (>10 mg/l) and hypoalbuminemia (<35 g/l) were allocated a score of 2. Patients in whom only one of these biochemical abnormalities was present were allocated a score of 1. Patients in whom neither of these abnormalities was present were allocated a score of 0. The GPS has recently been modified based on evidence that hypoalbuminemia, in patients with colorectal cancer without an elevated C-reactive protein concentration, had no significant association with cancer-specific survival. Therefore, patients with an elevated C-reactive protein were assigned a modified GPS score (mGPS) of 1 or 2 depending on the absence or presence of hypoalbuminemia.[12]

Patients received regular follow-up (3 and 6 months and then yearly to 5 years) with yearly CT scanning and regular colonoscopic surveillance until 5 years post surgery. Information on date and cause of death was checked with that received by the cancer registration system and the Registrar General (Scotland). The study was approved by the Research Ethics Committee, Royal Infirmary, Glasgow.

Statistics

Grouping of the variables was carried out using standard thresholds. Comparison univariate survival analysis and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P value had to be greater than 0.05. Deaths up to April 2009 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Three hundred and eighty-five patients undergoing potentially curative resection for colorectal cancer between 1997 and 2007 were studied. Two hundred and forty-five patients (64%) underwent surgery for colonic tumors, and 140 patients (36%) had surgery for rectal tumors. The majority of patients were 65 or older (65%), were male (55%), and had TNM stage I/II disease (55%). Median number of lymph nodes sampled was 14 (range 1–41) for TNM stage II tumors and 14 (range 3–34) for TNM stage III tumors. One hundred and sixty-seven patients (43%) had an elevated C-reactive protein concentration (>10 mg/l), and 65 patients (17%) had hypoalbuminemia (<35 g/l). Of the 50 patients with hypoalbuminemia, 45 (69%) had an elevated C-reactive protein. The majority of tumors had no evidence of peritumoral inflammatory infiltrate using Klintrup (66%) criteria. One hundred and ten patients (29%) received adjuvant therapy.

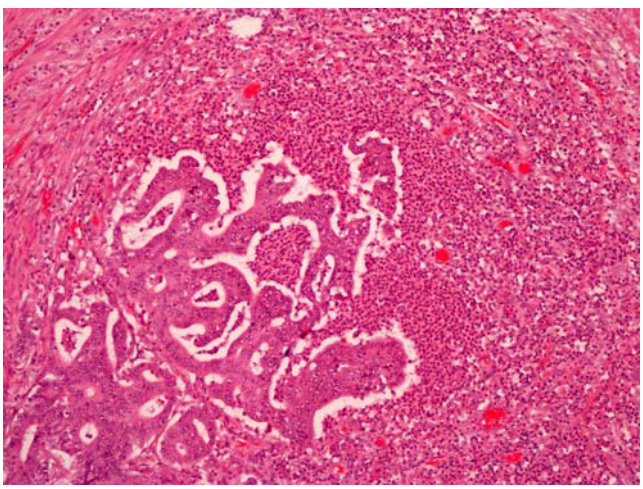

Figure 2 High-grade inflammatory cell infiltrate at the tumor's invasive margin.

The individual clinicopathological characteristics for patients undergoing surgery for colon and rectal cancers are shown in Table 1. Patients with colon cancers were older ($P<0.05$) and had higher T stage ($P<0.001$) and mGPS ($P\leq 0.001$) compared with rectal cancers. The proportions of patients with a high-grade tumor inflammatory cell infiltrate were similar in colon and rectal cancers.

The minimum follow-up was 25 months; the median follow-up of the survivors was 71 months. No patients were lost to follow-up. During this period, 105 patients died of their cancer, and a further 64 patients died of intercurrent disease. The relationship between clinical, pathological, and biochemical characteristics and cancer-specific survival in patients undergoing potentially curative resection for colon and rectal cancers is shown in Table 2. On univariate survival analysis in colon cancer patients, age ($P<0.01$), TNM stage ($P<0.001$), mGPS ($P\leq 0.001$), and a low-grade or absent peritumoral inflammatory cell infiltrate assessed by Klintrup criteria ($P\leq 0.001$) were associated significantly with cancer-specific survival (Table 2). On univariate survival analysis in rectal cancer patients, TNM stage ($P<0.05$), mGPS ($P<0.05$), and a low-grade or absent peritumoral inflammatory cell infiltrate assessed by Klintrup criteria ($P<0.01$) were associated significantly with cancer-specific survival (Table 2).

On multivariate survival analysis in colon cancer patients, TNM stage (HR 2.73, 95% CI 1.51–4.91, $P\leq 0.001$), mGPS (HR 1.56, 95% CI 1.03–2.38.60, $P<0.05$), and Klintrup criteria for inflammatory cell infiltrate (HR 2.12, 95% CI 1.05–4.30, $P<0.05$) were independently associated with cancer-specific survival (Table 3). On multivariate survival analysis in rectal cancer patients, mGPS (HR 1.76, 95% CI 1.00–3.10, $P<0.05$) and Klintrup criteria for inflammatory cell infiltrate (HR 5.74, 95% CI 1.34–15.60, $P<0.05$) were independently associated with cancer-specific survival (Table 3).

Table 1 Clinico-pathological Characteristics of Patients Undergoing Curative Resection for Colon Cancer and Rectal Cancer

	Colon $n=245$ (%)	Rectal $n=140$ (%)	P value
Age			
<65 years	81 (33)	53 (38)	
65–74 years	74 (30)	55 (39)	
>75 years	90 (37)	32 (23)	0.031
Sex			
Female	117 (48)	57 (41)	
Male	128 (52)	83 (59)	0.182
T Stage			
T1	2 (1)	8 (5)	
T2	11 (4)	19 (14)	
T3	140 (57)	84 (60)	
T4	92 (38)	29 (21)	<0.001
N stage			
N0	139 (57)	72 (52)	
N1	81 (33)	48 (34)	
N2	25 (10)	20 (14)	0.201
TNM Stage			
I	11 (5)	18 (13)	
II	128 (52)	54 (39)	
III	106 (43)	68 (48)	0.642
mGPS			
Low risk (0)	122 (50)	96 (69)	
Intermediate (1)	89 (36)	33 (23)	
High risk (2)	34 (14)	11 (8)	0.001
Klintrup criteria			
High-grade inflammation	82 (34)	47 (34)	
Low-grade inflammation	163 (66)	93 (66)	0.984
Adjuvant therapy			
No	175 (71)	100 (71)	
Yes	70 (29)	40 (29)	1.00

Table 2 The Relationship Between Clinical, Pathological and Biochemical Characteristics and Cancer-Specific Survival in Patients Undergoing Potentially Curative Resection for Cancer of the Colon (n=245) and Rectum (n=140): Univariate Survival Analysis

	Colon		Rectal	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age				
<65 years				
65–74 years				
>75 years	1.57 (1.19–2.07)	0.002	1.15 (0.72–1.85)	0.563
Sex				
Female				
Male	0.99 (0.63–1.55)	0.946	0.81 (0.39–1.66)	0.557
TNM Stage				
I				
II				
III	2.76 (1.77–4.32)	<0.001	2.02 (1.10–3.72)	0.024
mGPS				
Low risk (0)				
Intermediate (1)				
High risk (2)	1.65 (1.22–2.24)	0.001	1.77 (1.11–2.83)	0.017
Klintrup criteria				
High-grade inflammation				
Low-grade inflammation	2.79 (1.53–5.07)	0.001	5.74 (1.74–18.99)	0.004
Adjuvant therapy				
No				
Yes	1.01 (0.61–1.67)	0.967	1.43 (0.65–3.13)	0.374

The relationship between TNM stage, local (Klintrup), and systemic inflammatory (mGPS) responses and 3-year cancer-specific survival rates (%) in patients undergoing potentially curative resection for colorectal cancer is shown in Table 4. In TNM stage II disease, patients with a high- and low-grade tumor inflammatory cell infiltrate had a 3-year cancer-specific survival of 97% and 88%, respectively. In these patients with a low-grade inflammatory cell infiltrate, the 3-year cancer-specific survival were 95%, 82%, and 68% for a mGPS of 0, 1, and 2, respectively. In TNM stage III disease, patients with a high- and low-grade tumor inflammatory cell infiltrate had a 3-year cancer-specific survival of 85% and 70%, respectively. In these patients with a low-grade inflammatory cell infiltrate, the 3-year cancer-specific survival were 78%, 60%, and 60% for a mGPS of 0, 1, and 2, respectively (Table 4).

Discussion

The results of the present study show that, in patients with colon and rectal cancers, both local (Klintrup) and systemic (mGPS) inflammatory responses are independently associated with cancer-specific survival. Such routinely available inflammatory measures offer a new approach to staging the biologic phenotype of the tumor and, together with tumor staging, offer a more sophisticated and accurate approach to outcome prediction in patients with primary operable colon and rectal cancers.

For example, in the present study, the 3-year survival rate for TNM stages II and III colorectal cancer was 91% and 73%, respectively. However, within the TNM stage II disease, 3-year survival rate varied between 100% and 68% depending on the Klintrup and Glasgow Prognostic Scores. Similarly, within the TNM stage III disease, 3-year survival rate varied between 97% and 60% depending on the Klintrup and Glasgow Prognostic Scores. Therefore, we believe such measures should be incorporated within routine staging of primary operable colon and rectal cancers.

In the present study, it was of interest that, on multivariate survival analysis, although the hazard ratios for the mGPS were similar in both colon (HR 1.56) and rectal (HR 1.76) cancers, the corresponding hazard ratios for Klintrup criteria were 2.12 and 5.74, respectively. The basis of the increased hazard ratio for the Klintrup score in rectal cancer is not clear. However, it may be that the local inflammatory response is better at controlling tumor dissemination in rectal cancer. Indeed, there were fewer T4 stage rectal tumors compared with distribution in colonic tumors. This might explain why the observation that tumor inflammatory cell infiltration had prognostic value was initially made in rectal cancer.[6]

Table 3 The Relationship Between Clinical, Pathological and Biochemical Characteristics and Cancer-Specific Survival in Patients Undergoing Potentially Curative Resection for Cancer of the Colon (n=245) and Rectum (n=140): Multivariate Survival Analysis

	Colon		Rectal	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age				
<65 years				
65–74 years				
>75 years		0.258		0.274
Sex				
Female				
Male		0.207		0.630
TNM Stage				
I				
II				
III	2.73 (1.51–4.91)	0.001		0.275
mGPS				
Low risk (0)				
Intermediate (1)				
High risk (2)	1.56 (1.03–2.38)	0.038	1.76 (1.00–3.10)	0.033
lintrup criteria				
High-grade inflammation				
Low-grade inflammation	2.12 (1.05–4.30)	0.037	5.74 (1.34–15.60)	0.015
Adjuvant therapy				
No				
Yes		0.259		0.774

The interrelationships between the local and systemic inflammatory responses have previously been examined.[14] While C-reactive protein was not directly related to the local inflammatory cell response, it appears that the Klintrup and Glasgow Prognostic Scores are linked through an increase in circulating neutrophils and a decrease in circulating lymphocytes. However, the relationship is likely to be complex and that the two responses are part of the continuous interplay between the tumor and host. For example, a high-grade local inflammatory response represents an adequate host immune defense preventing tumor spread. As tumor growth continues, there may then be a switch from a local to a systemic inflammatory response.

The systemic inflammatory response is now an established indicator of poor prognosis in a variety of human cancers.[5,20] However, it remains to be determined which components of the systemic inflammatory response play pivotal roles. Of these, the value of C-reactive protein is most recognized being associated with cancer cachexia[21,22]), compromised cell-mediated immunity,[23,24] and upregulation of growth factors and angiogenesis.[25,26] Nevertheless, the mechanisms underlying the relationship between local and systemic inflammatory responses and cancer-specific survival are likely to be complex. These include extrinsic pathways such as nutritional and functional decline, immune dysfunction and tumor angiogenesis,

Table 4 The Relationship Between TNM Stage, Local (Klintrup), and Systemic Inflammatory (mGPS) Responses and 3-Year Cancer-Specific Survival Rates (%) in Patients Undergoing Potentially Curative Resection for Colorectal Cancer (n=366)

	TNM Stage II (n=182)			TNM Stage III (n=174)		
	Klintrup high grade	Klintrup low grade	Klintrup low/ high grade	Klintrup high grade	Klintrup low grade	Klintrup low/ high grade
mGPS 0	100% (n=34)	95% (n=65)	97% (n=99)	97% (n=26)	78% (n=71)	84% (n=97)
mGPS 1	94% (n=21)	82% (n=36)	87% (n=57)	77% (n=14)	60% (n=45)	64% (n=59)
mGPS 2	92% (n=13)	68% (n=13)	80% (n=26)	67% (n=3)	60% (n=15)	61% (n=18)
mGPS 0–2	97% (n=68)	88% (n=114)	91% (n=182)	85% (n=43)	70% (n=131)	73% (n=174)

growth, and dissemination. Recently, it has also been proposed that there are also intrinsic pathways involved in cancer-related inflammation, such as the induction of genetic instability by inflammatory mediators, leading to the accumulation of genetic alterations in cancer cells and progressive tumor growth and dissemination. Indeed, a recent review proposes that cancer-related inflammation represents the seventh hallmark of cancer.[27]

It has long been recognized that low circulating albumin concentrations or hypoalbuminemia before surgery are associated with poor outcome in patients with cancer. For example, in a cohort of over 400 patients with colorectal cancer, Heys and coworkers[28] showed that the presence of a low circulating concentration of albumin before surgery and the magnitude of the decrease were associated with poorer overall survival. However, there is increasing evidence that the prognostic value of albumin may be secondary to an ongoing systemic inflammatory response, as evidenced by elevated concentrations of C-reactive protein, in a variety of cancers.[19,29,30] Indeed, the GPS has been modified based on the observation that hypoalbuminemia, in patients with colorectal cancer without an elevated C-reactive protein concentration, had no significant association with cancer-specific survival.[12] Therefore, although many papers have documented the prognostic value of hypoalbuminemia in a variety of cancers, it may be that the prognostic value of hypoalbuminemia is in part reflecting the systemic inflammatory response[31] and therefore subordinate to the prognostic value of C-reactive protein. The prognostic value of hypoalbuminemia in the presence of an elevated C-reactive protein, i.e., a mGPS of 2, probably reflects a profound loss of lean tissue mass.[32] Therefore, although there is a direct relationship between an elevated C-reactive protein and hypoalbuminemia, it is likely that the relationship is complex, and other factors are important in determining hypoalbuminemia. In particular, in the USA, hypoalbuminemia is considered to mainly reflect poor nutritional status.

With the increasing evidence that host or immune responses are important prognostic indicators in addition to TNM stage, a variety of prognostic scores based on the presence of the systemic inflammatory response have been described.[12,33,34,35] Recently, Iimura and coworkers[36] have developed and validated a combined model of TNM stage and C-reactive protein, termed TNM-C for predicting outcome in patients undergoing surgery for renal cancer. The results of the present study indicate that a similar model based on TNM stage, Klintrup and mGPS scores would be of value in patients undergoing potentially curative surgery for colorectal cancer.

In summary, the results of the present study show that both local and systemic inflammatory responses are important independent predictors of survival in patients undergoing potentially curative surgery for colon and rectal cancers. These scores combined with TNM stage improve the prediction of survival in these patients.

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Dr. Kirk A. Ludwig (Milwaukee, WI): Congratulations to you and your colleagues on a very nice study. The data suggest that for patients with colorectal cancer, inflammatory changes at the level of the tumor are associated with increased survival, while systemic inflammatory responses are associated with a decrease in survival. Furthermore, both local and systemic inflammatory responses can be determined by routine pathologic examination and limited laboratory measurements.

Let me start by thanking the authors for providing me with a copy of the manuscript. In reviewing the manuscript, a number of questions come to mind but I have four that I wonder if you can answer for me.

First, it is known that colorectal tumors that arise via the mismatch repair gene mutation pathway or microsatellite unstable tumors often elicit a local inflammatory response known as a Chron's-like reaction. There is some evidence that these tumors have a better overall prognosis than those tumors that arise via the classic pathway. Given this, did you assess these tumors for microsatellite instability, and if so, is there a relationship between the microsatellite unstable tumors and the good prognosis tumors that you identify?

Second, it appears that 110 patients in your series received adjuvant chemotherapy, and I assume that most of these were stage 3 patients. Were the stage 3 patients receiving chemotherapy evenly distributed across your subgroups as defined by the Klintrup criteria and the modified Glasgow Prognosis Score? And could the use of chemotherapy, in some, but not all of these patients, have confounded your results?

Third, did you look to see if there was a relationship between the variable you measured and other commonly evaluated tumor characteristics, such as tumor grade or differentiation, number of involved lymph nodes in the stage three patients, or more detailed staging systems that break stage 2 and 3 patients into those with stage 2 or 3A or stage 3 or 3B?

Finally, given your data, are you ready to suggest that the power of these variables in predicting prognosis is strong enough to use them in making decisions about who is and who is not offered chemotherapy for their stage 2 or 3 disease?

Closing discussant

Dr. Campbell Roxburgh: The first question relates to microsatellite instability. Unfortunately, we do not perform microsatellite instability analysis on our colorectal cancer patients, and therefore, the data are not available in terms of whether those are related to local inflammatory responses. This is, however, a very interesting point and, potentially, something we will look at in the future.

However, the Crohn's-like reaction described previously with MSI-H tumors was also discussed in the original manuscript by Klintrup and colleagues in the development of their own score. So there is, potentially, a link there, and that is something I think we will take away and look at.

You asked about chemotherapy, and whether TNM stage and provision of chemotherapy was evenly distributed across the subgroups as defined by the Klintrup criteria and a modified Glasgow Prognosis Score.

I can tell you that they were. There were no significant differences between patients who had adjuvant chemotherapy within these different subgroups. In terms of whether chemotherapy was a confounder, we did not identify chemotherapy was a prognostic factor on univariate or multivariate analysis. Such analysis should control for potential confounding variables.

I do know, however, that for patients who did not receive chemotherapy, the prognostic value of the local and systemic inflammatory response still holds true within that group.

And you also asked if the individual criteria were related to any of the actual tumor characteristics. I can tell you that the GPS is not related to any of the actual tumor characteristics. However, in the Klintrup criteria there is a relationship between our low-grade local inflammatory response and increasing T stage. In addition, we have also looked at tumor budding or de-differentiation along the invasive margin. It appears that low-grade local inflammatory responses are related to an increased percentage of tumor budding at that invasive margin.

Finally, am I ready to recommend that the results of these data are ready for use in clinical practice for gauging adjuvant chemotherapy? I can tell you that, at our institution, we routinely measure the systemic inflammatory response in all of our patients.

It has been known for some time that these patients are at higher risk, and our oncologists take this into consideration when prescribing adjuvant chemotherapy. However, whether adjuvant chemotherapy is the most appropriate treatment for patients at high risk, as stratified by these means, remains to be seen. Maybe some other form of immunomodulation may be a more appropriate target.

However, regarding attempts at stratification, I would absolutely recommend that these scores can stratify high risk and have been validated at a number of centers now.

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2009 SSAT POSTER PRESENTATION

Circulating Cytokeratin 18 Fragment M65—A Potential Marker of Malignancy in Colorectal Cancer Patients

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Abstract Soluble cytokeratin 18 fragments (M30, M65) are released from human cancer cells during cell death and hold potential as biomarkers in colorectal cancer characterized by frequent metastatic spread. A total of 62 colorectal cancer and 27 control patients were included in the study. M65 (necrosis and apoptosis) and M30 (apoptosis) were quantified preoperatively (n=62) and postoperatively (n=31) using specific enzyme-linked immunosorbent assays. Presence of disseminated tumor cells (DTC) in the bone marrow was assessed by staining of A45-B/B3-positive cells in aspirates. M65 was significantly elevated in patients with International Union against Cancer stage I and IIA tumors compared to controls. A subgroup (19/31) exhibited a significant (p<0.05) decrease of M65 after tumor surgery (503.9±230.7 to 342.6+94.8 U/l; −32.0±16.5%), in contrast to 12 patients who revealed higher M65 levels postoperatively (386.5±128.5 to 519.1±151 U/l; +37.4±32.3%). DTC in bone marrow were found in 10% (2/19) of patients with decreasing and 50% (6/12) of the patients with increasing M65 serum concentrations after surgery (p=0.028). In conclusion, M65 as marker is likely to be valuable to identify patients with a high incidence of systemic disease.

Keywords Colorectal cancer · Cytokeratin 18 · M65 · Tumor marker · Disseminated tumor cells

Introduction

The majority of patients with colorectal cancer present at a stage when the primary tumor can be surgically removed with curative intent. However, despite the high resection rate of colorectal cancer, about 30–50% of these patients subsequently develop distant metastases, most frequently to the liver and lungs.[1] In these patients, cancer cells had been disseminated either before or during surgery of the primary cancer. Despite recent technical advancements, appropriate detection systems for the routine use to determine extent of pre- and intraoperative hematogenic tumor cell dissemination are still missing. However, a host of diverse tumor markers have been investigated in order to detect residual disease and to aid in prognosis and selection of further therapy.[2]

Among these markers, cytokeratins (CKs) belonging to the intermediate filament family are particularly useful tools for the surveillance of carcinomas. Out of the more than 20 different CKs known, CK 8, 18, and 19 are most abundant in epithelial cells and represent serum tumor markers that are released from proliferating or necrotic/apoptotic tumor cells.

The clinical value of determining soluble fragments of CK 8, 18, and 19 in body fluids lies in the early detection of recurrence and the fast assessment of the efficacy of therapy response in carcinomas.[3] Protein analyses revealed the abundant presence of CK8 and CK18 fragments

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truncated at the N-terminus in cancerous epithelial cells. CK18 is cleaved by caspases during apoptosis, and for detection of this fragment, termed M30, a specific monoclonal antibody is available, as well as another one recognizing total soluble CK 18 fragments (M30 and M65). Specific enzyme-linked immunosorbent assays (ELISA) using these antibodies distinguish between apoptotic (M30) or apoptotic and necrotic (M65) tumor cell death in serum samples.[4]

Assessment of soluble CK18 fragments has been shown to facilitate discrimination between non-cancer and cancer patients and to be of value in prognosis assessment and monitoring of response to treatment. Furthermore, patients with primary and recurrent breast cancer exhibited higher M30 serum levels than healthy individuals.[5] In patients with recurrent cancer, M30 concentrations correlated with the number of involved organs and performance status, although there was no relation between M30 antigen levels and patient prognosis.

Similarly, determination of the mode of cell death in response to chemotherapy was reported for endometrial tumors and prostate cancer.[6] Statistically significant differences in the levels of CK18 fragments were found for patients with lung cancer, benign lung disease, and healthy control individuals.[7] Patients with higher basal M30 serum levels had significantly shorter median survival than those with lower basal M30 concentrations. An approximately fourfold increase in M30 was observed in lung cancer patients in response to chemotherapy after 48 h.

These results suggest that M30 and M65 serum levels might be used as novel biomarkers for prediction of survival and monitoring of the efficacy of chemotherapy in cancer patients.[8,9] In a previous study, we found that patients with colon tumors of stages I and IV had significantly elevated M30 serum concentrations compared to controls.[10] Furthermore, determinations of serum M30 levels performed immediately prior to and 7 days after tumor surgery identified patients with persisting M30 elevation and with an increased risk of recurrence within 36 months.[10] In a report by Koelink et al., plasma concentrations of M30 and M65 levels of 49 colorectal cancer patients up to 50–60 days before and after surgical resection of the tumor, respectively, were found to be related to disease stage and tumor diameter and to be predictive of disease-free survival.[11] In order to further investigate the significance of M65 in individual colon cancer patients, we conducted a pilot study collecting blood samples immediately prior to tumor surgery and 7 days thereafter and evaluated a putative correlation of M65 concentrations with M30 levels, tumor parameters, and dissemination of tumor cells to bone marrow.

Materials and Methods

Study Population

The study population consisted of a total of 62 patients with colorectal cancer treated at the Donauspital SMZ Ost, Vienna, Austria between January 2002 and December 2005. Tumor stage was classified according to the 5th edition of the TNM classification of the International Union against Cancer (UICC).[12] Grading was performed according to WHO recommendations for tumors of the digestive system. None of the patients had received cytostatic chemotherapy and/or radiotherapy prior to surgery. Adjuvant treatment was administered according to recommendations for stage II and III patients. Patients who had no inflammatory, malignant, or cardiac and trauma conditions served as controls (n=27). Written informed consent was obtained from all patients. The study was approved by the local ethics committee and the institutional review board.

Collection of Bone Marrow and Serum Samples

Bone marrow aspirates (5 ml each) were obtained by needle aspiration from both upper iliac crests. Preoperative aspirations were performed immediately prior to the operation under general anesthesia. Blood samples were taken preoperatively and on day 7 postoperatively and centrifuged at 2,000 rpm for 10 min to obtain serum samples. All samples were stored at −20°C until processing.

Control Group for the Assessment of Disseminated Tumor Cells in the Bone Marrow

Fourteen patients served as controls. Six patients underwent surgery because of high-grade dysplasia (two patients), bowel necrosis (one patient), diverticulosis (one patient), diverticulitis (one patient), and ischemic colitis (one patient). Eight patients underwent bone marrow investigation because of abnormal hematological data. Of these, four had a normal bone marrow, two had Hodgkin's lymphoma (one with bone marrow involvement), one patient had an NK/T-cell lymphoma without bone marrow involvement, and one had lymphoplasmocytic lymphoma with minimal bone marrow involvement. All bone marrow samples of the control group were negative for A45-B/B3 epithelial tumor cells using the present method for immunohistochemical analysis.

Immunocytochemical Analysis and Scoring of DTC

Mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation. Cells (1×10^6) were placed on each glass slide. For immunocytochemical staining, the

monoclonal pan-cytokeratin antibody A45-B/B3 (Micromet, Munich, Germany) was used as primary antibody, followed by detection with IDetect Super Stain System Fast Red (ID Labs, London, ON, Canada). Two experienced pathologists reviewed the A45-B/B3-stained sections containing a minimum of 2×10^6 mononuclear cells in a blinded and independent manner. One tumor cell per 2×10^6 screened mononuclear cells was interpreted as a positive result of disseminated tumor cells (DTCs), indicative of minimal residual disease in bone marrow.

M30 and M65 ELISA

From all serum/plasma samples, the concentrations of M30/CK-18-Asp396-NE and M65/total soluble CK18 fragments were determined using the M30-Apoptosense® and the M65-ELISA® assays (Peviva AB, Bromma, Sweden), respectively. All determinations were done in duplicate according to the manufacturer's instruction. Units of the M30 ELISA were defined using a synthetic peptide (1 U= 1.24 pmol). Units of the M65 ELISA were based on these M30 units to allow for direct comparison of the assays. The coefficient of variance for the duplicate measurements of M30 and M65 was <7.5%.

Statistical Analysis

Comparison of control and colon cancer groups was done using ANOVA and Dunnett's tests ($p<0.05$) and distribution of DTC-positive patients was checked for statistical significance with Fisher's exact test (SPSS, Chicago, IL, USA).

Results

Patient Characteristics

Clinical data of the colon cancer patients involved in the present study is displayed in Table 1. DTC in bone marrow aspirates were detected using the monoclonal antibody A45-B/B3 directed to a common epitope of CK polypeptides. Out of the 62 colon cancer patients, 13 (21%) were positive for DTCs.

Preoperative Measurements of M30 and M65 Serum Concentration

M65 and M30 concentrations were determined in serum samples obtained from 62 colon cancer patients immediately prior to surgery and mean values of both parameters were calculated for the individual tumor stages (Fig. 1). Patients with tumors of UICC stage I and IIA had significantly higher preoperative values of M65 than controls ($p<0.05$). All other patients, including those with metastases, exhibited mean M65 values that could not be distinguished significantly from controls. Preoperative M30 serum concentrations were correspondingly lower but did not differ significantly from controls for all stages (Fig. 1). Data show that the controls tended to exhibit a higher M30/M65 ratio (4.4±1.6; $n=27$) compared to the colon cancer patients (3.7±0.6; $n=62$); however, this difference was not statistically significant. Similarly, there were no significant differences for the M30/M65 ratios between all stages and the control population (data not shown). Mean preoperative M30 and M65 serum concentrations calculated for the tumor patient groups with different tumor grading are shown in Table 2. In spite of a tendency for M65 to decrease with increasing tumor grade, differences between the groups did not reach statistical significance, as in the case of M30.

Table 1 Clinical Characteristic of 62 Patients with Colorectal Carcinoma and Preoperative Bone Marrow Investigation for Disseminated Tumor Cells (DTC)

Male/female	43/19	
Median age	68.17 (range 45–81)	
WHO grading		
G1/G2/G3	2/35/23	
ND[a]	2	
UICC staging	Disseminated tumor cells	
	Total number (*n*)	Positive/negative (*n*)
Tis	1	0/1
I	18	4/14
IIA	8	1/7
IIB	2	1/1
IIIA	2	1/1
IIIB	11	1/10
IIIC	5	1/4
IV	11	3/8
Recurrence[b]	4	0/4

[a] Not determined

[b] Local relapse

Relationship of M30 and M65 Serum Concentrations

Figure 2 displays a scatter plot of M30 and M65 measurements fitted by a polynomial regression curve (correlation coefficient $r^2=0.736$). Although M30/M65 ratios were variable, the M30–M65 relationship follows a course appearing like enzyme kinetics with saturation of the production/release of M30 by caspase-mediated cleavage at high concentrations of M65 (>800 U/l). A double-reciprocal graph of 1/M65 versus 1/M30 mimicking a Lineweaver–Burk diagram yielded good linear correlation

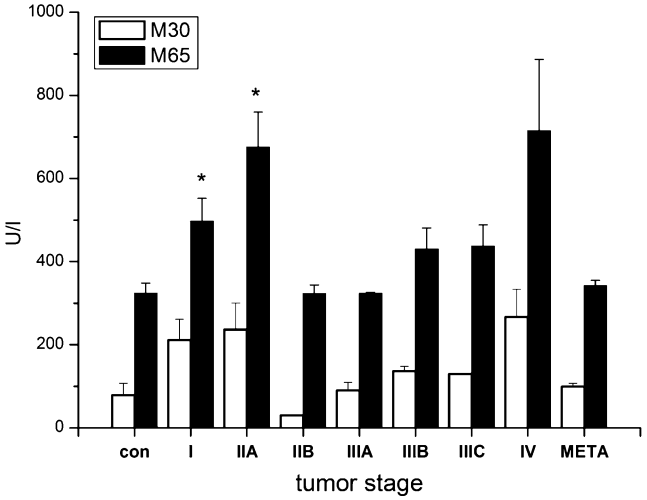

Figure 1 Preoperative serum concentrations of M30 and M65 in colorectal cancer patients for different tumor stages and control (mean± SEM). Mean values of groups that differ significantly from the control group are indicated by an *asterisk* ($p<0.05$).

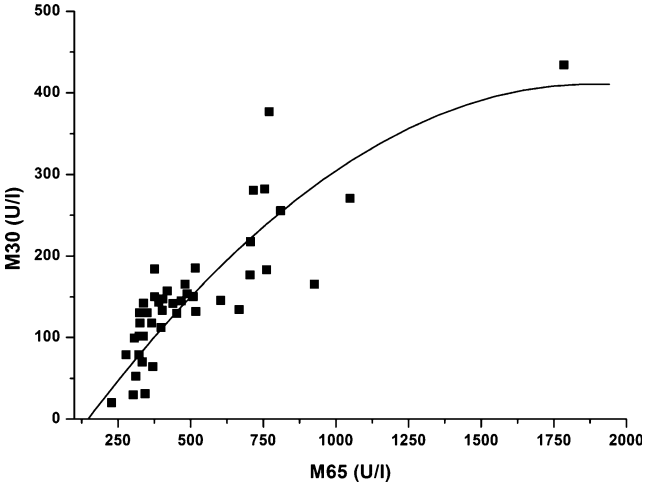

Figure 2 Scatter plot of preoperative M30 and M65 serum concentrations in colorectal cancer patients. A polynomial regression curve is included.

($r=0.781$, $p<0.001$) with an apparent V_{max} (rate of reaction) of 230 U/l M30 and an apparent Michaelis constant K_m of 250 U/l M65. The individual M30/M65 ratios revealed no correlation with tumor stage, grade, and other clinical parameters, respectively (data not shown).

Effect of Surgical Therapy on M65 Serum Concentrations

Since preoperative measurements of M65 seemed to be of limited significance, the possible influence of tumor surgery on serum concentrations of this marker were investigated further in 31 patients postoperatively in addition to the preoperative determinations. Serum samples of these 31 patients were obtained immediately prior to surgery and 7 days postoperatively and used for the determination of M65 concentrations. One group of patients exhibited a reduction of M65 levels (<100% of the corresponding M65 serum concentration prior to surgery) in response to the removal of the tumor, whereas the second group revealed no decrease; a significant increase in circulating M65 was observed despite surgery (Fig. 3A, B). While the first group responded with a drop of M65 from 503.9±230.7 to 342.6±94.8 U/l (−32.0±16.5%) to the removal of the tumor, the second group showed a mean increase of M65 from 386.5±128.5 to 519.1±151.0 U/l (+37.4±32.3%). The two groups of patients revealed no significant differences in age, tumor grade, tumor stage, and other clinical parameters (data not shown). Detection of DTC in these patient groups by assessment of CK-positive tumor cells in bone marrow aspirates proved a low incidence of minimal residual disease in patients with a reduction of M65 as a consequence of the removal of the tumor tissue. In contrast, the patients with still increasing M65 concentrations following surgery had a high incidence of tumor-positive bone marrow samples: 10% (2/19) versus 50% (6/12) patients ($p=0.028$). DTC-positive patients in the responding group were of UICC tumor stages I (1/2) and metastatic disease (1/2), DTC-positive patients in the group with persisting elevations of M65 were of UICC stages I (2/6), IIIA (1/6), IIIB (2/6), IIIC (1/6), and IV (1/6). The patient with UICC IV in the group with persisting elevations of M65 underwent synchronous resection of a single liver metastasis and was operated with curative intent. The bone marrow-negative group of this population comprised patients with tumors of stage 0/I (4/6), stage IIIB (1/6), and one case with metastatic disease (1/6). Therefore, M65 serum concentrations failing to show decrease upon removal of the tumor seem to point to extended and systemic disease.

Table 2 Mean Preoperative M30 and M65 Serum Concentrations (Mean±SD) Calculated for the Tumor Patient Groups with Different Tumor Grading

	N	M30	M65
G1	3	206±49.0	615±195.0
G2	35	190±27.3	534.8±61.9
G3	22	195.2±54.3	473.8±46.0
ND[a]	2	88.8±12.9	337.3±2.0

[a] Not determined

Discussion

Results of the present study revealed significantly increased preoperative values of M65 in colon cancer patients bearing UICC stage I and IIA tumors compared to a control group. Cell death and appearance of elevated serum levels of CK in early stage tumors may be associated with increased rates of cell death or other factors related to production,

degradation, release, and peripheral elimination of these cytokeratin fragments.

Patients with UICC stages IIB–III exhibited M65 serum concentrations similar to healthy controls, and patients with extended disease showed elevated M65 levels, however, with a highly variable distribution. Stabilization of tumor cells and decrease in apoptotic cell death have been demonstrated for higher tumor stages of breast cancer.[13] M30 is a neoepitope of CK18 generated by cleavage of the soluble CK18 fragment/M65 by caspases 9, 3, and 7.[14,15] Although M30, indicative of tumor cell apoptosis, was slightly increased in the patients with stages I and IIA mentioned above, differences to healthy controls did not reach statistical significance. An increase in the apoptotic cell fraction following the order normal mucosal tissue–adenoma–carcinoma was reported previously.[16] The percentage of the M30-positive tumor cell fraction was reported moreover shown to lack correlation with prognosis of colon cancer patients.[17] Tumor patients tended to exhibit lower M30/M65 ratios compared to controls; however, this difference again was not significant.

The relationship between M30 and M65 concentrations was similar to enzyme kinetics, revealing saturation-like kinetics for the conversion/release of M65 to the corresponding fragment M30 by caspases in tumor tissues. Although not dependent on the activity of single enzymes, but rather on production in heterogeneous tumor cell populations, intracellular degradation, and release as well as distribution in the circulation, serum concentrations of M30 showed no linear correlation with the parent polypeptide M65 in the presented colon cancer patients.

For 31 colon cancer patients, preoperative determinations of M65 were compared to measurements obtained 1 week following surgical removal of the tumor. Thus, two groups could be distinguished according to their courses of serum concentrations of M65: the first responded to surgery with a decrease of M65 to normal levels, while the second group failed to show any reduction in circulating M65 and even progressed to higher concentrations (Fig. 3A, B). We did not perform a postoperative bone marrow assessment after 7 days because we did not expect a change in the bone marrow status in a time frame of 1 week only. Although postoperative assessments of DTCs in bone marrow were shown to be of predictive value in breast cancer, these were not predictive in other solid tumors like prostate cancer and colorectal cancer.[18,19]

The group with normalizing M65 values postoperatively revealed M65 serum concentrations that cannot be distinguished from the M65 levels of the normal control group without surgery. Due to relative high variability of M65 measurements between individual patients, the course of M65 in individuals seems to have a greater prognostic value.

Recombinant CK18/M65 was reported to have a half-life of 2.3 days in normal human plasma at 37°C, and therefore, removal of the source of release of CK18/M65 is expected to be followed by a rapid drop of its concentration in the circulation during 1 week.[20] Wound-healing processes are advanced after 1 week and should therefore not account for increased levels of soluble CK18.[21] Hence, the remaining DTCs most likely seem to be responsible for the persistent production and release of M65. These data imply that most of the M65 antigen is produced and released by DTCs and not by the bulk of the tumor. In addition, tumor cells were shown to become mobilized during surgery and thus can be detected in peripheral blood. Increased concentrations of M65 1 week after operation was not a general observation but restricted to a subpopulation of the patients that underwent surgery.

Tumor dissemination can be proved by analysis of bone marrow aspirates using epithelial-specific markers for detection of cancer cells.[22] Since bone metastasis is less frequent in colon cancer, such tumor cells appearing in bone marrow may be a sign of dissemination to other sites.[21] The two colon cancer groups with divergent courses of M65 levels were

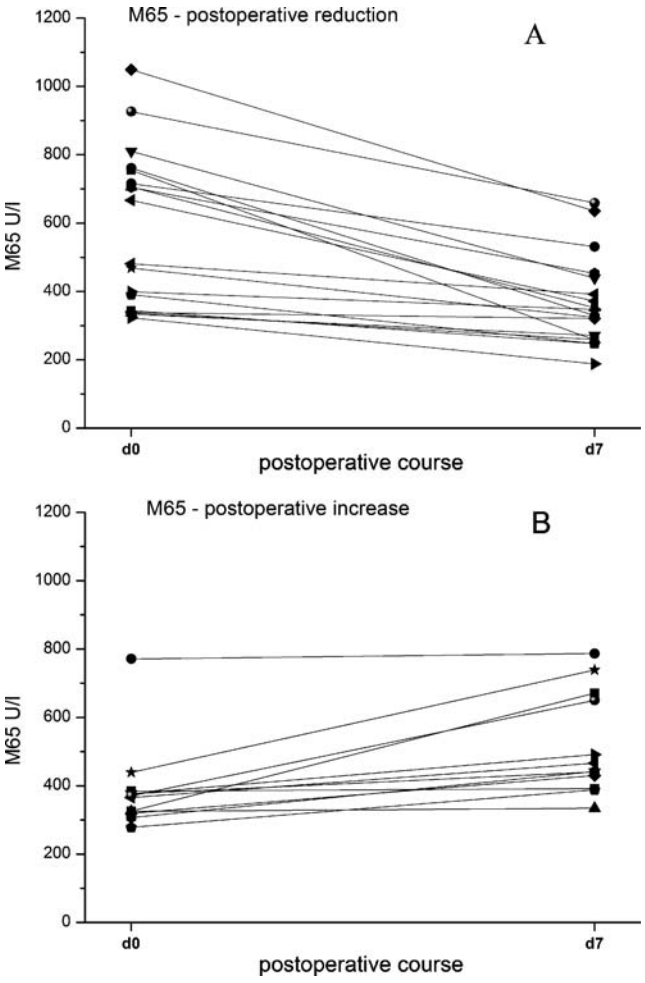

Figure 3 Comparison of preoperative (*d0*) serum concentrations of M65 with postoperative (*d7*) values in colorectal cancer patients exhibiting either a reduction (**a**) or an increase (**b**) (>100% of preoperative concentration) of this antigen.

found to differ significantly in their frequencies of DTC-positive bone marrow aspirates: 10% of the patients with decreasing M65 in response to surgery were DTC positive, whereas 50% of the patients with remaining or increasing serum M65 were DTC positive. DTC-negative cases associated with increasing M65 included two patients with extended disease and four patients with stage 0–I colon cancer. Assuming that persistent serum M65 levels are representative of remaining tumor cell dissemination, assessment of DTCs in bone marrow aspirates using the pan-CK monoclonal antibody A45-B/B3, as performed in the present study, will miss approximately 50% of the patients with minimal residual disease, since it may have affected other tissues and organs.[20] Although the significance of the remaining M65-releasing tumor cells is not clear, the presence of this tumor cell fraction may indicate an increased risk of tumor recurrence. This has to be demonstrated in a larger study for M65, whereas we have demonstrated a correlation of early recurrences with persisting perioperative serum concentrations of M30 in a similar patient population.[10]

Conclusion

The present pilot study indicates that serum concentration of M65 is elevated in low and advanced stages of colon cancer. The difference in preoperative and postoperative serum levels of this antigen seems to represent an interesting marker of tumor cell dissemination and further investigation is warranted. Tests for micrometastases involving bone marrow aspirates are tedious and other methods relying on identification of circulating tumor cells in blood or biochemical parameters have been investigated.[22,23] Perioperative serum measurements of M65 seem to be helpful to identify residual tumor cells in colon cancer patients. This marker is not restricted to DTCs of the bone marrow that have approved prognostic significance in breast cancer patients. Although M30 and M65 are increasingly investigated in diverse tumor entities and serum levels of these CK fragments seem to be correlated with tumor load and prognosis, larger studies are needed for confirmation of these markers.[24,25]

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2009 SSAT PLENARY PRESENTATION

Sympathetic and Parasympathetic Regulation of Rectal Motility in Rats

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Abstract
Introduction The colon and rectum are regulated by the autonomic nervous system (ANS). Abnormalities of the ANS are associated with diseases of the colon and rectum while its modulation is a putative mechanism for sacral nerve stimulation. The purpose of this study is to establish a rat model elucidating the role of the efferent ANS on rectal motility.
Materials and Methods Rectal motility following transection or stimulation of parasympathetic pelvic nerves (PN) or sympathetic hypogastric nerves (HGN) was measured with rectal strain gauge transducers and quantified as a motility index (MI). Colonic transit was measured 24 hours after transection by calculating the geometric center (GC) of distribution of ^{51}Cr
Results and Discussion Transection of PN and HGN decreased MI to 518±185 g•s ($p<0.05$) and increased MI to 5,029±1,954 g•s ($p<0.05$), respectively, compared to sham (975±243 g•s). Sectioning of PN and HGN decreased transit with GC=4.9±0.2 ($p<0.05$) and increased transit with GC=8.1±0.7 ($p<0.02$), respectively, compared to sham (GC=5.8±0.3). Stimulation of PN and HGN increased MI to 831±157% ($p<0.01$) and decreased MI to 251±24% ($p<0.05$), respectively.
Conclusion Rectal motility is significantly altered by sectioning or stimulating either HGN or PN. This model may be useful in studying how sacral nerve stimulation exerts its effects and provide insight into the maladies of colonic motility.

Keywords Rectum · Autonomic nervous system · Hypogastric nerve · Pelvic nerve · Sacral nerve stimulation

Abbreviations
ANS Autonomic nervous system
HGN Hypogastric nerves
PN Pelvic nerves
MI Motility index

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Introduction

Involuntary control of the colon and rectum is regulated by the autonomic nervous system (ANS) and enteric nervous system. The ANS can be divided into parasympathetic and sympathetic components. In humans, the parasympathetic innervations to the left colon, sigmoid colon, and rectum arise from preganglionic neurons whose cell bodies are located in the sacral spinal cord. Their axons leave the spinal cord through the S2, S3, and S4 ventral spinal roots and reach the colon via the pelvic nerves (PN) and inferior hypogastric plexus.[1]

The sympathetic innervation to the distal colon and rectum arises from cell bodies that lie within the dorsal horn of the lumbar spinal cord. The axons of these preganglionic neurons synapse in prevertebral ganglion. The axons then travel with the mesenteric arteries and their branches to

reach the effector. One set of axons runs along the inferior mesenteric artery to innervate the distal part of the transverse, left, and sigmoid colon as well as the proximal rectum. The distal part of the rectum and internal anal sphincter receive their innervations from neurons located within the inferior mesenteric ganglion. Their axons reach their effector via the hypogastric nerves (HGN). Both the pelvic and hypogastric nerves carry afferent sensory information in addition to efferent ANS outflow.[1] However, it remains unclear how the PN and HGN regulate rectal motility.

In humans, the pelvic plexus is compromised of many widely dispersed ganglia with multiple interconnections. The pelvic plexus of the rat, however, has a single major ganglion with distinct sympathetic and parasympathetic inputs the HGN and PN, respectively. The ganglia and its inputs are also readily identifiable with the aid of a dissecting microscope.[2,3] The anatomy has been extensively studied and the projections of the ganglion are well known.[4] These properties make the rat and ideal candidate for studying ganglionic activity regulating the descending colon and rectum.

Abnormalities of the ANS have been associated with several disease processes of the colon and rectum including low anterior resection syndrome, fecal incontinence, constipation, and irritable bowel syndrome (IBS). The extrinsic and intrinsic nervous system is subject to injury during anterior resection which may lead to postoperative defecatory disorders.[5] In patients with neurogenic fecal incontinence visceral afferent sensory pathways are abnormal as evidenced by an increase in sensory threshold of the rectum.[6] Small fiber sensory neuropathies have been reported in a subset of patients with idiopathic slow transit constipation.[7] Furthermore, hypersensitivity of the mechano-sensors for stretch and contractile tension are implicated as pain factors in IBS.[8] Also, it is well documented that patients with IBS are more sensitive to balloon distension of the rectosigmoid region.[9–11]

Sacral nerve stimulation involves the operative placement of temporary or permanent electrodes against the nerves of the sacral plexus to alter the physiological function of the bladder, rectum, and pelvic floor.[12] The manner in which sacral nerve stimulation exerts its effects remains poorly understood.[13,14] Originally, sacral nerve stimulation was thought to exert its effects at the level of the anal sphincter and pelvic floor. However, recent studies suggest that sacral nerve stimulation exerts its effects on multiple nerves within the sacral plexus including voluntary somatic, afferent sensory and efferent autonomic nerves.[12] Unfortunately, in humans, studying the effects of manipulation on the ANS, such as sacral nerve stimulation, is difficult. Both the methods for measuring changes to the colon and rectum as well as degree of manipulation to the ANS are limited.

The aims of our study were to elucidate the functional role of efferent autonomic nerves on rectal motility in the rat. We studied how damage to individual components of the autonomic nervous system affects rectal motility. We also studied how stimulation of the individual components of the ANS affects rectal motility.

Materials and Methods

Animals

All experiments used Sprague–Dawley rats maintained in the animal care facility at the Clement J. Zablocki VA Medical Center, Milwaukee WI. Rats were kept in a controlled environment (21±1°C, 30–70% humidity, 12-h light–dark cycle) and given free access to tap water and standard rat chow (LabDiet 5001-Rodent Diet, PMI International). All rats were housed at standard conditions for at least 7 days prior to any experimentation. Rats were individually caged following each procedure. All operative procedures were carried out under Isoflurane (2%) anesthesia. At the conclusion of each experiment rats were sacrificed with Isoflurane (5%). Protocols describing the use of rats were approved by the Animal Care and Use Committee of Clement J. Zablocki VA Medical Center (Milwaukee, WI).

Rectal Motility Recording After Denervation

As previously reported,[15] a strain gauge transducer (Kyowa Strain Gages, Japan) was implanted on the serosal surface of the rectum in nine male Sprague–Dawley rats. The animals were divided equally into three groups: transection of bilateral parasympathetic PN (n=3), transection of bilateral sympathetic HGN (n=3), or sham operation (n=3). In each rat, the abdomen was entered via a 2–3 cm lower midline incision. The abdominal contents were gently placed within the upper abdomen and kept in place with sterile moist gauze. Bilateral pelvic ganglia were identified in each animal using gentle manipulation with cotton-tipped applicators. The appropriate nerves were sectioned and hemostasis was obtained by direct pressure with a cotton-tipped applicator. A strain gauge transducer was then affixed to the serosal surface of the rectum perpendicular to the longitudinal axis using a 4–0 silk suture, 4 cm above the anus. The transducer lead was brought through the left abdominal wall and tunneled subcutaneously to the posterior neck. The incision was closed and the animal was awakened from the procedure. Rats were connected to a recording system (PowerLab, ADInstruments, Colorado Springs, CO) 30 min following the end of the procedure.

Rectal motility was recorded daily for 24 h after transducer placement and quantified by determining a motility index (MI; area under the curve expressed as

gram•second) of representative 30 min transducer recordings). Comparisons between groups were made using the Student's *t* test.

Colonic Transit Study After Denervation

Under Isoflurane anesthesia, 24 male Sprague–Dawley rats underwent placement of a silicone tube into the proximal colon, as previously reported.[15] The animals were divided into three groups (n=6–9): transection of bilateral parasympathetic PN, transection of bilateral sympathetic HGN, or sham operation. The silicone tube (ID=1/32 in., OD=3/32 in.) was placed within the proximal colon through an enterotomy made in the cecum. The catheter was secured to the cecum using 3–0 silk in a purse-string fashion. The proximal portion of the tube was brought through the left abdominal wall and tunneled beneath the skin to the posterior neck and fixed to the skin.

To evaluate colonic transit after nerve transection, 0.2 ml of ^{51}Cr (2.5 μCi/ml; Perkin Elmer, Waltham, MA) was injected and flushed with 0.2 ml normal saline via the catheter into the proximal colon on the postoperative day 1. Three hours after the administration of ^{51}Cr, the entire colon was removed and divided into ten equal segments. Feces excreted within the 3-h period were collected and referred to as segment 11. The radioactivity of each segment was ascertained by use of a gamma counter (Perkin Elmer-2470, Waltham, MA).

The distribution of radiochromium was quantified as a geometric center (GC) using the following calculation: GC=∑(fraction of ^{51}Cr per segment×segment number), as previously reported.[15] Groups were compared using the Student's *t* test.

Electrical Nerve Stimulation

Under Isoflurane anesthesia the abdomen was entered via a 2–3 cm lower midline incision. A strain gauge transducer was implanted on the serosal surface of the rectum in six rats. Rats underwent stimulation of the right PN followed by stimulation of the right HGN. Each nerve was stimulated at 10 V, 10 Hz for 30 s with pulse duration of 2 ms. There was a 5-min interval between stimulations.

After the first series of stimulations, rats were administered atropine or propranolol and stimulations were repeated. Comparisons between the Motility Index (MI) 30 s prior to stimulation and for 30 s following onset of stimulation were made using the paired Students *t* test.

Results

Rectal Motility Recording After Denervation

Differences were noted between the transducer tracings from each of our experimental groups. The sham group demonstrated normal rectal activity with a maximum contractile force of approximately 5 g. The PN transection group showed dramatically decreased rectal motility while the HGN transection group showed a dramatic increase in rectal motility with maximal contractile force of approximately 15 g (Fig. 1).

The data from the nine transducer recordings were quantified by integrating the area under the tracings and then expressed as a MI. Calculations were performed on representative 30-min segments of the recordings. Animals undergoing PN transection were found to have a signifi-

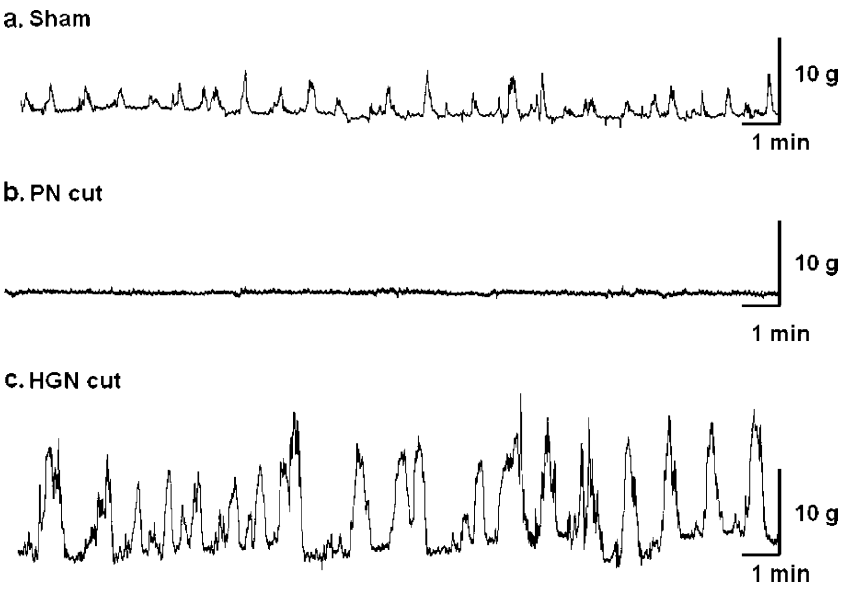

Figure 1 Fifteen-minute representative recordings in awake rats with rectal strain gauge implant. **a** The tracing of rectal contractions in sham operated rats. **b** The tracing of rectal contractions in rats with bilateral parasympathetic PN transected. **c** The tracing of rectal contractions in rats with bilateral HGN transected.

cantly reduced MI (518±185 g•s, n=3), compared to that of sham-operated rats (975±243 g•s, n=3, p=0.04). In contrast, the MI was significantly increased (5,029± 1,954 g•s, n=3, p=0.02) in rats who underwent HGN transection.

Colonic Transit Study After Denervation

Colonic transit was significantly different in both treatment groups when compared to the sham group. The animals in the sham group had an average geometric center of 5.8±0.3 (n=7). The animals with bilateral PN sectioned demonstrated an attenuation in colonic transit with an average geometric center of 4.9±0.2 (n=6, p=0.05). The animals with bilateral HGN transected showed an increase in colonic transit time with an average geometric center of 8.1±0.7 (n=9, p=0.01). Two of nine animals in the sham group were found to be outliers as the geometric center was calculated to be greater than the third quartile plus 1.5 times the interquartile range and were not used to calculate the average geometric center. It is possible these animals sustained injury to the HGN during visualization of the pelvic ganglion.

Electrical Nerve Stimulation

Stimulation of a unilateral PN caused rectal contraction while stimulation of a unilateral HGN caused relaxation (Fig. 2). After cessation of HGN stimulation a contraction was noted and classified as a rebound contraction. There was an average 831±157% (n=6, p<0.01) increase in MI during PN stimulation while there was an average 251±24% (n=6, p<0.05) decrease in MI during HGN stimulation.

The contractions induced by PN stimulation were abolished by the administration of atropine, while the biphasic responses (relaxations followed by rebound contractions) to the HGN stimulation were abolished by propranolol administration (Fig. 2).

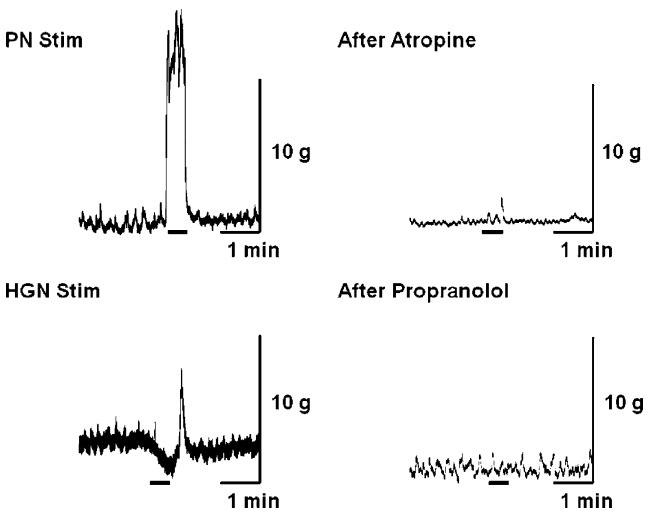

Figure 2 Rectal transducer tracings during stimulation of a unilateral PN (*PN Stim*) and a unilateral HGN (*HGN Stim*). A potent contraction was seen during PN Stim, while relaxation followed by a rebound contraction was seen during HGN Stim. The effects of the administration of atropine and propranolol on PN Stim and HGN Stim are also shown. A *bar* beneath the tracings indicates the duration of stimulation.

Discussion

The rat is an ideal model for studying ganglionic activity regulating the descending colon and rectum. In higher mammals the pelvic plexus is compromised of many widely dispersed ganglia with multiple interconnections. The pelvic plexus of the rat, however, has a single major ganglion with distinct sympathetic and parasympathetic inputs the HGN and PN, respectively. The ganglia and its inputs are also readily identifiable with the aid of a dissecting microscope.[2,3] The anatomy has been extensively studied and the projections of the ganglion are well known.[4] Manipulation of the ANS and measuring its effects are relatively straightforward in the rat model. Furthermore, many models for specific disease states of the colon and rectum exist in the rat including but not limited to, inflammatory bowel diseases, IBS, and pelvic nerve damage induced by pregnancy.

The effects of autonomic denervation on colorectal motility and transit still remain unclear. Within the Order Carnivora, sacral parasympathetic innervation has a major role in the generation of colonic propulsive activity, especially during defecation, initiated either by rectal distension or electrical stimulation of afferent axons running in the PN.[1] Transection of the PN in dogs led to a long-lasting decrease in contractile complexes in the colon.[16] An identical mechanism is also presumably present in humans, since it has been observed that defecation is impeded after surgical section of the PN.[17] In fact the opposite phenomenon of increased bowel activity is seen in patients who have undergone anterior resection of the rectum in which much of the sympathetic input to the descending colon is removed.[18]

Patients who have undergone anterior resection of the rectum are particularly likely to experience defecatory disorders such as urgency, soiling, and diarrhea. Our current findings may help to explain the hyper-contractility of the distal colon following the low anterior resection.[5,19,20] Stimulation of sympathetic axons is known to inhibit colonic motility.[21,22] In anesthetized cats, transection of sympathetic efferents enhances rectal motility.[23] We have recently shown that surgical denervation of the left colon results in a significant increase in motility in rats in vitro. This increase seems to be the result of destruction of an inhibitory sympathetic pathway.[24] This suggests the pres-

ence of a tonic inhibitory nervous influence on colonic motility.

Our study adds to this evidence as the HGN sectioning led to a dramatic increase in rectal motility as well as acceleration of colon transit. A tenant of colon resection for cancer is to base the extent of resection on vascular inflow. The vascular inflow of interest for low anterior resection is the inferior mesenteric artery (IMA).[5] As previously described, the majority of sympathetic innervation to the transverse colon, descending colon, and rectum runs along the IMA. As the IMA is divided so is the sympathetic innervation. This may lead to a relative overabundance of parasympathetic innervation leading to increased motility which may be observed as urgency, soiling, and diarrhea.

The extrinsic nervous system does not seem to play such a major role in colonic transit for all species. For instance, in the guinea pig colon, fecal transportation may be entirely organized by the enteric nervous system.[25] This does not appear to be the case in rats as our PN transection group showed a significant decrease in rectal motility as well as decreased colon transit while the HGN transection group showed a significant increase in rectal motility as well as increased colon transit. It appears that rats may more closely resemble the colonic nervous control seen in dogs and humans

In contrast to the upper gastrointestinal tract, the colon displays very complicated motility patterns such as segmental, and anterograde or retrograde propagating contractions. Furthermore, contractile patterns of the colon have been defined as colonic migrating motor complexes and colonic non-migrating motor complexes.[18] In order to characterize colonic contractions into one of these two categories, it is necessary to measure contractile patterns in at least two distinct segments. A limitation of our study was that only contractile patterns from the rectum were measured. We therefore are unable to be certain about the propagative nature of these contractions. However, we believe that the majority of the contractions are indeed propagative as there was a direct correlation between rectal contractions and colonic transit as measured by MI and GC, respectively.

PN stimulation induces contractile responses throughout the entire colon including the proximal colon in cats,[26] while others showed that in dogs only the left colon and rectum responded to PN stimulation.[27] A recent retrograde labeling study showed that the left colon receives parasympathetic input from the PN in cats.[28] Although our current study demonstrated that stimulation of the PN causes contractions in the rat rectum, it remains to be determined whether the PN extends its innervation to the entire colon of rats. Our preliminary data suggests that PN stimulation causes contractions in the mid colon, distal colon as well as rectum in rats (unpublished observations). Our current study demonstrated that atropine treatment almost completely abolished the contractile responses evoked by the PN stimulation. This suggests that the PN regulates rectal contractions via muscarinic receptors in rats.

The affects of the HGN stimulation on rectal motility has not been fully studied. Hedlund and colleagues[29] reported that sympathetic nerves to the rectum exert both excitatory and inhibitory responses. Excitatory responses to HGN stimulation have only occasionally been observed in cats.[30] The present study revealed that HGN stimulation induced rectal relaxations followed by rebound contractions, suggesting a predominant inhibitory effect of HGN innervation in the rat. The relaxations and rebound contractions induced by HGN stimulation were antagonized by propranolol. This supports the previous finding that the inhibitory responses elicited by sympathetic nerve stimulation is a result of activation of postganglionic fibers acting on beta adrenoceptors of colorectal smooth muscle cells.[29]

Sacral nerve stimulation has been used successfully in patients with fecal incontinence, for which conventional treatment has failed. In these patients, an 80% improvement in incontinence rates has been reported.[31–34] Sacral nerve stimulation has also been applied to the treatment of functional idiopathic constipation with encouraging results.[35–37] Treatment with sacral nerve stimulation resulted in a significant improvement in Wexner constipation scores as well as increased quality of life.[38] Sacral nerve stimulation was found to provide a significant reduction in diarrhea predominant IBS and improvement in quality of life.[39] Other studies have shown a modest improvement in external and internal anal sphincter function as measured by anal squeeze pressure.[34,40,41] Sacral nerve stimulation also increases rectal mucosal blood flow and heighten sensation to rectal distension.[12]

The manner in which sacral nerve stimulation exerts its effects remains poorly understood. Originally, sacral nerve stimulation was thought to exert its effects at the level of the anal sphincter and pelvic floor. However, recent studies suggest that sacral nerve stimulation exerts its effects on multiple nerves within the sacral plexus including voluntary somatic, afferent sensory, and efferent autonomic nerves.[12] This study was designed to investigate how the efferent autonomic nerves regulate colorectal motility. We demonstrated that the parasympathetic PN have a stimulatory effect while the sympathetic HGN have an inhibitory effect on colorectal motility in rats. In future studies, we hope to implant a sacral nerve stimulator into the sacral foramen of the rat and observe how changes caused by sacral nerve stimulation are modified by altering the PN and HGN. There are undoubtedly differences in how the pelvic organs in humans and rats respond to alterations in the ANS. This straightforward readily available model allows for testing of hypotheses and elucidation of the mechanisms by which sacral nerve stimulation, an effective but incompletely

understood method of treatment, exerts its effects on colorectal function in humans.

Conclusion

In conclusion, manipulation of the ANS can have a significant impact on rectal motility. Transection of parasympathetic innervation leads to a dramatic decrease in rectal motility whereas transection of sympathetic innervation causes an increase in rectal motility. This is evidenced by both changes in motility index and transit time. Stimulation of the parasympathetic PN causes rectal contraction while stimulation of the sympathetic HGN causes rectal relaxation. The rat is a suitable model for studying how manipulations of the ANS affect rectal motility. This model may prove to be useful in studying the manner in which sacral nerve stimulation exerts its effects and provide further insight into maladies of colonic motility.

Conflicts of interest No conflicts of interest exist.

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Discussant

Dr. Jonathan Critchlow (Beth Israel Deaconess Medical Group, Boston, MA): This group has developed a novel model for the study of colonic and rectal motility. They have demonstrated that damage to sympathetic nerves increases motility, parasympathetic symptom, behaves in a contrary manner. Stimulation provokes the opposite response adrenergic and musculogenic blockers cancel all the effects.

The findings are really not totally new. My former chief would talk about giving a high spinal to treat a colonic lleus, and the affects of sympathetic blockade have been demonstrated by other investigators.

As you mention, sacral nerve stimulators show great promise in the treatment of constipation and probably act mostly in a motility, although they may have some effect on the sphincters themselves.

I think the key here is the model itself. It is a beautiful model. It will allow us to pars out some of the effects of motility in very complex disorders. In your manuscript you did mention low anterior syndrome. We know of several contributing factors, trauma to the sphincter, loss of sensation, radiation therapy, and loss of rectal capacitance. Motility is probably a big player as well, where a hyperfunctioning sigmoid denervated by high ligation of the intense mesenteric artery is pitted against a small rectal stump made reluctant by damage to either small or large nerve fibers during total mesorectal excision. So here is your model and we wish to see how this turns out.

Two questions. First, if motility is at play in low anterior syndrome, which of the nervous systems do you think is the most important?

Second, if you could share your plans for the next step and whether you have any preliminary results in using this model, because this is really where the key is.

Closing Discussant

Dr. Timothy J. Ridolfi: Thank you for your questions. As most people know, there are two nervous systems that control the colon; the enteric nervous system, which is considered the intrinsic nervous system, and the autonomic nervous system, which is considered the extrinsic component. There is debate whether the intrinsic or the extrinsic component plays a larger role during low anterior resection syndrome.

During low anterior resection syndrome the sympathetic fibers traveling along the inferior mesenteric artery are taken. Those nerves are known to innervate the distal portion of the transverse colon to the most proximal portion of the rectum. After taking those fibers, the remaining most distal portion of the colon, has a preponderance of parasympathetic innervation. We feel that this sympathectomy may be a large component of how low anterior resection syndrome is caused.

As far as your second question, we have utilized this model in looking at the extent of innervation of the pelvic nerves within the colon. We stimulated the pelvic nerves and hypogastric nerves and placed transducers along the colon in four different places. Interestingly, we have found that the most proximal extent of pelvic nerve stimulation is the transverse colon in this rat model, which is similar to the scenario that is found in humans.

We are also very interested in implanting a sacral nerve stimulator into this rat model and observing how manipulating the different components of the autonomic nervous system could change the effects of sacral nerve stimulation.

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Unexpected Identification of Gallbladder Carcinoma During Cholecystectomy

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Keywords Gallbladder carcinoma · Incidental gallbladder cancer · Laparoscopic cholecystectomy · Stop rules

There is no clear understanding of what proportion of all gallbladder (GB) cancers are identified incidentally, but certainly, most of the 8,500 cases per annum in the USA present with signs and symptoms. However, while most incidental gallbladder carcinomas are first realized *after* cholecystectomy for presumed benign biliary conditions, some are discovered *during* an operative endeavor.

Overall, incidental gallbladder cancer occurs in roughly 1% of all cholecystectomies performed, but the identification of such appears to be on the rise since the advent of laparoscopic cholecystectomy in the early 1990s.[1] These tumors are usually early-stage malignancies (T1 or T2) that are not obvious but are instead recognized post hoc during histologic evaluation of the specimen. The outcomes for such tumors are generally favorable, demonstrating superior 5-year survival (50%) over non-incidentally diagnosed tumors (20%).[2] One important exception to this advantageous prognosis is when violation of the gallbladder during the laparoscopic dissection allows intraluminal tumor cells to disseminate throughout the ceolomic cavity. The management and prognosis of early-stage incidental gallbladder carcinoma is featured elsewhere in this symposium.

On the other hand, perhaps, only 35% of all incidental gallbladder carcinoma falls into the T3 and T4 categories. By definition, these tumors have at least penetrated the serosal layer and *practically* are evident to the naked eye. This monograph focuses on the circumstance when a surgeon will embark on a cholecystectomy (laparoscopic or open), or any other abdominal operation for that matter, only to encounter suspicious findings in the right upper quadrant. This exceedingly rare event may, in fact, be a once-in-a-career event for any given surgeon but requires a thoughtful game plan, coupled with skilled execution. Given the better prognosis expected in the incidental setting, yet the inherent aggressive nature of this particular malignancy, this particular scenario falls under the category of *surgical jeopardy*—where the conduct and approach of the operation has important ramifications for success.

In 2005, Strasberg proposed the concept of "Stop Rules," as they pertain to preventing biliary injury during cholecystectomy. This idea, adapted from the aviation and nuclear power industries (among others), emphasizes the application of applying a "braking" mechanism to a planned sequence or conventional course when there is sufficient doubt as to a successful outcome. By this reasoning, a process must be stopped before it continues into a zone of great risk. For example, a pilot working without the luxury of an automatic navigation system should not risk landing without first securing identification of the runway. If not achieved, the landing must be aborted

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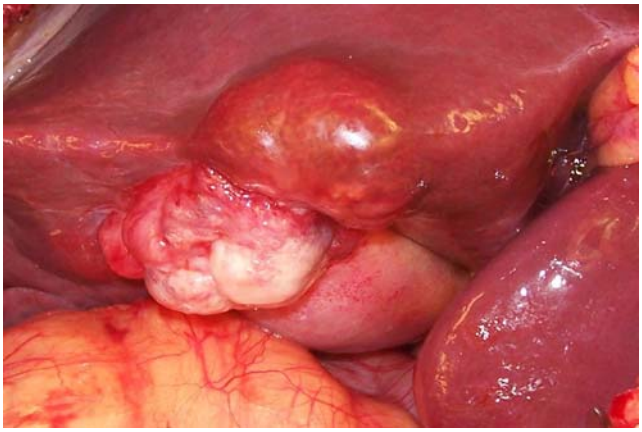

Figure 1 An incidentally identified, 2.5 cm, exophytic gallbladder malignancy evident at the time of a laparoscopic cholecystectomy for biliary colic.

because "the negative effects of diversion are considered minor compared to the risk of a dangerous landing and its disastrous consequences."[3]

Strasberg paints an analogous story in terms of laparoscopic cholecystectomy urging that safety is the chief consideration that governs intraoperative decision making. He proposes that (1) failure to progress, (2) anatomic disorientation, (3) difficulty in visualizing the field, and (4) inability of laparoscopic equipment to carry out usual tasks, like grasping or separating, be considered stop rules for routine cholecystectomy. While these concepts were initially proposed in order to mitigate the risk of biliary injury, I might propose that a fifth parameter be invoked—suspicion of malignancy.

There are many reasons *not* to proceed with laparoscopic cholecystectomy in this setting. First, it is known that bile spillage from *routine* cholecystectomy occurs frequently (25–36%).[4] This rate could be expected to be even higher in a more technically challenging case involving an infiltrating mass, which is often adherent to surrounding structures. One experience with this problem (mostly T2 tumors) revealed that there was a 44% spillage rate, 71% recurrence rate, and numerous positive margins (R1/R2 resections).[4] Second, port site recurrence is common (around 20%), even in a non-perforated dissection, and is analogous to an incurable scenario. Third, survival (35–40% at 3 years) is inferior to that achieved with historical accounts of open cholecystectomy. Survival has been shown to be even more clearly compromised when perforation occurs during laparoscopic cholecystectomy for GB cancer.[1] Finally, Pawlik et al.[2] has shown that the incidence of *any* residual disease after initial cholecystectomy for GB carcinoma ranges from 38% for T1b to 77% for T3 tumors, suggesting that the original scope of resection is inadequate. For these reasons, laparoscopy may convert a curable scenario to incurable.

What, then, can be done when an unexpected lesion is identified (Fig. 1)? First, survey the terrain. Do a thorough diagnostic peritonoscopy which visualizes both the visceral and parietal surfaces. Assess for signs of advanced malignancy like ascites, liver metastases, and/or carcinomatosis. Gallbladder malignancies present with metastatic disease in well over 50% of the cases, and staging laparoscopy has been proven useful in those cases where the tumor is suspected preoperatively.[5,6] Identification of these findings, with immediate frozen-section confirmation of malignancy, is a simple means of diagnosis and should abandon any ideas of attacking the primary mass itself. Avoid the temptation to perform cholangiography, given the attendant risk of tumor dissemination. Biopsy of the mass is also dissuaded for the same reason. Furthermore, frozen section analysis of the primary is unreliable. Alternatively, ultrasound is quite effective at delineating malignancy vs benign adenomyomatosis vs polyps (which are largely dysplastic, depending on size). Reserve biopsy for the scenario where the tumor is clearly unresectable for cure, or the patient is unfit for a radical resection, and a definitive diagnosis is required for the oncologist. Always stage upward if possible (i.e., metastases>nodes>primary) and perform the biopsy of the GB mass through an acoustic window of liver parenchyma to avoid bile peritonitis.

Ultrasound is an invaluable adjunct to laparoscopy for staging this disease. The classic sonographic characteristics of GB cancer (Fig. 2) include large size (2–5 cm), eccentric and/or segmental wall involvement, fibrotic appearance, stromal hypervascularity, and invasive qualities. While ultrasound is limited in delineating degree of GB wall invasion required to call T1 vs T2 disease, it is quite good at defining serosal penetration into the liver, hepatic vascular invasion, and intraparenchymal liver metastases, which upstage the tumor to T3 or T4.

Once staging information has been obtained, the surgeon must make a reasonable decision regarding his/her capabilities

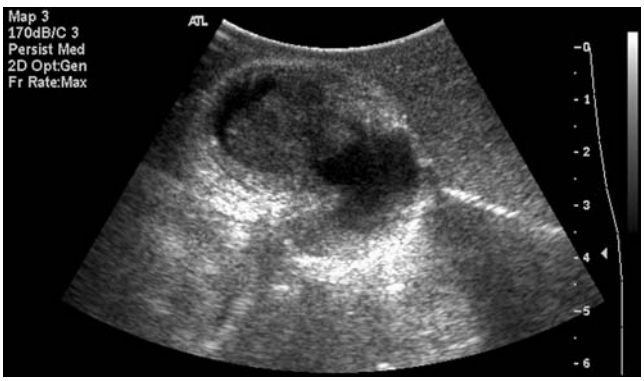

Figure 2 Ultrasonic appearance of an eccentric, intralumenal mass consistent with gallbladder cancer.

to properly address the problem. If not well versed in advanced hepatobiliary resection techniques, it is appropriate to back out at this point, before any definitive bridges are burned, and refer to a colleague or specialist with such experience. If the surgeon is indeed proficient in this realm, it is appropriate to proceed directly to a radical cholecystectomy, realizing that liver resection and portal lymphadenectomy are required to achieve better survival. The bile duct does not need to be routinely resected but should be saved for those cases of a positive cystic duct margin (which should always be analyzed intraoperatively given involvement in up to a third of cases).[2]

In summary, the unexpected finding of an overt gallbladder malignancy is a rare event and, by definition, indicates advanced stage disease. The stakes are high, and careful consideration should be given when deciding whether resection, in the moment, is appropriate. "Stop Rules" should be considered. Decisions should be governed by the surgeon's capabilities—both in their experience as well as with technical skills for hepatobiliary malignancies. Definitive radical resection is required for long-term oncologic success, and this may require portal lymphadenectomy, central bile duct excision, and reconstruction, or even en-bloc adjacent organ removal. If the surgeon is not prepared for such endeavors, cholecystectomy should be abandoned, but more thorough staging can be obtained minimally before referral to a specialist in hepatobiliary surgery.

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What to Do When the Pathology from Last Week's Laparoscopic Cholecystectomy is Malignant and T1 or T2

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Keywords Gallbladder cancer · Laparoscopic cholecystectomy · Malignancy · Prognosis · Reresection

Laparoscopic Conduct does not Degrade Prognosis

In 1% to 2% of patients, the pathology of the gallbladder removed during routine laparoscopic cholecystectomy (LC) will reveal cancer. It is important to realize and counsel the patient that having had a LC does not degrade their prognosis. Donahue et al. analyzed data from the National Cancer Database comparing patients having cholecystectomy before and after the introduction of LC and found that there was no change in either survival or frequency of early stage tumors.[1] In addition, while analysis of the larger SEER database revealed a shift in the incidence of gallbladder cancer (GBC) to patients under the age of 50, there was also a slight but significant improvement in GBC survival with the introduction of LC.[2] Another recent study compared 24 patients diagnosed with GBC following LC with 40 diagnosed at open cholecystectomy (OC) and found no difference in the 5-year survival between the two groups.[3] We have analyzed data for 44 patients at Oregon Health and Science University over the past 10 years comparing patients who had an LC with those who did not and found no difference in survival for all stages (unpublished). In a separate Japanese study, patients diagnosed at LC were compared to those diagnosed after LC, and survival was also the same for each stage.[4] In this study, patients with T1 cancer who underwent only LC had a 100% 5-year survival.[4] Another case series from Johns Hopkins University revealed a similar lack of difference in survival between patients who had one definitive procedure compared to those who had laparoscopic cholecystectomy followed by later definitive resection.[5] In this study, 82% of patients diagnosed incidentally had stage I or II disease. Surprisingly, when stage II patients who were diagnosed incidentally were compared to stage II patients not identified incidentally, those diagnosed incidentally with GBC had significantly improved 5-year survival.[5]

Completion of Staging

After incidentally diagnosed GBC, the case should be reviewed with the pathologist to determine if the malignancy was on the side of the gallbladder fossa, the status of the cystic duct margin, and nodal status.

We next perform additional workup to complete staging. There is a paucity of level I evidence in this regard. However, the best available literature suggests that magnetic resonance imaging with magnetic resonance cholangiopancreatography and magnetic resonance angiography has the greatest sensitivity and specificity for vascular invasion and is probably more accurate than computed

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tomography for clinical staging. Positron emission tomography has been used but is not recommended as its use does not often alter management.[6,7]

Operative Decision Making

Port site recurrence of GBC has been a concern since LC started. A port site recurrence is a harbinger of poor prognosis. However, wound recurrence also occurs in OC. A Swedish registry study from 2002 showed a 6.5% wound recurrence in OC with 75% of these representing T3 or T4 disease. Median survival after wound recurrence was 10 months.[8] Other studies have shown that patients who have wound or port site recurrence have additional metastatic disease in 89% to 100% of cases.[9,10] Wound recurrence, therefore, represents M1 disease and resection will not improve survival. Whether prophylactic port site resection will prevent recurrence is unknown. What we do know is resection creates wounds that are difficult to manage and often slow to heal. We will occasionally perform port site resection in patients who have had known spillage during laparoscopic cholecystectomy since these patients have a known increased risk of port site recurrence.[11] However, on the basis of available data, we no longer perform routine prophylactic port site resection.

Several studies have showed that cholecystectomy is the procedure of choice for Tis and T1a disease. LC alone for this stage provides a 100% 5-year survival in most studies. However, some conflict exists over T1b disease. While some studies show that cholecystectomy alone is adequate, there are now six case series in which cholecystectomy only, for T1b disease, was associated with decreased survival.[12–17] As these studies suggest that T1b represents a higher risk lesion, we perform reresection in healthy patients with T1b disease.

T2 disease warrants reresection. During LC, operative dissection is in the subserosal plane of the perimuscular connective tissue. This violates the tissue plane infiltrated by T2 tumors. In these patients, 57% will have residual disease, 30% will have nodal disease, and 16% will have metastatic disease.[14,18] For this reason, we start all cases with exploratory laparoscopy to assess for occult metastatic disease. Operative conduct during reresection must include assessing for metastasis, and if none are found, resection of residual disease and clearance of nodal drainage. Shirai has definitively demonstrated an improvement in long-term survival for patients who undergo reresection after cholecystectomy for T2 disease (90% if reresected vs 40.5% if cholecystectomy alone) and other studies have agreed with this finding.[3,5,18–20]

Another important consideration is to determine the extent of resection. Resection strategies vary from right hepatic lobectomy, en bloc bisegmentectomy of only segments IVb and V, to wedge resection of only the gallbladder bed. Evidence supports that the type of resection performed is not critical as long as it results in an R0 resection.[14,21] Independent of the extent of hepatic resection, if the cystic duct margin is positive on the LC specimen, resection of the bile duct should be performed as there is a 42% chance that the patient will have residual disease. Bile duct resection will also facilitate lymphadenectomy and may make operative conduct safer. However, routine resection of the bile duct has not been shown to improve median 5- or 10-year survival.[5,14,21]

In summary, prognosis is not degraded with an incidental diagnosis of GBC during the course of LC. The available evidence supports definitive reresection to achieve RO margins in patients who have T1b or T2 disease. This will improve the long-term survival of the patient. Unfortunately, a recent review of the SEER database shows that radical reresection for T2 GBC disease is not being performed in the majority of patients in the United States.[22]

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Treatment of T3 Gallbladder Cancer

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Abstract Until recently, advanced stage gallbladder cancer has been viewed with great pessimism and many patients abandoned as without potentially curative treatment option. Recent results have significantly improved due to a number of advances. Improvements in radiologic staging including positron emission tomography now allow selection of patients with disease treatable by local regional resection. With improvements in surgical and anesthetic techniques, aggressive surgery has proven T3 and T4 tumors to be resectable with safety and result in long-term survival.

Keywords T3 gallbladder cancer · Tumor · Surgery · Radiologic staging

Introduction

T3 gallbladder cancer comprises those tumors that perforate the serosa (visceral peritoneum) of the gallbladder and may invade adjacent organs including the surrounding liver. Once the tumor penetrates the muscularis layer of the gallbladder, tumor cells have access to the lymphatics. Tumors growing through the serosa also have a high propensity for peritoneal dissemination. In a recent series, cases of T3 gallbladder cancer were found to have lymphatic metastases in 58% of patients, and peritoneal metastases in 42%.[1] This would explain the low (27%) likelihood of surgical resection for all T3 tumors encountered. Thus, one of the challenges is identification of disseminated disease both preoperatively and by minimally invasive methods to avoid the morbidity of laparotomy for those with unresectable disease.

Improvements in Tumor Staging

Preoperative Imaging Cross-sectional imaging has improved tremendously over the last decade to allow definitive diagnosis of a majority of cases of unresectable disease. With a good computed tomography angiogram, invasion of the perihepatic vasculature can be well defined. Not only can the portal venous involvement by tumor be seen but involvement of the hepatic arteries can also be noted. Of particular importance is the status of the right hepatic artery, which passes behind the common bile duct in the region of the neck of the gallbladder. Patients who

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are jaundiced from a biliary obstruction at this level are highly likely to have invasion of the hepatic artery. The reason this is important is that jaundiced patients tolerate hypoxia poorly. Thus, in patients with clear right hepatic arterial involvement, a right hepatic lobectomy may be necessary in order to achieve an R0 resection. Alternatively, a preoperative biliary drainage to relieve jaundice may be prudent to improve safety of the subsequent resection.

Magnetic Resonance Cholangiopancreatography This technique may also be useful in the patient who is jaundiced in helping define the level of biliary obstruction. We have found, however, that this test is most useful before any biliary drainage, when the bile ducts are very dilated. With improvements in multidetector computer tomography units in the last decade that now allow very precise assessment of liver, vascular, and even peritoneal involvement by tumor, the need for the more expensive magnetic resonance scanning has greatly diminished.

Fluorodeoxyglucose Positron Emission Tomography This type of scanning has evolved to become an important test in the management of gallbladder cancer. It is capable of confirming lymphatic metastases in this population of patients with high likelihood of such metastases. It is also capable of identification of peritoneal disease, including laparoscopic port involvement.[2] In a recent series of 126 patients with biliary or gallbladder cancers, 24% of PET scans performed as preoperative staging influenced therapy.[2]

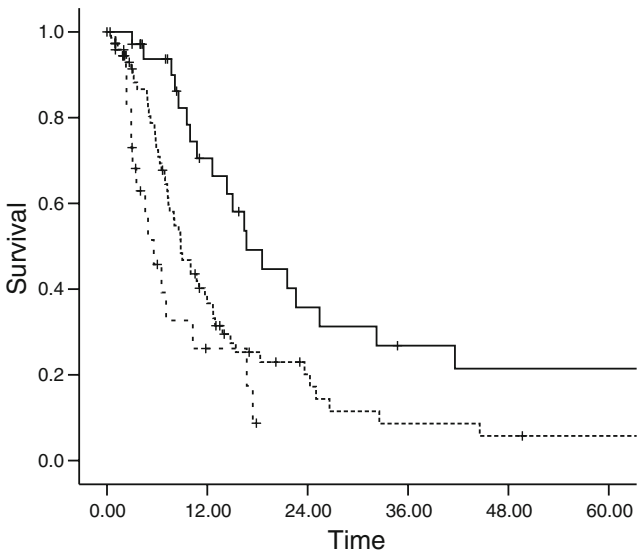

Figure 1 Survival of 137 patients with T3 gallbladder cancer. Survival for those treated with radical resection (n=35; *solid line*), with cholecystectomy (n=74; *dashed line*), and with only biopsy (n=24; *long dashed lines*) are shown. The 5-year survival rates were 0%, 5%, and 21%, respectively. P<0.0001.

Table 1 Results of Surgical Management of Advanced Gallbladder Cancer

Author	Year	Parameter (N)	Survival (%)				
			Median	1 year	2 years	3 years	5 years
Kohya[5]	2008	29	13	50	32	17	17
Coburn[3]	2008	71	19	60	45	42	20
Reddy[6]	2007	12	38	58	50	50	15
Fong[1]	2000	36	17	71	49	27	21

For T3 or T4 gallbladder cancer, we now consider fluorodeoxyglucose positron emission tomography an important staging tool in patient selection for radical surgery.

Extent of Surgery

Extent of Liver Resection By definition, T3 cancers transgress the gallbladder serosa. The cystic plate is the gallbladder serosa on the liver side. Thus, the minimal resection necessary is the gallbladder fossa, consisting of segments 4B and 5 of the liver. There are times that a bigger liver resection is necessary. The most clear-cut case is involvement of the vasculature of the right lobe, most commonly the right hepatic artery. In patients who have had a recent exploration and cholecystectomy for presumed cholelithiasis, a right hepatic lobectomy may also be necessary because the recent surgery may make it difficult to distinguish tumor from scars. This approach is supported by data. Figure 1 demonstrates the outcome for 123 patients with T3 gallbladder cancer treated either with no resection, simple cholecystectomy, or radical resection. All patients without surgical resection died by 18 months with a median survival of 6 months. The 5-year survival of patients subjected to simple cholecystectomy was 5%, while that for radical resection was 22%.

Extent of Lymph Node Dissection The likelihood of lymphatic dissemination is 58%[1] for a T3 gallbladder cancer. Therefore, from a theoretical standpoint, resection of the perihepatic lymph nodes is not only important for staging but also possibly important as therapy. This has been borne out by data. Recently, Coburn et al. examined surveillance, epidemiology, and end results data and analyzed the difference in outcome for the 1,114 patients not treated with a radical lymph node dissection compared with the 71 with documented lymphadenectomy. There was a survival advantage for those treated with lymphadenectomy.[3]

Recommendation In cases of T3 gallbladder cancer, the minimal operation with curative intent is cholecystectomy with segments 4B and 5 resections and a portal lymphadenectomy. For those with prior cholecystectomy, a right hepatic lobectomy

and additional resection of port sites may be necessary because of the high risk of port implantation of tumor.[4]

Outcomes

The perioperative mortality for such radical resections has greatly improved over the last two decades. Most recent series report an operative mortality less than 5%.

The long-term outcome of patients after radical resection clearly is superior to those treated with simple cholecystectomy or no surgery.[5] Nevertheless, it is sobering that recent data still demonstrate that long-term survival and cure only occur in a minority of patients even after radical resection (Table 1). Only 20% seem to be long-term survivors. This is partly due to the resistance of these tumors to chemotherapy and radiotherapy. The search for effective adjuvant systemic and biologic therapies is, therefore, the most important issue in further improvements of outcome for patients afflicted with this dismal disease.

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2008 SSAT QUICKSHOT PRESENTATION

Downregulation of Adiponectin/AdipoR2 is Associated with Steatohepatitis in Obese Mice

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Abstract
Background Recent evidence suggests that obesity is associated with hypo-adiponectinmia and chronic inflammation. Adiponectin regulates fat storage, energy expenditure, and inflammation. We propose that high fat diet induces steatohepatitis, reduces serum adiponectin, and liver adiponectin receptors.
Methods A 4-week-old C57BL male mice were fed high fat diet (n=8) or regular chow (control; n=6) for 7 weeks. Body weight, liver weight, and serum adiponectin were measured. Liver sections were stained with hematoxylin and eosin and oil red for fat content. Liver homogenates were used for protein (immunoblotting) and mRNA (reverse transcription PCR) of Toll-like receptor 4 (TLR4), tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, sterol regulatory element-binding proteins (SREBP)-1c, and adiponectin receptors (AdipoR1/AdipoR2) in addition to nuclear phorsphorylated p65NF-κB. Gels were quantified using densitometry; t test was used, and $p<0.05$ was significant.
Results High fat diet increased body (50%) and liver weight (33%), as well as hepatocyte fat content and ballooning. Mice fed high fat diet exhibited reduced serum adiponectin and liver AdipoR2. High fat diet increased hepatic levels of SREBP-1c, TLR4, TNF-α, and IL-6 protein and mRNA and increased activation of p65NF-κB.
Conclusions Diet-induced liver steatosis is associated with increased lipogensis, upregulation of pro-inflammatory cytokines, and transcription factors as well as downregulation of AdipoR2. Reduction in serum adiponectin suggests that adiponectin signaling may be the crosslink between high fat diet, hepatic inflammation, and nonalcoholic fatty liver disease.

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Keywords Adiponectin receptor · Steatohepatitis · Lipogensis · Inflammation · Obesity

Introduction

The prevalence of obesity has increased dramatically in recent years. Obesity is the most important factor contributing to insulin resistance,[1] diabetes,[2] nonalcoholic fatty liver disease (NAFLD),[3] and cardiovascular disease. Adipokines that originate in adipose tissue play a key role in glucose and lipid metabolism; specifically, adiponectin serum levels are decreased in obesity;[4–6] whereas increasing adiponectin levels reversed obesity-induced insulin resistance in mice via activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR)-α.[7,8] In addition, adiponectin exhibits anti-inflammatory properties by inhibiting pro-inflammatory transcriptional factors such as nuclear factor κB (NF-κB) and by upregulating anti-inflammatory transcriptional factors such as PPAR-γ.[7]

Adiponectin receptors AdipoR1 and AdipoR2 are ubiquitous; disruption of AdipoR1 abrogates adiponectin-induced AMPK activation,[8] whereas that of AdipoR2 decreases PPAR-α activity. Both AdipoR1 and AdiopR2 play important roles in the regulation of glucose and lipid metabolism, inflammation, and oxidative stress in mice livers.[9]

Ligand-promoted (TLR4 and NF-κB) signaling has been recognized to be the most important inflammatory pathway; recent reports demonstrate that deletion of TLR4 can prevent obesity-induced nonalcoholic steatohepatitis (NASH) and improves insulin sensitivity in mice.[10]

We hypothesize that high fat diet (HFD) induces steatohepatitis, reduces adiponectin, and its receptors and upregulates TLR4–NF-κB–TNF pro-inflammatory signaling pathways.

Materials and Methods

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

High fat diet Four-week-old c57BL male mice were maintained in light- and temperature-controlled environments (12-h light/12-h dark, 20–24°C). One group of mice was fed regular (RD) chow containing 5% fat by weight; another group was fed HFD (Harlan) containing 40% fat for 7 weeks. Subsequently, animals were scarified, body weight and liver weight were measured, and tissues and blood samples were also harvested.

Reverse Transcription–Polymerase Chain Reaction Briefly, total cells mRNA was isolated by Trizol solution (Invitrogen, Carlsbad, CA). One to five micrograms of RNA was primed using oligo (dT) (Gibco, Gaithersburg, MD) and subsequently reverse transcribed (Gibco, Gaithersburg, MD). Complementary DNA production was amplified in the presence of mouse-specific TLR4, IL-6,TNF-α, SREBP-1c, AdipoR1, AdipoR2, and β-microglobulin (βMG) primers for 25 cycles of PCR in a UNO-Thermo block (Biometra, Tampa, FL). The sequences for primers were as follows: TLR4, sense 3′ACCTGGCTGGTTTACACGTC3′ and antisense 5′CAGGCTGTTTGTTCCCAAAT3′; TNF-α, sense 5′GGCAGGTCTACTTTGGAGTCATTGC3′ and antisense 5′ACATTCGAGGCTCCAGTGATTTCGG 3′; IL-6, sense TTGCCGAGTAGACCTCATAGTTGACC and antisense CAAGAGACTTCCAGCCAGTTGC; AdipoR1, sense 5′CTGGGAATCTTGACGATGCTG3′ and antisense 5′CGAAGCTCCCCATAATCAGT3′; AdipoR2, sense 5′GGCTTTATTATTCTTTCTACTG3′SREBP1c: sense 5′AGAATCTCCTGGTGACAATGCTTATT3′ and antisense 5′AAGCGGATGTAGTCGATGG3′; and βMG, sense 5′CTCCCCAAATTCAAGTGTACTCTCG3′ and antisense 5′GAGTGACGTGTTTAACTCT-GCAAGC3′. The PCR products were separated with electrophoresis in 2% agarose gel and photographed digitally (UVP, GDS 8000 Upland, CA) and quantified by densitometry.

Immunoblotting Cells were lysed in RIPA buffer [phosphate-buffered saline (PBS) with 0.1% sodium dodecyl sulfate (SDS), 1% NP40, 0.5% sodium deoxycholate]; 50–100 μg samples of protein was fractionated by 10% SDS polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, blocked for 1 h with PBS (5% instant non-fat dry milk; 0.1% Tween-20), then incubated for 2 h with antibodies (0.05 μg/ml) to either TLR4 (BD Biosciences, San Diego, CA), TNF-α, SREBP-1c, phosphorylated p65NF-κB, and β-actin (for whole cells protein loading control) and histone 1 (for nuclear extract loading control; Cell Signaling technology, Beverly, MA). Bound primary antibody was detected by incubating with horseradish peroxide goat anti-mouse or anti-rabbit-IgG (0.0125 μg/ml). Membranes were developed using Super Signal (Pierce, Rockford, IL) ECL reagent and quantified by densitometry.

Nuclear translocation of p65NF-κB and nuclear level of 68 KD SREBP-1 Nuclear and cytoplasmic extracts were prepared as described previously.[11] Nuclear phorsphoylated p65 NF-κB levels and 68 KD SREBP-1c were determined by Western blotting; histone 1 was used as nuclear extract loading control.

Immunofluorescent staining for protein co-localization Briefly, formalin-fixed liver sections were de-paraffinized/hydrated with xylene, ethanol, PBS, and treated with 0.1–0.2% trypsin in 0.4% $CaCl_2$ for 1 h and then incubated

with either anti-F4/80 (macrophage marker), AdipoR1 or AdipoR2 antibodies (1:200 in PBS plus 10% normal goat serum) for 2–4 h. The slides were washed with PBS+0.1% Triton X-100, incubated with fluorescent isothiocyanate goat anti-mouse or rabbit IgG and mounted with anti-fade solution containing DAPI. The slides were examined by Nikon microscope, and the images were merged by Image-Pro-Express software (Image Processing Solutions Inc., North Reading, MA).

Enzyme-Linked Immunosorbent Assay IL-6 protein levels were measured by using rat IL-6 enzyme-linked immunosorbent assay (ELISA) kit (Biosource international, Camarillo, CA), as described by the manufacturer (Quantikine, Minneapolis, MN).

Data Analysis All experiments were repeated at least in triplicates. A *t* test was used to compare means; $p<0.05$ was significant. Data are mean±SD.

Results

HFD increased body weight by 50%; similarly, liver wet weight increased by 33%. HFD increased the number of fat droplets and hepatocyte ballooning(data not shown).

SREBP-1c expression The expression of SREBP-1c (68 kDa), one of the key transcriptional factors in glucose and fat metabolism, increased in mice fed HFD (protein, 4,543±37 vs. 2,574±26; mRNA, 3,456±23 vs. 1,528±10; all $p<0.001$; HFD vs RD, Fig. 1).

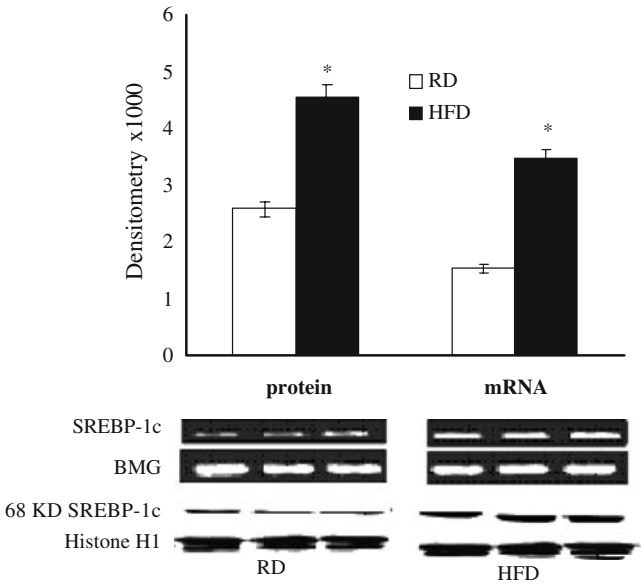

Figure 1 High fat diet (HFD) increased the expression of SREBP-1c in the liver [(*$p<0.001$ vs regular diet (RD)]. Representative gels are shown *below* the bar graph.

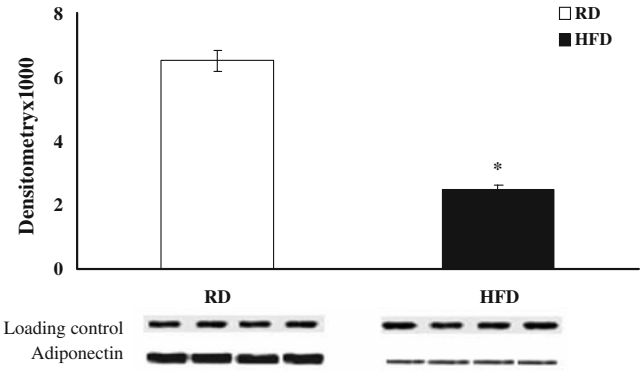

Figure 2 High fat diet significantly decreased serum adiponectin [(*$p<0.001$ vs regular diet (RD)]. Representative gels are shown *below* the bar graph.

Adiponectin and Adiponectin receptors Adiponectin serum levels decreased in mice fed HFD as compared to controls (2,500±20 vs. 6,500±30; $p<0.001$ vs. RD, Fig. 2).

AdipoR2 expression in the liver dramatically decreased in mice fed HFD as compared to control (protein, 1,308±10 vs. 3,045±18; mRNA, 1,981±15 vs. 4,738±20; all $p<0.001$ vs. RD, Fig. 3a); however, AdipoR1 was not changed (data not shown). We confirmed these findings by immunofluorencet staining; HFD decreased immunostaining for AdipoR2 in the liver. In addition, the majority of cells that stained for AdipoR2 stained also for the macrophage marker F4/80, suggesting that Kupffer cells express adiponectin receptors (Fig. 3b).

Pro-inflammatory cytokine and signaling HFD increased the expression of TLR4 (protein, 4,678±35 vs. 2,675±15; mRNA, 6,789±35 vs. 3,458±29; Fig. 4a), the activation of nuclear phorsphorylated p65 NF-κB (5,438±30 vs. 2,560±21; Fig. 4b), the expression of TNF-α (protein, 4,429±35 vs. 2,390±25; mRNA, 3,200±24 vs. 1,301±25; Fig. 4c), and the expression of IL-6 (protein, 2.3±0.1 vs. 1.3±0.1; mRNA, 2,350±24 vs. 1,201±15; Fig. 4d); all $p<0.01$ vs RD.

Discussion

NAFLD is characterized by accumulation of excess lipid in the liver and is associated with obesity and the metabolic syndrome. NAFLD represents a spectrum of histological changes that range from steatosis and steatohepatitis to fibrosis and cirrhosis. The factors implicated in the progression of liver steatosis to fibrosis and cirrhosis are poorly understood. Recent studies emphasize the role of insulin resistance,[3,12] oxidative stress,[13] lipid peroxidation, and inflammatory cytokines[14] in the development of

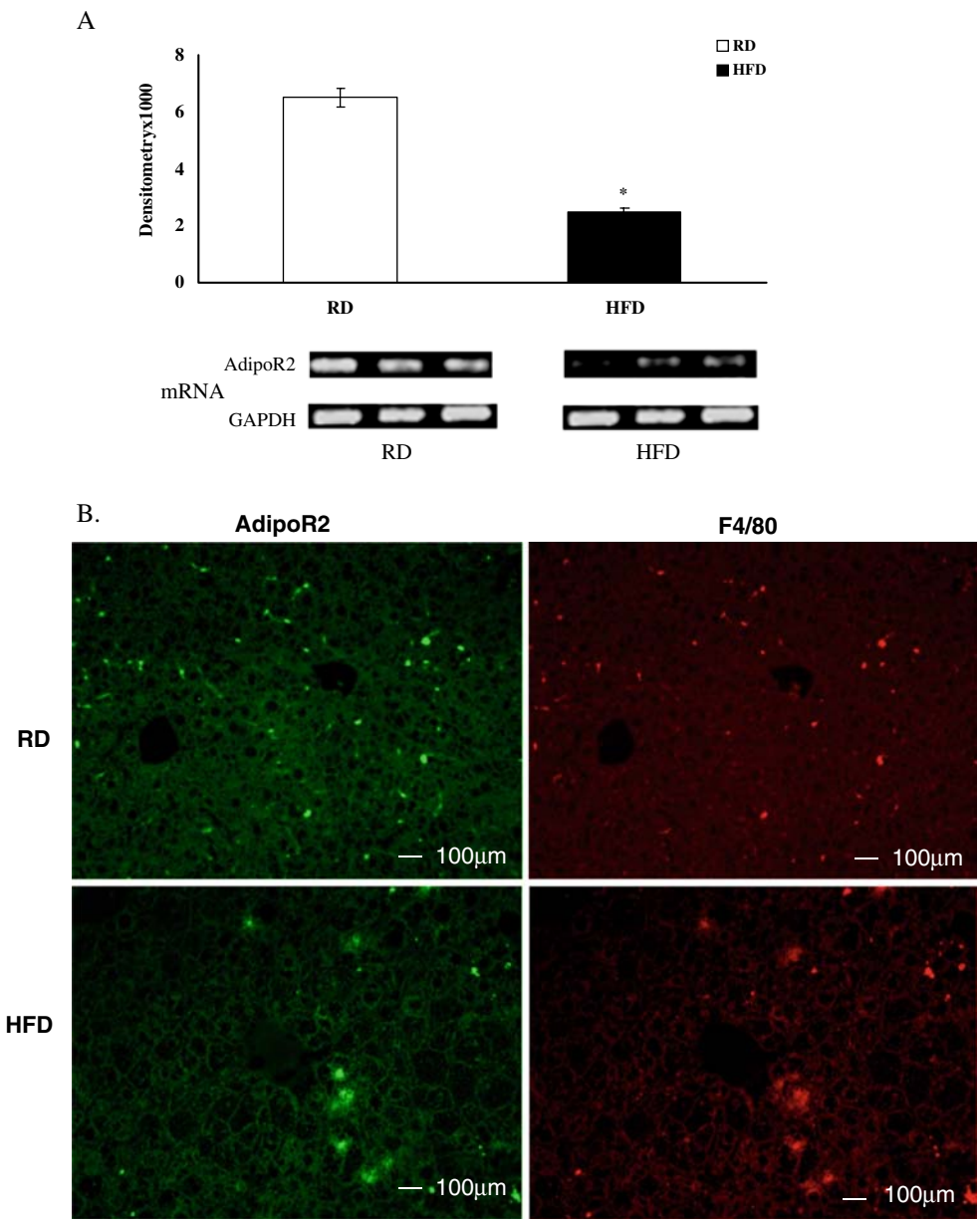

Figure 3 **a** High fat diet (HFD) decreased adiponectin receptor AdipoR2 [*$p<0.001$ vs regular diet (RD)]. Representative gels are shown *below* the bar graph for AdipoR2 mRNA. **b** HFD decreased immunofluorescent staining for AdipoR2 protein in liver sections. *Right panel* images are stained with macrophage marker F4/80, suggesting that adipoR2 is expressed in Kupffer cells in RD and diminished in HFD animals.

steatohepatitis. According to the two hit theory, Kupffer cells play a central role in progression of steatosis to steatohepatitis via production of inflammatory cytokines.[15] The role of Kupffer cells in the pathogenesis of other inflammatory conditions such as acute pancreatitis,[16–18] trauma, and sepsis[19] is well established.

Adiponectin is a protein hormone (30 kDa) secreted by adipocytes and circulates in the serum as three major oligomers: low, medium, and high molecular weight multimers.[7] The link between obesity and adiponectin continues to be explored specially that recent evidence suggests that obesity is associated with hypo-adiponectemia and that surgically induced weight loss increases serum adiponectin in humans.[20,21] Additionally, adiponectin reduces insulin resistance via its receptors AdipoR1 and AdipoR2. Additional signaling between adipose tissue and the liver is important as evidence emerges that low adiponectin levels are associated with steatohepatitis and fibrosis in rodents and humans.[22,23]

More importantly, adiponectin plays a central role in alcohol-induced liver injury in laboratory animals. Chronic ethanol exposure downregulates adiponectin expression, enhances hepatic lipogenesis, and impairs fatty acid oxidation via inhibiting key hepatic transcriptional regulators such as AMPK, sirtuin 1 (SIRT1), PPAR-γ coactivator alpha, PPAR-α, and SREBP-1c. In ethanol-fed mice, resveratrol administration markedly increased circulating adiponectin levels and enhanced mRNA expression of hepatic adiponectin receptors (AdipoR1/R2).[7]

In mice, AdipoR1 is ubiquitously expressed, whereas AdipoR2 is abundantly expressed in the liver. AdipoR2

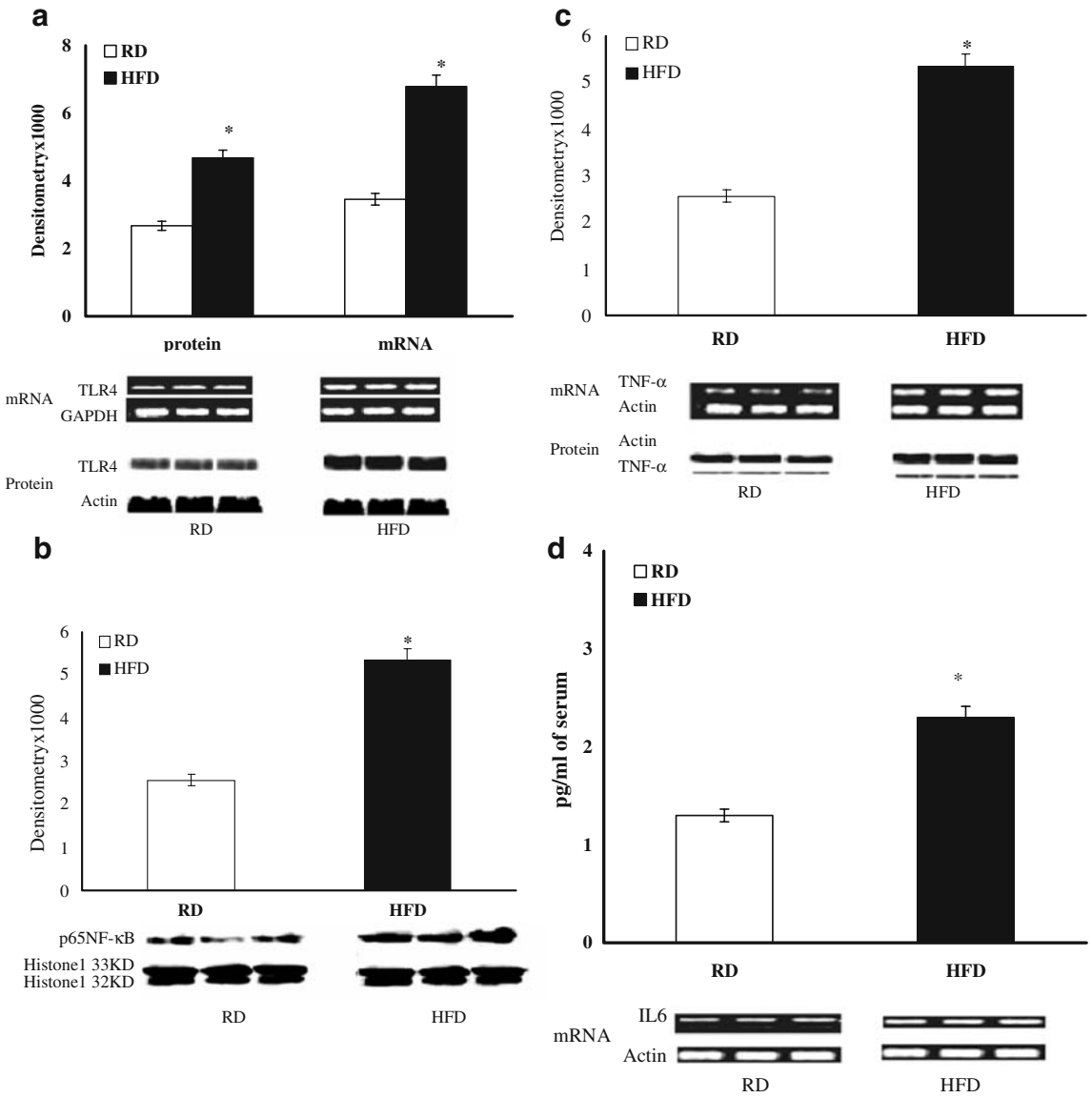

Figure 4 Pro-inflammatory cytokine and signaling: **a** High fat diet (HFD) induced TLR4 expression in livers [all *$p<0.001$ vs regular diet (RD)]. Representative blots are shown below bar graph. **b** High fat diet (HFD) induced activation of p65NF-κB in livers [*$p<0.001$ vs regular diet (RD)]. Representative gels are shown *below* the bar graph. Histone 1 was used as nuclear protein loading control. **c** High fat diet induced TNF-α expression in livers [all *$p<0.001$ vs regular diet (RD)]. Representative gels are shown *below* the bar graph. **d** High fat diet induced IL-6 expression in livers (RT-PCR for mRNA) and in serum (ELISA for protein); all *$p<0.001$ vs regular diet (RD). *Bar* graph shows serum IL-6; representative gels of IL-6 mRNA are shown *below* the bar graph.

serves as a receptor for the globular and full-length adiponectin molecule and mediates fatty acid oxidation, glucose uptake via AMPK, and activates PPAR-α.[7] The C-terminal extracellular domain of adipoR1 and R2 interacts with adiponectin and mediates its cellular effects.[24]

Because of adiponectin's anti-inflammatory and anti-lipogenic properties and because of its role in liver injury, we hypothesized that HFD decreases adiponectin and is associated with upregulation of inflammatory cytokines in the liver as well as the development of steatosis.

In our model, HFD-induced generalized adiposity and liver steatosis. These histological changes were accompanied by upregulation of SREBP-1c, which is a transcriptional factor that regulates lipogenesis and triglyceride synthesis. The active SREBP-1c (68 kDa) induces transcription of key genes such as ACC, FAS, GPTA, and DGAT. Moreover, SREBP-1c is required for the induction of pancreatic beta-cell genes and is positively regulated by insulin and negatively regulated by AMPK. Moreover, SREBP-1c is the predominant isoform in the liver; reduction of SREBP-1C

activity by adiponectin via AMPK leads to decreased lipogenesis in the liver.[8,25–27] Our data confirm these findings; in obese mice, hypoadiponectinemia is associated with increased lipogenic cell signaling and liver steatosis.

Obesity also contributes to the pathogenesis of steatohepatitis by upregulating key inflammatory signaling pathways. We and others have demonstrated that Kupffer cells are the major source of cytokines in the liver during sepsis and acute pancratitis.[16,19] HFD upregulated TNF-α, IL-6, and TLR4 and increased activation of p65NF-κB. This pro-inflammatory profile has common features with the pattern of hepatic cytokine production during acute pancreatitis and sepsis, which originates within Kupffer cells.[28,29]

We confirmed that HFD is associated with reduction of serum adiponection as well as reduction in AdipoR2 in the liver. AdipoR2 localized to cells that stain with the macrophage marker F4/80, suggesting that Kupffer cells may be the link between obesity-induced hypo-adiponectemia and inflammatory changes in the liver. This is an agreement with recent observations of the role of adiponection in alcoholic liver disease[7] and the role of inflammation in obesity-related diseases.[30]

Our finding that AdipoR2 is expressed in Kupffer cells is novel and warrants further investigation. Our findings that HFD induces steatohepatitis are supported by others[24] and by reports that down-regulation of AdipoR2 promotes hepatic inflammation. Moreover, our findings are in agreement with the concept that obesity is associated with hepatic inflammation.[14,21,30,31]

This study has several limitations; although our intervention was to induce obesity, the observational nature of these data cannot be overstated; additionally, further experiments are needed to confirm that the location of AdipoR2 is within Kupffer cells and not in the other non-parenchymal liver cells.

Conclusion

HFD is associated with increase adiposity and lipogenesis in the liver. Reduction of serum adiponectin and its liver receptors is associated with an upregulation of pro-inflammatory cytokine production. Science adiponectin is produced by adipose tissue, and adiponectin R2 is expressed in Kupffer cells, The complex relationship of signaling between peripheral adipose tissue and the liver warrants further studies.

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SSAT POSTER PRESENTATION

Predictive and Prognostic Value of CA 19-9 in Resected Pancreatic Adenocarcinoma

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Abstract
Background Preoperative serum values of CA 19-9 have been reported to be associated with survival in patients undergoing resection of pancreatic adenocarcinoma.
Hypothesis Preoperative CA 19-9 levels are associated with margin and/or lymph node status in patients undergoing pancreatoduodenectomy for pancreatic carcinoma.
Methods We conducted a review of 143 patients undergoing pancreatoduodenectomy for pancreatic adenocarcinoma from July 2001 through April 2006 at our institution. Preoperative serum values of CA 19-9 and total bilirubin, pathologic findings, and survival were analyzed. A cutoff value for CA 19-9 (120 U/ml) was determined using a Cox proportional hazards model for survival.
Results Overall survival at 1, 3, and 5 years for patients with CA 19-9≤120 U/ml was 76%, 41%, and 31%, respectively, versus 64%, 17%, and 10% for patients with CA 19-9>120 U/ml (p=0.002). CA 19-9>120 U/ml was not associated, however, with a greater chance of an R1 or R2 resection (p=0.86), tumor involving the SMA margin (p=0.88), tumor at the portal vein groove (p=0.14), or lymph node metastases (p=0.89).
Conclusions Our findings do not support a cutoff value for CA 19-9 that is associated with margin or lymph node involvement. Preoperative CA 19-9≤120 U/ml is, however, associated with increased overall and recurrence-free survival.

Keywords Pancreatic carcinoma · Pancreatic ductal carcinoma · CA 19-9 antigen · Survival analysis · Predictive value of tests

Introduction

The serum assay of CA 19-9 based on monoclonal antibodies raised against tumor-associated carbohydrate antigen 19-9 was defined originally in the culture medium of a colorectal cancer cell line.[1] Clinically, CA 19-9 is used most often in patients with upper gastrointestinal malignancies, especially pancreatic neoplasms. In patients with pancreatic cancer, CA 19-9 is used typically to follow response to either operative or medical therapy and as a diagnostic adjunct in selected cases or specific conditions.[2,3]

The relationship between increases in CA 19-9 and poor survival is well documented.[4–7] Numerous studies have shown that CA 19-9 is associated not only with resectability but also with tumor stage in pancreatic cancer.[8–10] In fact, Schlieman et al.[8] suggested that an increased CA 19-9 level is predictive of unresectability at the time of exploration even when there is no evidence of unresectability on preoperative imaging studies. Whether an increased CA 19-9 is associated with malignancy present at the operative margins (thus R1 or R2 resections) or with lymph node metastases is not known.

Because CA 19-9 is secreted actively into the biliary system, the specificity of CA 19-9 levels is decreased in the presence of hyperbilirubinemia caused by biliary obstruc-

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tion.[11] Using the adjusted or corrected CA 19-9 level (c-CA19-9) in the presence of hyperbilirubinemia may improve the accuracy and usefulness of the assay. Kang et al.[12] showed that an increased preoperative c-CA 19-9 may predict recurrence.

We hypothesized that preoperative CA 19-9 levels are associated with margin and nodal involvement in patients undergoing pancreatoduodenectomy for pancreatic ductal adenocarcinoma and that c-CA 19-9 improves this predictive capacity. Our aim was to calculate a threshold at which CA 19-9 or c-CA 19-9 would be associated with an R1 or R2 resection, malignant involvement of the margin at the superior mesenteric artery (SMA), presence of tumor at the portal vein groove (this includes patients with tumor at the portal vein groove who had an R1 or R2 resection and those who had an R0 resection by portal vein resection/reconstruction), or lymph node metastases. Additionally, we assessed the association between CA 19-9 and c-CA19-9 with survival and recurrence-free survival.

Methods

The medical records of patients who underwent a pancreatoduodenectomy for ductal adenocarcinoma at the Mayo Clinic in Rochester, MN, USA between July 2001 and April 2006 were reviewed. We analyzed patient demographics, follow-up, type of pancreatic resection, preoperative biliary stenting, use of adjuvant or neoadjuvant therapy, preoperative serum levels of CA 19-9 and total bilirubin, status of the surgical margin, lymph node involvement, T-stage, and tumor size. CA 19-9 levels were measured in our institution's medical laboratory using the Siemens AVIDIA Centaur CA 19-9 Assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Corrected CA 19-9 was calculated by dividing serum CA 19-9 level by serum total bilirubin concentration (mg/dl) from the same specimen.

Surgical margins were assessed initially by intra-operative frozen section analysis and then by routine permanent section. This approach allowed re-resection to achieve negative margins during the initial operation. Resected specimens underwent pathologic evaluation in accordance with the sixth edition of the American Joint Committee on Cancer guidelines. The surgical margins evaluated routinely included the proximal common hepatic duct, pancreatic neck, margin at the uncinate process or the SMA, posterior, inferior, and superior (soft tissue) pancreatic margin, portal vein groove, and proximal duodenal margin if the patient was undergoing a pylorus-preserving resection.

Survival and recurrence were reviewed primarily via information from the medical record and our cancer registry. The Accurint system, a commercially available database that contains more than 20 billion records from 400 sources and includes dates of death, was used to augment information regarding death.[13] Recurrence was judged to have occurred if specific reference to recurrence was elucidated in the medical record or if there were radiographic findings of recurrence.

There were six outcomes of interest primarily investigated: death, recurrence or death, positive margin at any location, positive SMA margin, tumor at the portal vein groove, and lymph node metastases. Kaplan–Meier curves were constructed for the outcomes of survival and recurrence-free survival, and the associations of covariates with these outcomes were investigated using Cox proportional hazards models. Logistic regression models were constructed for the binary outcomes. Receiver operating characteristics (ROC) curves were constructed and area under the curve (AUC) statistics were calculated to assess the discriminatory ability of CA 19-9 and c-CA 19-9 for malignant involvement at particular margins and lymph node involvement.

Both univariate and multiple-variable models were constructed. Covariates in the multiple-variable model were selected for inclusion via univariate statistical significance and/or clinical judgment. When highly confounded covariates, such as total bilirubin and preoperative stent, were being considered, only one of them was included in the multiple-variable model according to clinical judgement.

The prognostic effects of CA 19-9 and c-CA 19-9 on patient survival were studied using a changepoint method[14]. This method is used to find the best cutoff values such that the continuous variables, CA 19-9 and c-CA 19-9, could be dichotomized according to survival. The Cox proportional hazards models were utilized with CA 19-9 or c-CA 19-9 dichotomized at every possible cut point in 60% of the data (training data set) and then validated by the remaining 40% of the data set. The final cutoff values for CA 19-9 (120 U/mL) and c-CA 19-9 (60 U/mL) were chosen at the highest Chi-squared value bracketed at every cut point within four units, rounded to the nearest quartile, and then rounded to the nearest increment of five to avoid overspecification.

SAS v9.1 (SAS Institute, Cary NC, USA) was utilized. All tests were two-sided, and p values less than 0.05 were considered statistically significant.

Results

Patient Characteristics

Both preoperative CA 19-9 and serum total bilirubin were available for 143 of 222 patients reviewed; these 143 patients comprised the study population. The mean age at operation was 65±11 years (range=37–89) with 62% being male. Median follow-up in the 143 patients treating deaths as censored values was 3.3 years (13 days to 5.9 years). Patient characteristics are detailed in Tables 1 and 2.

Table 1 Summary of Patient Characteristics (CA19-9)

Characteristic/feature	All (n=143)	CA 19-9≤120U/ml (n=73)	CA 19-9>120U/ml (n=70)	p value
Age at surgery				
Mean (SD)	65.3 (10.6)	64.2 (10.8)	66.5 (10.5)	0.17
Sex, no. (%)				
M	89 (62)	45 (62)	44 (63)	0.88
Total bilirubin				
Mean (SD)	6.4 (7.6)	5.5 (7.2)	7.4 (8.0)	0.15
Bile duct stenting before surgery, no. (%)				
Not known	2		2	
Yes	103 (73)	51 (70)	52 (77)	0.38
Adjuvant therapy, no. (%)				
Not known	10	3	7	
Yes	109 (82)	57 (81)	52 (83)	0.87
Neoadjuvant therapy, no. (%)				
Yes	9 (6)	5 (7)	4 (6)	0.78

Survival

Overall survival at 1, 3, and 5 years for patients with CA 19-9≤120 U/ml was 76%, 41%, and 31%, respectively, compared to 64%, 17%, and 10% for patients with CA 19-9>120 U/ml (p=0.002; Fig. 1a; Table 3). Similarly, for c-CA 19-9, survival at 1, 3, and 5 years for patients with c-CA 19-9≤60 U/ml was 78%, 37%, and 28%, compared to 60%, 20%, and 11% for patients with c-CA 19-9>60 U/ml (p=0.006; Fig. 1b; Table 3).

Recurrence-free survival at 1, 3, and 5 years for patients with CA 19-9≤120 U/ml was 67%, 36%, and 30% respectively, compared to 52%, 16%, and 16% for patients with CA 19-9>120 U/ml (p=0.007; Fig. 1c; Table 3). Similarly, for c-CA 19-9, recurrence-free survival at 1, 3, and 5 years for patients with c-CA 19-9≤60 U/ml was 64%, 34%, and 29%, compared to 54%, 16%, and 16% for patients with c-CA 19-9>60 U/ml (p=0.02; Fig. 1d; Table 3).

Margin Status

R0 versus R1 or R2 resection One hundred ten (77%) patients underwent R0 resections vs. 32 (23%) who underwent R1 or R2 resections. Despite being associated with a lesser overall and disease-free survival, CA 19-9> 120 U/ml and c-CA 19-9>60 U/ml were not associated with R1/2 resection in univariate logistic regression models (p=0.86 and p=0.63, respectively; Table 4).

Table 2 Summary of Patient Characteristics (c-CA19-9)

Characteristic/feature	All (n=143)	c-CA 19-9≤60U/ml (n=80)	c-CA 19-9>60U/ml (n=63)	p value
Age at surgery				
Mean (SD)	65.3 (10.6)	64.7 (11.5)	65.9 (9.5)	0.49
Sex, no. (%)				
M	89 (62)	51 (64)	38 (60)	0.67
Total bilirubin				
Mean (SD)	6.4 (7.6)	8.4 (8.2)	3.8 (5.9)	0.0003
Bile duct stenting before surgery, no. (%)				
Not known	2	1	1	
Yes	103 (73)	59 (75)	44 (71)	0.62
Adjuvant therapy, no. (%)				
Not known	10	3	7	
Yes	109 (82)	62 (81)	47 (84)	0.61
Neoadjuvant therapy, no. (%)				
Yes	9 (6)	4 (5)	5 (8)	0.47

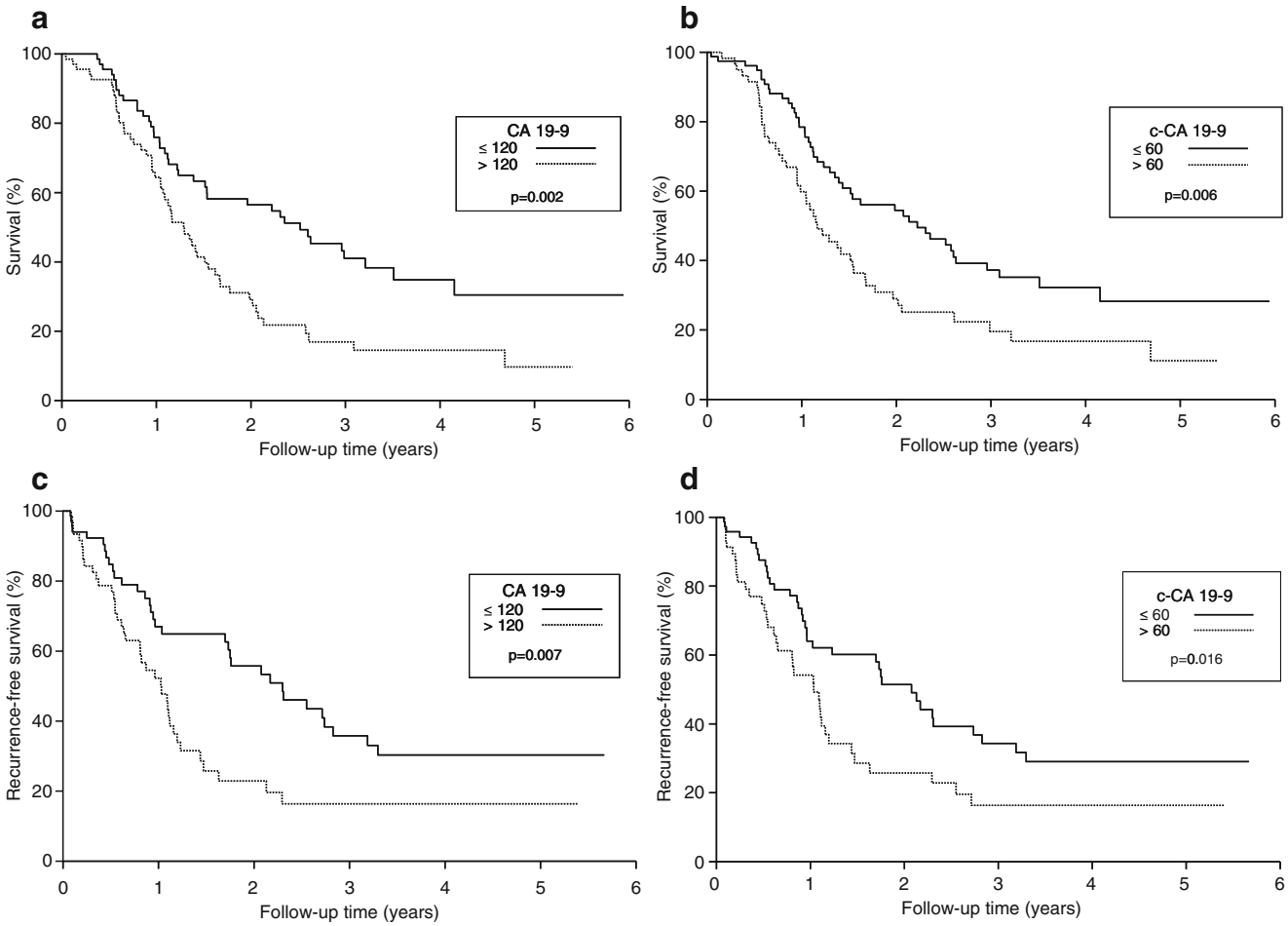

Figure 1 Kaplan–Meier curves depicting overall survival for CA 19-9>120 vs. ≤120 (**a**), overall survival for c-CA 19-9>60 vs. ≤60 (**b**), recurrence-free survival for CA 19-9>120 vs. ≤120 (**c**), and recurrence-free survival for c-CA 19-9>60 vs. ≤60 (**d**).

Tumor at SMA margin One hundred eighteen (83%) patients had a negative SMA margin vs. 24 (17%) with a positive margin (one patient had an unknown status). In univariate logistic regression models, again CA 19-9> 120 U/ml and c-CA 19-9>60 U/ml were not associated with a positive SMA margin (p=0.88 and p=0.77; Table 4).

Tumor at portal vein groove One hundred twenty-one (85%) patients had no tumor present at the portal vein groove vs. 21 (15%) who did have tumor present at the portal vein groove (one patient had an unknown status). Of the 21 patients with tumor at the portal groove margin, 14 (67%) underwent portal vein resection and reconstruction, thereby achieving an R0 resection in eight patients. In univariate logistic regression models, again CA 19-9> 120 U/ml and c-CA 19-9>60 U/ml were not associated with tumor at the portal vein groove (p=0.14 and p=0.88; Table 4).

Lymph Node Status

Fifty-eight (41%) patients did not have any positive lymph nodes vs. 85 (59%) with positive lymph nodes. In univariate logistic regression models, CA 19-9>120 U/ml and c-CA 19-–9>60 U/ml were not associated with positive lymph nodes (p=0.89 and p=0.85; Table 4).

T Classification

Forty-three patients (30%) had T1 or T2 lesions vs. 100 (70%) with T3 lesions. In univariate logistic regression models, CA 19-9>120 U/ml and c-CA 19-9>60 U/ml were not associated with T3 lesions (p=0.46 and p=0.98; Table 4).

Mass Size

The mean mass size was 34±12 mm. In univariate linear regression models, CA 19-9>120 U/ml was associated with an

Table 3 Cox Proportional Hazards Models for Survival and Recurrence

Variable	Hazard ratio	95% CI for hazard ratio	*p* value
Survival			
Age (per 10 years)	1.1	(0.9–1.3)	0.33
Male sex	1.0	(0.7–1.5)	0.99
T-stage (1/2 vs. 3)	1.5	(0.9–2.4)	0.11
Mass size (per 10 mm)	1.2	(1.0–1.4)	0.02
Positive nodes	1.5	(1.0–2.3)	0.07
Positive margin	2.0	(1.2–3.1)	0.004
Positive SMA margin	1.7	(1.0–2.8)	0.04
Tumor at portal vein groove	1.3	(0.8–2.4)	0.32
CA 19-9 (≤120 vs. >120)	2.0	(1.3–3.0)	0.002
c-CA 19-9 (≤60 vs. >60)	1.8	(1.1–2.7)	0.006
Recurrence			
Age (per 10 years)	1.0	(0.8–1.3)	0.68
Male sex	0.8	(0.5–1.3)	0.41
T-stage (1/2 vs. 3)	1.6	(0.9–2.8)	0.08
Mass size (per 10 mm)	1.2	(1.0–1.5)	0.03
Positive nodes	1.5	(0.9–2.5)	0.08
Positive margin	2.2	(1.3–3.7)	0.006
Positive SMA margin	1.9	(1.0–3.4)	0.04
Tumor at portal vein groove	1.6	(0.8–2.9)	0.15
CA 19-9 (≤120 vs. >120)	1.9	(1.2–3.1)	0.007
c-CA 19-9 (≤60 vs. >60)	1.8	(1.1–2.8)	0.02

increase in mass size (mean=4 mm, 95% confidence interval 0.1 to 8.0 mm, $p=0.05$). Corrected CA 19-9>60 U/ml, however, was not associated with increasing mass size ($p=0.16$).

Receiver Operating Characteristic Curves

ROC curves were constructed and AUC were calculated to evaluate the capability of CA 19-9 and c-CA 19-9 to predict specific pathologic variables outside of the constraints imposed by the cutoff values of 120 U/ml for CA 19-9 and 60 U/ml for C-CA 19-9 (Fig. 2). CA 19-9 and c-CA 19-9 as continuous variables were not associated with presence of tumor at the SMA margin, tumor at the portal vein groove, R0 resection, or positive lymph nodes ($p\geq 0.15$). In each curve, no cut point was found that provided sensitivity and specificity greater than 60%.

Multivariate Analysis

Survival and Recurrence-Free Survival

CA 19-9 and c-CA 19-9 were analyzed independently in multivariable models for survival and recurrence-free survivals after adjusting for mass size, overall margins, and positive nodes. After adjusting for these covariates, CA 19-9>120 U/ml was associated with both death (HR=1.8, $p=0.01$) and recurrence or death (HR=1.9, $p=0.02$); c-CA 19-9>60 U/ml was also associated with both death ($p=1.8$, $p=0.007$) and recurrence or death (HR=1.8, $p=0.02$).

Margins, Mass Size, and Lymph Node Ratio

After adjusting for positive lymph nodes and an R0 versus R1 or R2 resection, the mass size in patients with CA 19-9> 120 U/ml was an average of 4 mm greater than the mass size in patients with CA 19-9≤120 U/ml ($p=0.03$). After adjusting for T classification and positive lymph nodes, however, CA 19-9>120 U/ml was not associated with an R0 resection versus R1 or R2 ($p=0.96$). After adjusting for mass size, c-CA 19-9>60 U/ml was not associated with lymph node ratio ($p=0.09$).

Analysis for Preoperative Stenting

Due to the association between increased total serum bilirubin concentrations, CA 19-9, and the effect of preoperative bile duct stenting, the relationship between stenting and CA 19-9 and c-CA 19-9 was assessed. In our patient population, neither CA 19-9>120 U/ml nor c-CA 19-9> 60 U/ml was associated with stenting ($p\geq 0.38$).

Table 4 Cox Proportional Hazards Models for Pathology Findings

Variable	Odds ratio	95% CI for odds ratio	*p* value
R0 versus R1 or R2			
T-stage (1/2 vs. 3)	5.5	(1.6–19.3)	0.007
Mass size (per 10 mm)	2.1	(1.4–3.1)	0.0001
Positive nodes	2.5	(1.0–6.1)	0.04
CA 19-9 (≤120 vs. >120)	1.1	(0.5–2.4)	0.86
Adjusted CA 19-9 (£60 vs. >60)	0.8	(0.4–1.8)	0.63
Tumor at SMA margin			
T-stage (1/2 vs. 3)	5.9	(1.3–26.2)	0.02
Mass size (per 10 mm)	1.9	(1.3–2.7)	0.002
Positive nodes	3.1	(1.1–8.9)	0.03
Tumor at portal vein groove	3.1	(1.1–8.7)	0.04
CA 19-9 (≤120 vs. >120)	1.1	(0.4–2.6)	0.88
Adjusted CA 19-9 (£60 vs. >60)	0.9	(0.4–2.1)	0.77
Tumor at portal vein groove			
T-stage (1/2 vs. 3)	3.0	(0.8–10.7)	0.10
Mass size (per 10 mm)	1.4	(1.0–2.1)	0.06
Positive nodes	1.5	(0.5–3.9)	0.45
SMA margin	3.1	(1.1–8.7)	0.04
CA 19-9 (≤120 vs. >120)	0.5	(0.2–1.3)	0.14
Adjusted CA 19-9 (£60 vs. >60)	0.9	(0.4–2.4)	0.88
Lymph node status			
T-stage (1/2 vs. 3)	1.9	(0.9–3.8)	0.09
Mass size (per 10 mm)	1.5	(1.1–2.0)	0.005
Margin	2.5	(1.0–6.1)	0.04
SMA margin	3.1	(1.1–8.9)	0.03
Tumor at portal vein groove	1.5	(0.5–3.9)	0.45
CA 19-9 (≤120 vs.>120)	1.0	(0.5–2.0)	0.89
Adjusted CA 19-9 (£60 vs. >60)	1.1	(0.5–2.1)	0.85
T classification			
Mass size (per 10 mm)	1.4	(1.0–1.9)	0.028
Positive nodes	1.9	(0.9–3.8)	0.09
Margin	5.5	(1.6–19.3)	0.007
SMA margin	5.9	(1.3–26.2)	0.02
Tumor at portal vein groove	3.0	(0.8–10.7)	0.10
CA 19-9 (≤120 vs. >120)	1.3	(0.6–2.7)	0.46
Adjusted CA 19-9 (£60 vs. >60)	1.0	(0.5–2.0)	0.98

Analysis on Patients with Total Bilirubin<2 mg/dl

There were 59 (41%) patients with a total serum bilirubin< 2 mg/dl. The median survival of these patients was 1.7 years and median recurrence-free survival was 1.2 years. In this subset, CA 19-9>120 U/ml was associated with recurrence or death (p=0.02), and patients with CA 19-9>120 U/ml had an average mass size of 6 mm greater than patients with CA 19-9≤120 U/ml (p=0.04). The association between CA 19-9>120 U/ml and survival approached significance (p= 0.06). CA 19-9>120 U/ml was not associated with an R1 or R2 resection on univariate analysis (p=0.26), involvement of the SMA margin (p=0.45), tumor at the portal vein groove (p=0.78), T3 lesions (p=0.23), or lymph node metastases (p=0.10).

Discussion

Our findings do not support a cutoff value for either serum CA 19-9 or c-CA 19-9 that is associated with unresectability for cure because of specific pathologic findings of any positive margin (R1 or R2 resection), a positive SMA margin, tumor present at the portal vein groove, or positive lymph nodes.

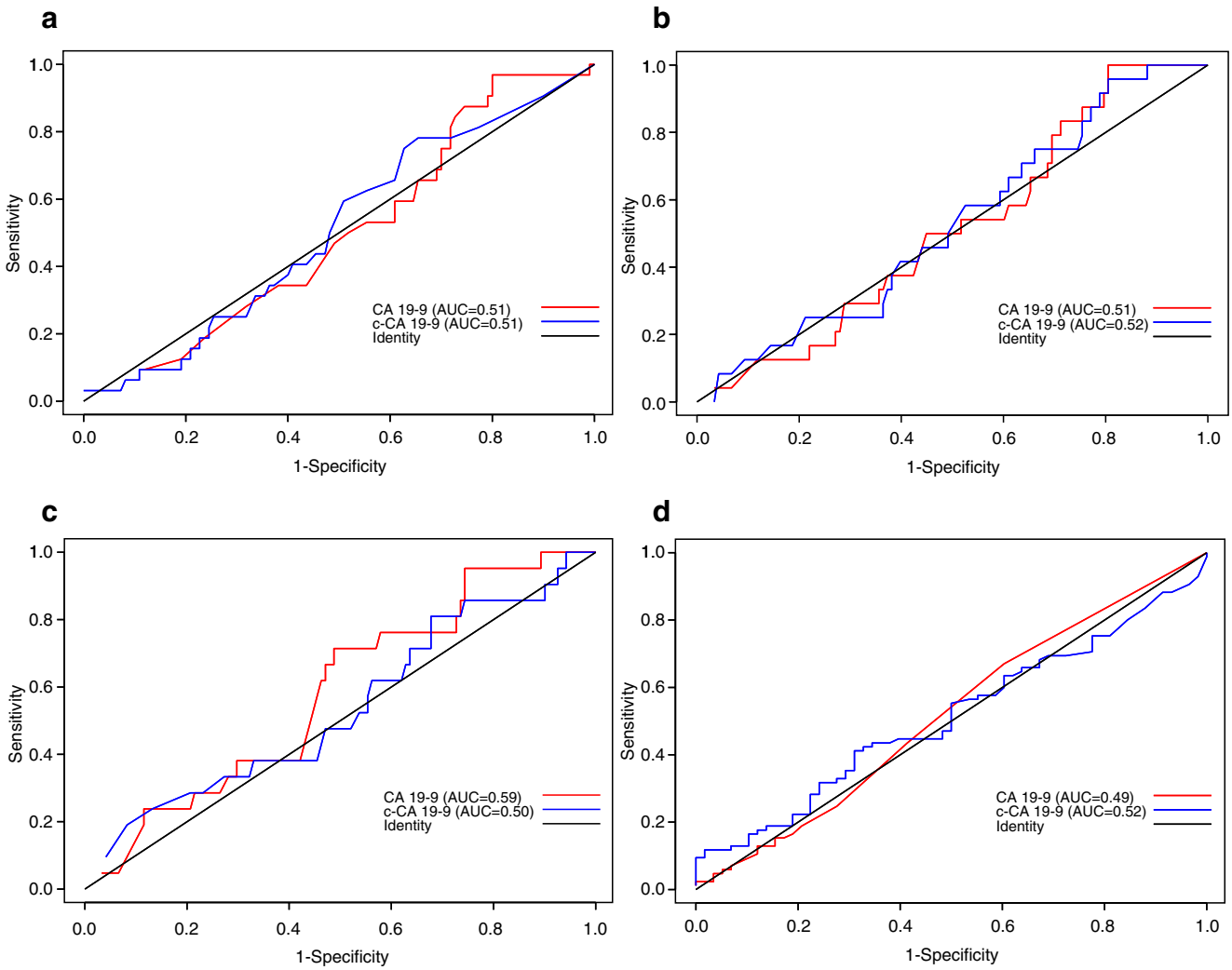

Figure 2 Receiver operating characteristic curves depicting the ability of CA 19-9 and c-CA 19-9 to predict a positive margin at any location (**a**), positive SMA margin (**b**), tumor at the portal vein groove (**c**), and lymph node metastases (**d**).

Furthermore, when analyzed as continuous variables via ROC curves, neither CA 19-9 nor c-CA 19-9 appears to have any other cutoff values that are predictive of resectability for cure. These observations persist despite reaffirming the association between preoperative CA 19-9 and c-CA 19-9 for both survival and recurrence-free survival demonstrated in previous studies. These findings are especially interesting given that both lymph node involvement and margin status, like CA 19-9, are known to be predictors of survival in pancreatic cancer patients.[15,16] CA 19-9>120 U/ml was associated with an increased mass size on univariate and multivariable analysis. Similar to CA 19-9, correcting CA 19-9 does not appear, unfortunately, to produce a significant association with any specific pathologic parameter indicative of unresectability for cure.

There is an emerging role for preoperative neoadjuvant radiation and chemotherapy in borderline resectable pancreatic cancer. In the experience of the MD Anderson Cancer Center, Varadhachary et al.[17] suggested that "patients with favorable responses to preoperative therapy (radiographic evidence of tumor regression and improvement in serum tumor marker levels) are the subset of patients with the best chance for a potential R0 resection and a favorable long-term outcome." Because the method used by Varadhachary et al. selects patients most likely to benefit from neoadjuvant treatment, not only can a non-curative resection and its obligate recovery and morbidity be avoided, but the overall survivability of patients with resected pancreatic cancer might improve.

In our study, when present, the positive margin was located at either the SMA margin and/or portal vein groove in 93% of patients. The ability to control the SMA margin is definitively limited by the SMA itself. Obtaining a margin free of malignancy at the portal vein groove is usually limited by the feasibility and morbidity of portal vein resection and reconstruction. If evidence for borderline resectability as

described by Varadhachary et al. could be augmented by a serum marker that predicts a positive margin (especially the SMA and portal vein groove margins) after an otherwise "curative resection" and even lymph node metastases, the indications for neoadjuvant therapy could be broadened beyond its current use in patients with findings of borderline resectability on imaging only. Unfortunately, CA 19-9 does not accomplish this potentially important goal when looking at the subset of patients with positive SMA margins or even the subset of patients with tumor present at the portal vein groove regardless of R0 resection by portal vein resection and reconstruction. While there was an association between CA 19-9 and tumor size, this association, however, adds little to information obtained typically by computed tomography or magnetic resonance imaging.

Our study has several limitations. First, different CA 19-9 assays, of which there are many, are known to produce quite disparate values at high serum CA 19-9 concentrations. Therefore, because our focus has been on CA 19-9 levels well above normal values, the cutoff values found in our study may not be applicable in institutions using a CA 19-9 assay other than the Siemens Diagnostics AVIDIA Centaur product. Also, preoperative biliary stenting was used in 73% of the patients in this study. This figure reflects the referral pattern inherent at our institution, is not our preferred approach to the patient[18] with mild to moderate jaundice (serum bilirubin≤15 mg/dl), and was not used in any formalized fashion. Such stenting undoubtedly affected our data, given the effect of biliary stenting on total serum bilirubin and, hence, on serum CA 19-9 levels. We could not control this effect because of the retrospective nature of our study. There is, however, no demonstrable association between CA 19-9 and the presence of a biliary stent in our study.

Several studies analyzing CA 19-9 have focused only on patients with normal or near-normal total serum bilirubin levels. Concern remains, however, that despite efforts to correct or adjust serum CA 19-9 levels, CA 19-9 may have a low predictive value in the presence of hyperbilirubinemia. Our subset analysis of patients with total serum bilirubin concentrations less than 2 mg/dl does not appear to have improved the association between CA 19-9 and any specific pathologic findings. Furthermore, increased serum CA 19-9 levels were associated with a lesser survival even when considering patients with hyperbilirubinemia. Correcting or adjusting CA 19-9 does little to alter this association.

Conclusion

Our findings do not support the use of CA 19-9 as a predictor of positive margins or lymph node status at resection and, therefore, CA 19-9 does not appear to be able to be used as a direct indicator of borderline resectability. We reaffirmed the strong association between preoperative CA 19-9 and both survival and recurrence-free survival in patients who undergo pancreatoduodenectomy. Although this knowledge can serve as an adjunct in the preoperative decision-making process, CA 19-9 and c-CA 19-9 do not appear to be precise enough to serve as a major or especially the sole decision point either for denying exploration for potential curative resection or for selecting patients for neoadjuvant therapy. While the ability to reliably predict lymph node and margin status preoperatively is desirable, CA 19-9 neither in its absolute nor corrected form appears to be useful for this role.

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A Standardized Technique for Laparoscopic Rectal Resection

Rolv-Ole Lindsetmo · Conor P. Delaney

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Abstract
Introduction Laparoscopic rectal resection (LRR) has not gained the same acceptance as laparoscopic segmental colonic resection because of technical challenges, increased operating time and costs, and concerns about the oncological outcome.
Discussion One way to overcome these challenges is by standardizing the laparoscopic technique in the same way as has been done with the open rectal cancer surgery. We have established a standardized, stepwise laparoscopic procedure for rectal resections that enhances the transformation of laparoscopic skills, identifies indications for conversion early in the operation, and makes the operation predictable and reproducible for the whole surgical team.
Conclusion We believe this saves time in the operating room and builds up laparoscopic team expertise.

Keywords Laparoscopy · Stepwise technique · Standardization · Rectum

Introduction

Laparoscopic surgery for rectal and rectosigmoid pathologies that require transabdominal resection has not gained the same popularity among colorectal surgeons as segmental laparoscopic colon resection. The feasibility and safety of laparoscopic rectal surgery has been documented repeatedly the last 15 years.[1–4] The lower frequency of laparoscopic rectal resections (LRR), with total or partial mesorectal excision (TME and PME), is probably due to the technical challenges of the rectal dissection inside the narrow pelvis and to the fact that only recently have data been published showing that the laparoscopic approach has the same short and long-term results as open surgery for rectosigmoid and rectal cancer.[5–8] By standardizing the laparoscopic technique in the same way as has been done with open rectal cancer surgery, the learning curve might be shortened and the technical challenges easier to overcome without compromising patient safety, operation time, and oncological outcome.[9–12]

We have established a standardized, stepwise laparoscopic procedure for rectal resections that enhances the transformation of laparoscopic skills, identifies indications for conversion early in the operation, and makes the operation predictable and reproducible for the whole surgical team. In this way, laparoscopic team expertise can be built up, and the time in the operating room will be closer to open rectal resections. The conversion rate can be kept below 5%, and patients will have the postoperative advantages of less pain, shorter recovery, and hospital stay as shown for laparoscopic colectomy.[13,14]

The purpose of this paper was to describe the standardized laparoscopic technique for patients undergoing rectal resections.

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Material and Methods

Recommended Equipment

As described previously, instruments can be standardized to reduce costs.[9] We favour the use of atraumatic 5-mm bowel

graspers. Dissection is performed with scissors cautery, but hook cautery may be required, especially at the right lower pelvic sidewall to avoid electrical short-circuiting into the pelvic sidewall. Energy-based devices as Ligasure or Harmonic scalpel are not required as the dissection plane in the pelvis is avascular. Indeed, these instruments can complicate surgery because their sealing properties can easily make a non-bleeding and incorrect plane into the mesorectal tissue. Reusable ports, scissors, graspers, and hook cautery reduce the variable equipment costs related to the operation.

The standardized, stepwise procedure includes the following:

1. Setup and positioning of the patient. The patient is positioned on a bean bag and the legs placed in yellowfin stirrups, with the perineum at the break of the table. An orogastric tube and a Foley catheter are inserted. Both arms are tucked, except if patients are too obese in which case the left arm will be kept out.
2. Port placement (Fig. 1). A 10-mm umbilical port is inserted with open technique. Intra-abdominal pneumoperitoneum pressure is limited to 15 mmHg. A 12-mm port is placed in the right lower quadrant, about 2.5–5 cm medial and superior to the right anterior superior iliac spine, choosing the site to ensure that instruments would reach the low pelvis without being limited by the right pelvic sidewall and pelvic brim. If an ileostomy is being placed, this port can be placed at the ileostomy site. A 5-mm port is placed in the left lower quadrant at the same position as the planned site for specimen extraction and a 5-mm port in the right upper quadrant. The abdominal cavity is inspected for metastatic or concomitant disease.
3. Exposure of the operating field. The patient is placed in steep Trendelenburg position and rotated to the right. The omentum is lifted over the transverse colon and placed in the upper abdomen. Small bowel loops are carefully pushed upwards and to the right, exposing the retroperitoneum from the sacral promontory toward the ligament of Treitz.
4. Identification of the inferior mesenteric artery (IMA) and the left ureter (Fig. 2).The sigmoid mesentery is grasped and drawn anteriorly to demonstrate the groove behind the IMA at the level of the sacral promontory. This maintains the hypogastric nerves, ureters, and gonadal vessels posteriorly behind the congenital peritoneum of the retroperitoneum. A medial to lateral dissection and mobilization of the upper mesorectum and the mesosigmoid are performed and the left ureter identified. In cases where the left ureter cannot be found, a lateral mobilization can be performed, so that it is identified in all cases before division of the IMA.
5. Division of the IMA. In cases of malignancy, a high ligation is performed, 1 cm distally to the origin of the IMA. In cases of benign disease, a low ligation can be performed, preserving the left colic artery. The artery is generally divided by a vascular stapler or clips, keeping the stapler for more obese cases. When a high ligation is performed, the left colic artery is also divided from the IMA to permit adequate mobilization of the descending colon for coloanal anastomosis.
6. Division of the inferior mesenteric vein (IMV) and mobilization of the left colon and splenic flexure. In patients having a high ligation, the IMV is divided at the level of the ligament of Treitz (Fig. 3). The left colon is dissected from the retroperitoneal tissue in the avascular plane anterior to Toldt's fascia. The splenic flexure is mobilized using scissors cautery, generally commencing with the lateral mobilization and then coming medial to enter the lesser sac between the greater omentum and the transverse colon, before completing the dissection to release the flexure.
7. Mobilization and division of the rectum. The mesorectum is dissected by cautery scissors or hook down to the distal resection level. The principle of traction and counter-traction is applied using 5-mm atraumatic forceps. In assisting the dissection in cases with low rectal cancer, a laparoscopic 10-mm fan retractor placed through the left lower quadrant port is useful for the anterior traction of the mesorectum reducing the risk for iatrogenic tearing of the mesorectal fascia and breaking into the mesorectal tissue. In cases with bulky tumors, narrow pelvis, or fatty mesorectum, some authorities favor placing a vessel loop around the

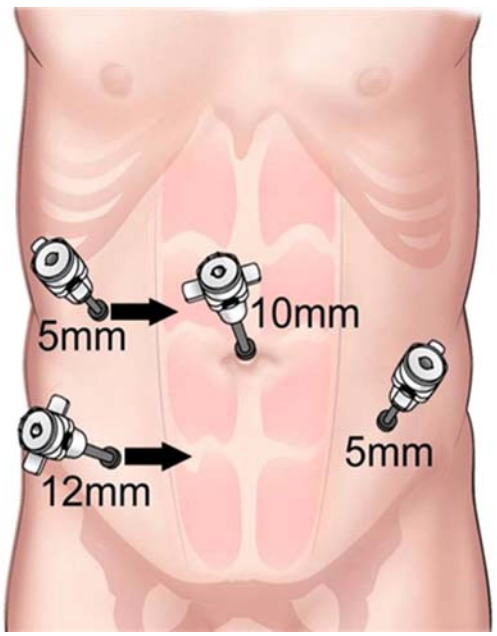

Figure 1 Trochar placement for laparoscopic rectal resections.

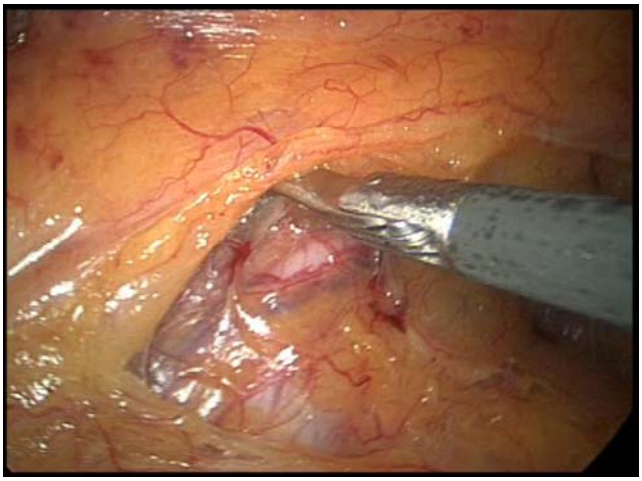

Figure 2 Identification of the inferior mesenteric artery and the left ureter.

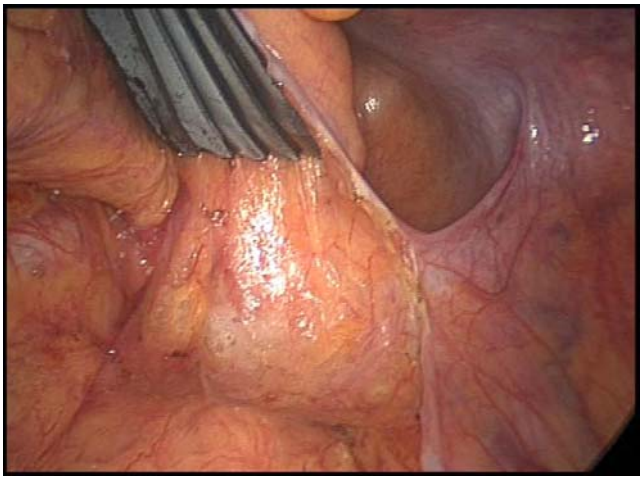

Figure 4 Anterior and superior traction by a fan retractor facilitates sharp dissection down to the pelvic floor.

mesorectal junction for anterior traction. In females, the uterus is elevated using a 0 prolene on a Keith needle through the anterior abdominal wall. The pelvic dissection is divided into three steps:

(a) Posterior dissection. The pneumoperitoneum helps open the avascular, areolar tissue between the mesorectal fascia and the presacral fascia. Anterior and superior traction by the vessel loop method or by gently lifting the mesorectum with an open grasper or fan retractor (Fig. 4), facilitates sharp dissection down to the pelvic floor.

(b) Lateral dissection. The mesorectum is drawn laterally away from the side of dissection using a grasper or the fan retractor and using the rigid pelvic sidewall to give counter-traction. The electrocautery dissection continues with the mesorectal fascia drawn medially. Hook cautery is often useful for the low, right lateral dissection.

(c) Anterior dissection. The peritoneum is incised just above or at the peritoneal anterior reflection joining the two lateral dissection planes. An open grasper gently pushes the rectum downwards in order to open the anterior dissection plane above the Denonvillier's fascia. Very low in the pelvis additional lateral dissection is frequently necessary to assist the anterior mobilization. By lifting the posterior vaginal wall in women or the seminal vesicles and prostate in men anteriorly with the open grasper or fan, the dissection continues down to the anorectal junction. If preoperative MRI shows threatened lateral margins even after preoperative chemoradiotherapy, a more radical approach is performed in the tumor area to achieve an at least 2 mm free circumferential resection margin (CRM). An open surgical approach is considered for these patients if the cancer is located below the peritoneal reflection and particularly for anterior tumors.

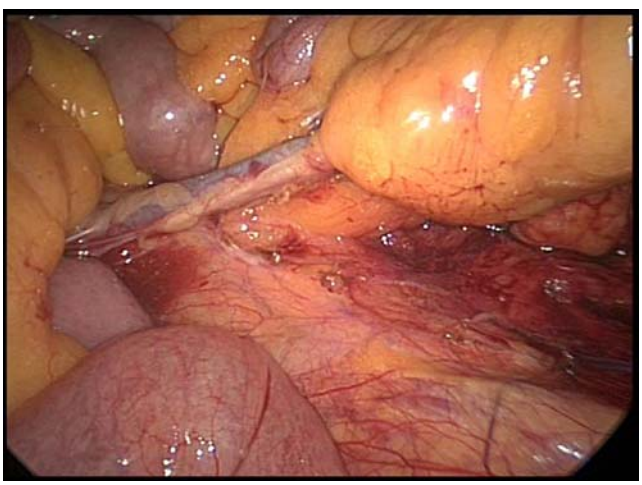

Figure 3 Inferior mesenteric vein before division with vascular stapler, electromechanic device or clips.

Transection of the rectum is done with a linear stapler. A perpendicular transection is achieved by traction of the fully mobilized mesorectum to the left side of the pelvic cavity. Any residual mesorectum is divided with electrocautery in cases of PME. Vessels are secured with clips for higher mesorectal transections. For very low tumors, a transanal intersphincteric dissection is performed to link with the abdominal dissection from above. In these cases, the specimen is removed through the anal canal and a hand-sewn anastomosis is performed.

8. Exteriorization of the specimen. The specimen is extracted using a wound protector through a 4–5-cm

muscle splitting incision in the left lower quadrant. Transection of the proximal colon and ligation of the mesocolon are performed extracorporeally. The marginal arteries are ligated after confirming pulsatile blood flow. A colonic J pouch or end-to-end anastomosis is prepared. The extraction site is closed in layers and pneumoperitoneum re-established.

9. Creation of the anastomosis. The anastomosis is performed with a circular stapler and tested by insufflating air into the rectum with the pelvic cavity filled with water. The completeness of the donuts is always checked.
10. Closure of port incisions. Fascial closure of all port sites >5 mm is done with a Carter–Thompson suture passer and absorbable 0 polyglycolic acid ties. All port skin incisions are closed with interrupted subcutaneous 4-0 absorbable suture.

Patients are tilted to the right and into Trendelenburg position during steps 3–6, except during mobilization of the splenic flexure when reverse Trendelenburg position is used. During the pelvic dissection, a steep Trendelenburg position is used to keep small bowel loops from falling into the operative field. The primary surgeon stands on the patient's right side during the whole operation with an assistant to his left. A second assistant is used selectively on the left side who subsequently inserts the stapler transanally.

Conversion is performed when adequate progress cannot be maintained. The left ureter should be identified in all cases. Not being able to identify the left ureter is an indication for conversion to open surgery but has never been necessary to date in the author's experience.

Discussion

The most challenging task in laparoscopic rectal cancer surgery is to maintain adequate short- and long-term oncological results. Although several series have been published with excellent outcomes,[3–5,15] data from high quality randomized clinical trials are scarce at the current time.[5–8] Significant concerns exist as to whether these results will be transferable to all surgeons performing rectal surgery, as rectal mobilization is a much more complex procedure than segmental colectomy. We believe that by standardizing the procedure as described in this paper, a quality control can be built into each step of the operation. The technical quality aspect of the procedure can be assessed by evaluating blood loss and operative times as well as by grading the TME specimen.[16] An intact, shiny, and bilobed fascial envelope surrounding the mesorectal fatty tissue, graded as 3, is ideal for every case. Grade 2, shallow breaks into the mesorectum, but not in areas near tumor, and grade 1, deep breaks that go down to the rectal wall, imply bad surgical technique or a technically very demanding dissection (perhaps obesity, deep pelvis with large tumor, or significant post-radiation change) and probably increase the risk of local recurrence of the cancer.[17]

By the time the pathology report is received, two more quality measures can be obtained: the circumferential resection margin and the number of lymph nodes detected in the specimen. If at least a 2-mm circumferential resection margin has not been achieved but should have been possible according to preoperative MRI information, the quality of the surgery may be in question and may have a direct impact on local recurrence and patient survival.[18–21] If this is shown to be a documented pattern, the surgeon may need to undergo further training. Such guidelines should apply to both open and laparoscopic surgery and are currently being developed in several European countries.

Performing a complete LRR with mobilization of the left colon, take down of the splenic flexure and creation of a colorectal or coloanal J pouch anastomosis should usually be completed within a mean operation time of 4–5 hours. The operation time will decrease with increasing experience and may even match the operation time for open rectal surgery, showing that the argument of prolonged use of operating room time associated with laparoscopic surgery can be rejected.

A prospective quality control system should be a part of any oncological procedure but is especially important regarding rectal cancer surgery and the issue of laparoscopic procedure and local recurrence. The CLASICC trial had an increased rate of involved CRM in the laparoscopic arm for anterior resections but had generally high rates of involved circumferential margins in both open (6%) and laparoscopic operations (12%).[22] Fortunately, this did not translate into an increased clinical local recurrence rate, and survival was equivalent in laparoscopic and open patients.[7] Nevertheless, there may be room for improvement of surgical quality even among experienced surgeons.

The learning curve for laparoscopic colectomy is probably longer than previously anticipated.[23] The learning curve is undoubtedly even longer for LRR, and these dissections should not be performed until the surgeon is familiar with the principles of TME, and the laparoscopic colon curve has been ascended, particularly for cases of neoplastic disease. Laparoscopic rectal cancer surgery is one of the most technically challenging laparoscopic procedures to perform and requires advanced laparoscopic experience and skills. Hospital and surgeon case load should therefore be considered before laparoscopic rectal cancer surgery is offered to patients.

One of the main arguments against laparoscopic rectal surgery is the technical difficulties associated with the

procedure resulting in high conversion rates. By making such decisions as early as possible in the procedure, conversion can be performed early if the ureter is not found, without negative impact on patient morbidity and length of hospital stay compared to primary open cases.[24]

Once the laparoscopic procedure has been performed, patients are managed with standardized perioperative care pathways to accelerate their recovery after surgery. The clinical results of combining this standardized, stepwise laparoscopic technique with standardized perioperative care protocols for rectal resections are reported elsewhere.[25]

Conclusion

By systematically applying standardized procedures based on best available evidence, quality improvements in all parts of patient care can be achieved. We have developed a standardized, stepwise approach to LRR in order to facilitate the teaching of laparoscopic skills and improve our own performance. We recommend others do similarly in order to achieve the necessary team expertise, making it possible to perform LRR safely with optimal short and long-term results.

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CASE REPORT

Aberrant Insertion of the Right Subclavian Artery: an Unusual Cause of Dysphagia in an Adult

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Abstract
Introduction Within this report, we present a patient with difficulty of swallowing caused by an aberrant right subclavian artery.
Discussion This is a congenital anomaly with the right subclavian artery originating from the dorsal part of the aortic arch and coursing through the mediastinum between the esophagus and the vertebral column. The diagnosis and treatment of this disorder is discussed based on the findings from the literature.

Keywords Dysphagia · Lusoria · Esophagus · Vascular anomaly · Subclavian

Introduction

Usual causes of difficulty of swallowing or dysphagia in adults include malignancy, esophageal motility disorders, or esophageal strictures. An uncommon cause of dysphagia is compression of the esophagus by a vascular structure. The clinical syndrome of dysphagia in association with an aberrant right subclavian artery compressing the esophagus was first discovered by David Bayford (1739–1790) and reported as dysphagia lusoria after "lusus naturea" (freak of nature) in 1787.[1] An aberrant right subclavian artery is the most common embryologic abnormality of the aortic arch and occurs in 0.5% to 1.8% of the population, but it is usually asymptomatic.[2,3] Within this report, we present a patient with dysphagia lusoria and discuss the anatomical abnormality, diagnosis, and treatment of this rare entity.

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Case Report

A 74-year-old woman was referred to our university hospital because of intermittent dysphagia with difficulty swallowing solid foods. Her medical history included a breast carcinoma for which she underwent breast-conserving treatment 3 years earlier. She had been put on proton pump inhibitors since several years without relieving her symptoms. At physical examination, no abnormalities were found. By report from the referring hospital, an endoscopy revealed no abnormalities besides a hiatal hernia without the aspect of a Barrett's esophagus or gastric inflammation. A barium contrast examination demonstrated a compression of the proximal esophagus at the level of the aortic arch suggesting of a mass compressing the esophagus (Fig. 1). Therefore, a computed tomography (CT) angiography scan was performed, which revealed an aberrant right subclavian artery arising from the aortic arch causing compression of the esophagus (Fig. 2). Because of the severity of symptoms and the inability to eat had caused weight loss, surgical treatment was indicated.

Under general anesthesia, she was placed in a half supine position and via a right supraclavicular approach, the right carotid and vertebral artery were identified. The aberrant right subclavian artery was dissected free from the esophagus and mobilized into the mediastinum. After administration of 5,000 IU of heparin intravenously, the proximal right subclavian artery was transected with an endostapler (Multi-fire Endo TA 30, Covidien, MA, USA) and cut. The distal

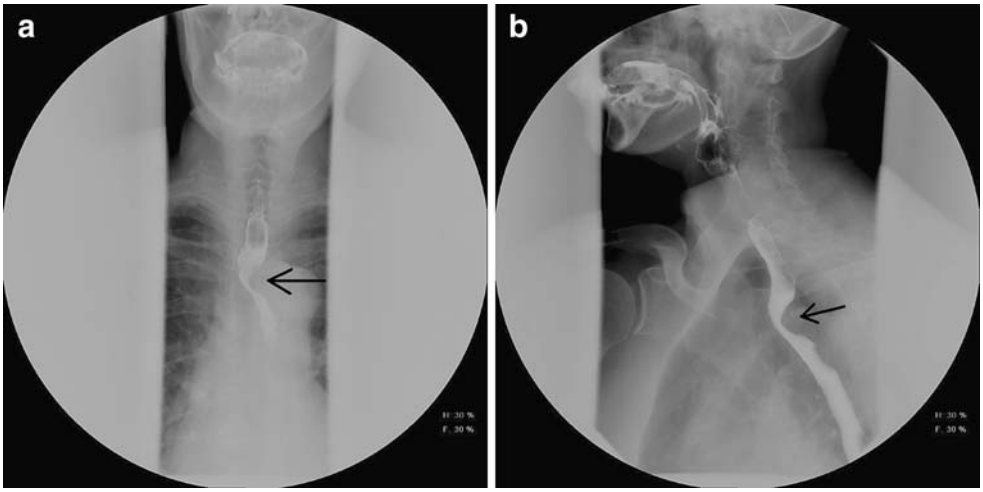

Figure 1 A barium contrast examination revealing a filling defect at the level of the aortic arch. In the posterior–anterior view, the parts of the esophagus proximal and distal to the defect are displaced to each other (*arrow*, **a**). In the lateral projection, a wedge-shaped impression on the dorsal esophagus is seen (*arrow*, **b**).

artery was anastomosed end to side to the right aortic artery (Figs. 3, 4, and 5). Immediately after the operation, the dysphagia had disappeared. The patient uneventfully recovered and was discharged 2 days after surgery. At 6 weeks of follow-up, a CT angiography showed a patent arterial reconstruction (Fig. 6).

Discussion

During early embryologic development, the aortic arches start as a duplicate system. The right aortic arch disappears proximally to form the right subclavian and common carotid artery. These latter vessels fuse to form the brachiocephalic trunk (or innominate artery) which is usually the first branch of the aortic arch. Abnormal involution of the right aortic arch with a persisting (seventh) intersegmental artery results in the evolution of an aberrant right subclavian artery.[3] The aberrant right subclavian artery arises from the dorsal part of the aortic arch with a broad base formed by a remnant of the persisting primitive right aorta, the so-called Kommerell's diverticulum.[4] The aberrant subclavian or lusoria artery passes through the mediastinum between the esophagus and the vertebral column to reach the right axilla in the majority of cases.[5,6] An aberrant right subclavian artery is in approximately one third of cases associated with carotid artery anomalies with a common origin of the left and right carotid artery (bicarotid truncus).[6]

The majority of patients with an aberrant right subclavian artery will remain asymptomatic during lifetime.[2,3,6] In symptomatic infants, it usually presents with respiratory signs. This is most likely due to the absence of tracheal rigidity, allowing its compression to lead to airway obstruction with recurrent pulmonary infection.[5–7] It is not clear

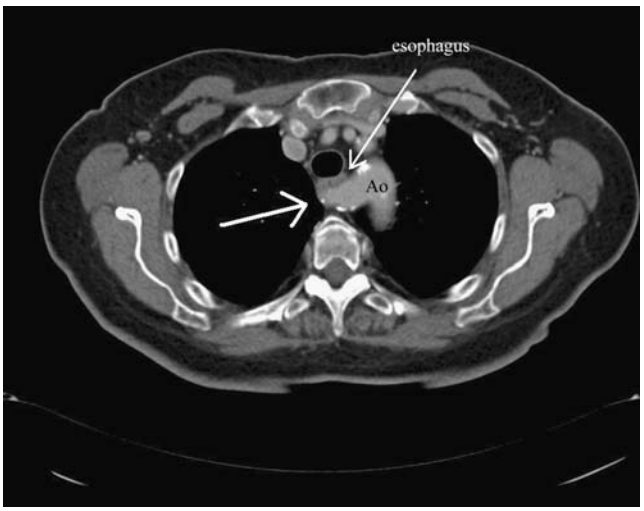

Figure 2 A CT angiography which shows the aberrant origin of the right subclavian artery (*white arrow*). Note the close relationship with the esophagus. *Ao* aortic arch.

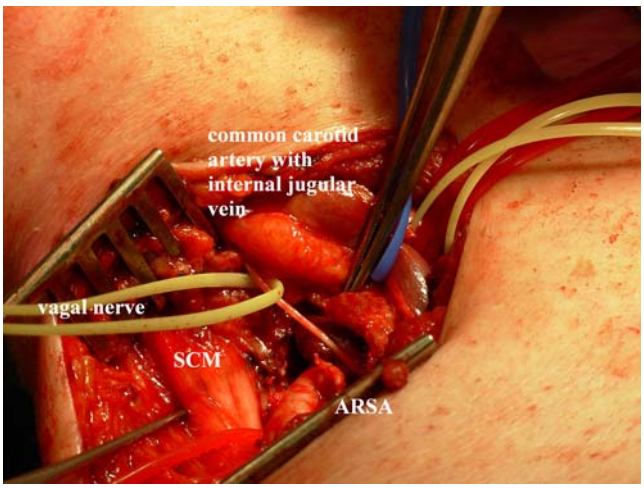

Figure 3 An intraoperative view showing the aberrant right subclavian artery crossing dorsally to the common right carotid artery. *SCM* sternocleidomastoid muscle.

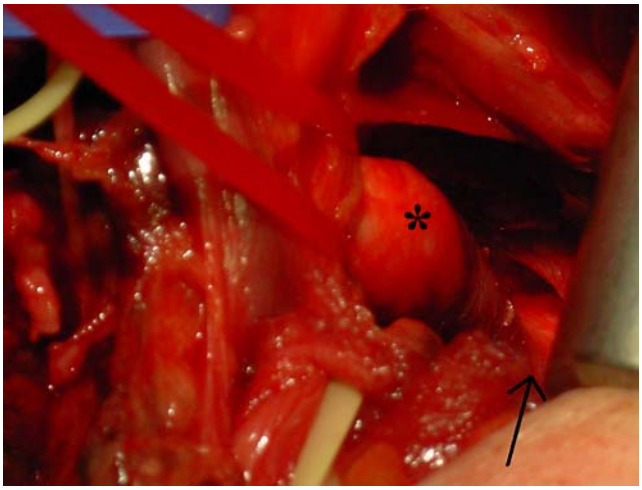

Figure 4 An intraoperative view showing the aberrant right subclavian artery (*asterisk*) crossing dorsally to the esophagus (*arrow*).

why dysphagia occurs in older patients. It could be attributed to increasing rigidity of the esophagus or arterial elongation and thickening due to atherosclerosis during lifetime.[7] Similarly, it is uncertain whether dysphagia occurs primarily due to esophageal impression or secondary to motility changes.[7] Others hypothesized that the coincidence of a common carotid origin and an aberrant right subclavian artery can give rise to compression of the esophagus between these vessels.[6,7]

In adults with dysphagia, an upper gastrointestinal endoscopy is usually performed. In case of an aberrant right subclavian artery, a pulsating impression can sometimes be seen, but it usually is normal.[5,7] A barium contrast examination will show a filling defect at the level

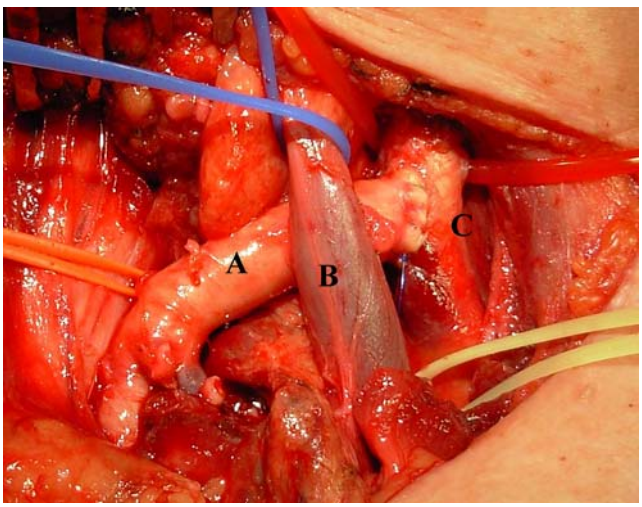

Figure 5 An intraoperative view showing the end-to-side anastomosis between the right subclavian artery (*A*) and the right common carotid artery (*C*). *B* Internal jugular vein.

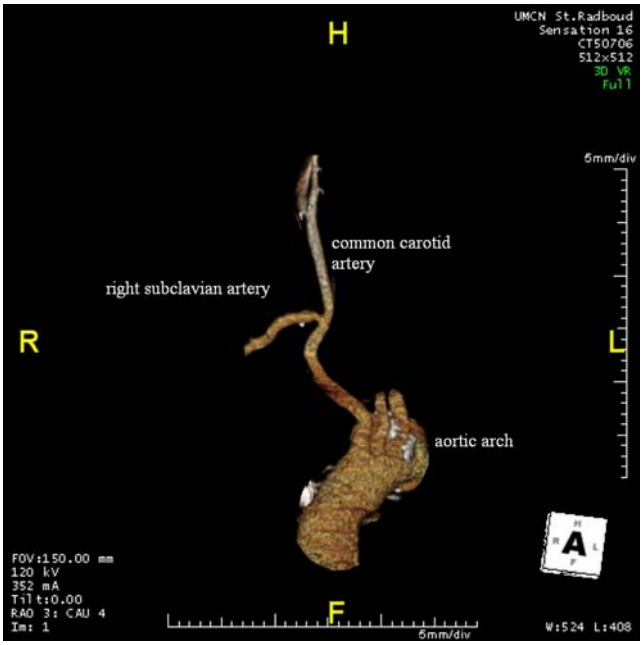

Figure 6 A 3D reconstruction of a postoperatively performed CT angiography showing the end-to-side anastomosis between the proximal right subclavian artery and the right common carotid artery.

of the aortic arch, as illustrated in our patient (Fig. 1). In the posterior–anterior view, the parts of the esophagus proximal and distal from the defect appear displaced (Fig. 1a).[6] In the lateral projection, a wedge-shaped impression on the dorsal esophagus is seen (Fig. 1b).[6] CT or magnetic resonance (MR) angiography is considered as the gold standard for the diagnosis of an aberrant right subclavian artery.[8]

The management of these patients depends on the severity of their symptoms. Janssen and colleagues reported three out of six patients with dysphagia lusoria who became free of symptoms after dietary changes or acid inhibition or promotility agents.[7] In case of severe or persistent symptoms, surgical intervention is warranted to remove the aberrant vessel and reconstruct the vascular supply. The surgical approach depends on the vascular anatomy: An isolated supraclavicular approach can generally be used if the aortic arch is normal without aneurysm formation of the proximal aberrant right subclavian artery, as in the presented patient. In patients with associated lesions, a combined cervical and thoracic approach might be more appropriate.[9] Simple ligation and division of the aberrant subclavian artery is likely to cause ischemia of the upper limb or a "subclavian steal" syndrome; therefore, subclavian carotid transposition is advocated (reimplantation of the right subclavian artery to the right common carotid artery), as we used in our patient.[10] The results of surgical treatment are excellent with relieve of symptoms in nearly all reported patients.[7,9]

In conclusion, dysphagia in an adult patient can be attributed to an anomaly of the right subclavian artery and this should be included in the differential diagnosis. A barium swallow examination can give a clue toward the diagnosis, but CT or MR imaging angiography is the golden standard for diagnosing this anomaly. In cases with severe symptoms resistant to medical therapy, surgical intervention should be considered. If no aneurysm or aortic disease is present, a supraclavicular approach with diversion of the proximal aberrant artery and an end-to-side anastomosis of the distal right subclavian artery to the right common carotid artery are recommended.

Conflicts of Interests For this manuscript, there was no financial support nor are there any conflicts of interests.

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HOW I DO IT

How I Do It: Gastrointestinal Cutaneous Fistulas

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Abstract
Introduction Gastrointestinal cutaneous fistulas are among the more complex surgical conditions, with mortalities in the current series between 6% and 20%, and in some non-U.S. series, up to 40%. The series of principles of recognition, preparation of the abdominal wall, enteral and parenteral nutrition, and support, is outlined. Diagnosis in the absence of signs of sepsis is usually obtained by a fistulagram done by collaboration between the senior surgeon and the senior radiologist and followed to make certain that there is no intestinal obstruction. If spontaneous (nonoperative) closure does not occur in 5 to 6 weeks, it is unlikely to occur and an operation will be required. In our experience, obliterative peritonitis does not subside until a minimum of 4 months, and so an elective operative approach should take place when required after 4 months since the previous operation (when the fistula occurred).
Methods A technical approach to operation is described. Avoiding enterotomies is critical. The abdomen should be entered in a fresh area, either by an extended incision, or in a virgin area transversely, if the previous incision was vertical and occupied the entire length of the abdomen. It often takes between 1.5 and 2 h to get into the abdomen without making additional enterotomies. The goal is to dissect laterally in one area until one enters a free lateral space which is free of adhesions. One then proceeds from lateral to medial to take down the adhesions from the previous incisions. When one is finished taking down these adhesions, it is usual that only 12 to 18 in. of bowel of the fistula and the surrounding enterotomies requires resection. An end-to-end anastomosis should be performed. Our practice is a two-layer silk-interrupted anastamosis. Adjunctive steps following the operation usually include a gastrostomy and a catheter jejunostomy. In order to be successful, the best results are obtained with a native abdominal wall closure with either component separation or an Abrahamson-type closure. If this cannot be achieved, multiple layers of vicryl are used, which usually enables the fistula to heal; a hernia usually results, but that can be dealt with at some future time. Using these principles, the last 50 cases at our personal series have been done without mortality.

Keywords Gastrointestinal fistulas · Technique

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Introduction: Causes of Gastrointestinal Cutaneous Fistula

Before we address surgical technique, we should discuss the antecedent causes of gastrointestinal cutaneous fistulas. At least 85% to 90% of gastrointestinal cutaneous fistulas occur post-operatively. Most follow lysis of adhesions for intestinal obstruction or resections for cancer or inflammatory bowel disease. Rarely, probably between 5–10%, spontaneous gastrointestinal fistulas occur as complications of inflammatory bowel disease or cancer. In cancer, it is usually at a late stage of the disease and associated with a poor prognosis. When the fistula follows operation it is usually the result of an unrealized enterotomy or an anastomosis, which leaks

often because of poorly prepared or distended bowel, or an emergency operation in a patient who is nutritionally or immunologically in suboptimal condition. This may also involve inadequate blood supply to the bowel, or in many situations, distended small bowel due to delay in relieving partial or near total intestinal obstruction.

Recently, there appears to be an increased new cause of postoperative fistulas, open or laparoscopic herniorhaphy. The occurrence of fistula occurs in two ways: the first involves an inadvertent enterotomy. The second is the insertion of permanent mesh, which may become infected and/or erodes into the bowel. In this case, the infected mesh must be removed in order for this fistula to close. Rarely, removal of the mesh alone is sufficient, without bowel resection. At the present apparent rate of occurrence, this may become one of the more common causes of gastrointestinal cutaneous fistula.

The post-operative course in a patient who ultimately develops a gastrointestinal cutaneous fistula is fairly typical. The patient does not do well following operation; the abdomen remains distended, there is lack of progression and recovery from the original operation, a low-grade fever, sometimes up to 101°F or 102°F and more abdominal tenderness than should occur following an uneventful procedure. On the fifth or sixth post-operative day, a wound infection presents, which is then drained with defervescence and some relaxation on the surgeon's part. Hopefully, the patient will now do well. However, within 24 h, enteric contents appear; one is dealing with a gastrointestinal cutaneous fistula.

There are two ways of dealing with this situation; the first is to reoperate immediately as there is good evidence that operation within 10 days postoperatively or after 120 days has an average mortality of about 10% while operation in between that interval has an average mortality of 20%.[1] This is data from an experienced center.[1] However, operation on the patient in suboptimal condition (that is if some of the conditions that have led to this patient getting a fistula in the first place still exist: poor nutritional condition, distended bowel, a tendency to obliterative peritonitis, and immunologic suboptimal condition) may likely result in another fistula. The second alternative is to accept the fistula, not re-operate and prepare for the prolonged preparation of a patient with a gastrointestinal cutaneous fistula for either non-operative closure, or after at least 4 months of nutritional and other preparation, for re-operation.

Nutritional Support

As soon as one discovers the fistula, one should place the patient NPO and initiate nutritional supplementation if this has not already been initiated. One can use either enteral or parenteral nutrition. The spontaneous closure rate is slightly greater with parenteral nutrition in most series, and enteral feeding may be difficult without an established feeding jeujunostomy to get an adequate amount of nutritional supplementation entirely by gut. It is advantageous to use the enteral route both for increased acute hepatic protein synthesis, a better condition of the liver[2] and more thickness of the gut wall in the event re-operation is required if spontaneous closure does not occur. Complications such as acalculous cholecystitis are less likely and hypo-albuminemia or a deficit in synthesis of acute phase proteins seem more rapidly corrected with enteral nutrition.

Absorption of enteral nutrition requires at least 4 ft of intact bowel below the entry of the feeding. One can put the feeding into the high jejunum provided there is 4 ft between the feeding entry and the fistula; alternatively, one can place a feeding tube into the fistula provided there is 4 ft of intact small bowel below the fistula. In a high fistula, one should feed below the fistula, into the fistula, as long as the distal bowel is not obstructed and in continuity.

Putting enteral feeds into the stomach is probably not as safe as feeding into the small bowel. Gastric motility may stop when the patient becomes septic and aspiration may occur. If one must use the stomach, the patient's head should be elevated, and to avoid aspiration, feedings should only be carried out between 7 A.M. and 9 P.M.

The technique of feeding varies as to whether the feedings enter the stomach or the small bowel. When hyperosmolar feedings enter the stomach, the stomach stops emptying and secretes free water until the gastric contents become isosmolar; gastric peristalsis resumes and the stomach empties in 2–4-cc aliquots transversing the pylorus, which opens rhythmically every 30 s. One then increases volume until the target is reached.

The small bowel cannot dilute enteral feedings. Consequently, one begins with diluted feedings. Our own practice is begun with small bowel tube feeding at an osmolality of 180, gradually increase volume and then slowly increase osmolarity. The small bowel may not tolerate anything more concentrated than 280 mosmol/cc. More concentrated feeding may result in a secretory diarrhea. Thus, one gives isosmolar jejunal feeding at increasing volumes until the target is reached or until secretory diarrhea occurs.

Elderly patients, patients with impaired cardiac output, or mesenteric vascular disease may be particularly prone to pneumatosis and for ischemic and/or necrotic bowel, perforation, and one must increase tube feeding slowly.

Radiographic Study

The next step is getting the patient to the point where s/he can undergo a fistulagram.[3] In the absence of sepsis; this is

the only radiological exam that is needed if one can establish patent distal bowel and no distal intestinal obstruction. The senior surgeon should perform this with a senior radiologist. Early films are most important. The fistulagram allows one to determine the condition of the bowel, intestinal discontinuity, whether the fistula is enteral or colonic, and, most important, whether there is distal obstruction. Fistulas will not heal spontaneously in the event of intestinal discontinuity or distal obstruction and are less likely to do so with an adjacent abscess or bowel, that is strictured, or in poor condition. Inflammatory bowel disease may result in spontaneous closure, but it will soon reopen. Remicaid or a similar medication and antibiotics may result in permanent closure, but the rate of reopening is considerable. If, however, a patient continues to be febrile or septic, a CT scan is appropriate using intravenous contrast and when possible oral contrast to look for an intrabdominal abscess.

Attempts to Heal the Fistula Nonoperatively

Normally, as soon as the fistula is identified, a sump is placed around the fistula and the adjacent protected skin. We prefer to place duoderm or some other protectant around the fistula and to place the sump, the diameter of which should vary with the thickness of the contents, over the fistula. We use a latex nephrostomy tube with an intracath to the tip breaking the suction. An extra hole cut 1 cm proximal may help break the suction. The sump will protect the skin and may, in some situations where the anatomy is appropriate, result in spontaneous closure of the fistula. Others may use a vacuum-assisted closure (VAC), but one must be aware of the fact that with a VAC there is an opportunity for the causation of new fistulas even after the original fistula may close.[4]

At times, when the fistula is a small lateral fistula, a pouch may be placed. Moreover, with a lateral fistula, it has a higher rate of spontaneous closure

Persistence in nonoperative closure of a fistula beyond 5 or 6 weeks in a sepsis-free patient generally means the fistula will likely not close non-operatively but does not mean that the operation should take place at that time. Our practice is to wait at least 4 months when subsidence of the obliterative peritonitis allows safer operation. The decision to operate is based on several criteria, including the patient's nutritional status. One can follow the adequacy of nutritional support by using serum transferrin, retinol-binding protein, and thyroxine-binding prealbumin[5]. Transferrin has a half-life of 5 to 8 days and it will respond more rapidly than albumin, which has a half-life of 20–23 days, although some have argued that half-life of the rapidly turning-over pool of albumin is shorter. Thyroxine-binding prealbumin and retinol-binding protein have also been shown to be prognostic of mortality.[5] The two are somewhat different in responding to either calories and or protein. However, one can also make the decision as to when to operate from the end of the bed by evaluating how the patient looks and the condition of the abdominal wall. If the abdominal wall has a large defect, one may consult the plastic service unless one is comfortable themselves performing a component separation or an Abrahamson procedure (in an Abrahamson closure, one divides the external oblique fascia laterally over the rectus muscle and transposes it medially, "flips it over" to cover the defect); otherwise, a muscular cutaneous flap may be necessary. If neither is possible, we use many thicknesses of vicryl mesh and accept (as often happens) a late hernia rather than risk infection, an abscess, and recurrence of the fistula.[6,7]

The Operation

At operation, one usually finds that even multiple fistulas usually involve only 12–18 in. of bowel, which is resected and continuity restored with an end-to-end anastomosis.[6] Lysis of adhesions of the entire small bowel from the ligament of Treitz to the ileocecal valve may be performed if distal obstruction has not been ruled out. In addition, one should identify all the components of the colon to make certain that the colon is not involved in the fistula.

The most critical aspect of the operation is getting into the abdomen without doing too much damage. One must carefully inspect the previous incisions as to whether or not they are adequate. Often, the previous incision is too small for what needs to be accomplished, leaving the surgeon at a disadvantage as far as visualizing exactly what is required and thus putting the patient at risk. The length of the incision is not particularly important provided there is adequate exposure. What is important is entry into the abdomen without making enterotomies. In order to do so, one must either enter the abdomen safely with a much longer incision, usually superiorly beginning at the xiphoid process or, if the previous incision is the entire vertical length at the abdomen, perhaps consider a transverse incision beginning in a "virgin area".

The Dissection of the Bowel

Our own practice is not to schedule any other case or any patients in the office on the day of attempted repair of an enterocutaneous fistula. The operation may take as long as 6 to 7 h, or longer, and one is fatigued. Secondly, these operations require time and it is a bad idea to have to rush through them, especially when one gets into difficulty.

Once one identifies the area of the fistula and the relevant area of the small bowel, the affected segment is usually relatively small and may have a number of enterotomies either previously made or when one tries to take it down from the abdominal wall to which it is invariably stuck. In a very difficult abdomen, it may take several hours to safely get into the abdomen avoiding an enterotomy (Figs. 1, 2 and 3). The technique is a combination of knife dissection and scissor dissection. The purpose is to enter the abdomen safely, and very tediously take care early in taking down the abdomen, taking down the bowel from the anterior wall of the abdomen without injuring the bowel (Fig. 3). Sooner or later, working methodically and carefully, one should be able to get to the lateral part of the abdomen where there are no adhesions and the bowel is relatively free. This is almost always the case and once one is there, one can then proceed laterally and begin to take down the bowel from the wound (Figs. 4a and b) and free up the required small bowel. Our preferred technique is to probe for soft areas from lateral to medial and get behind the adhesions, dissecting from the side not directly in front of the adhesions. At this point, one is prepared to dissect free the entire small bowel from the ligament of Treitz to the ileocecal valve if necessary. Finding the ligament of Treitz is important as our practice following such a prolonged procedure is to place a feeding jejunostomy for enteral nutrition as well as a gastrostomy for the post-operative period, in case the ileus is prolonged so that the patient does not have to have an indwelling nasogastric tube, which is uncomfortable and may contribute to pulmonary complications.

The technique of taking the bowel down from the abdominal wall is perhaps the most important aspect of the operation. There is a tendency to attack the adhesions directly from in front; however, this is the wrong approach. Once the free lateral space is entered, one can get one's hand behind the adhesions and to then bring the adhesions forward attacking the adhesions not directly, but laterally (Fig. 4a and b). One should not be hasty and persist in an area in which adhesions are difficult and will not allow careful dissection. If there is no progress in a particular area, one then takes a fully soaked laparotomy pad saturated with antibiotic solution (Cefzol or kanamycin) and places it in this area, leaving the bowel to get edematous, which then aids the dissection as the area between the loops of bowel becomes more edematous and reveals the plane between the loops of bowel. In the interim, one attacks a different area where dissection may be easier.

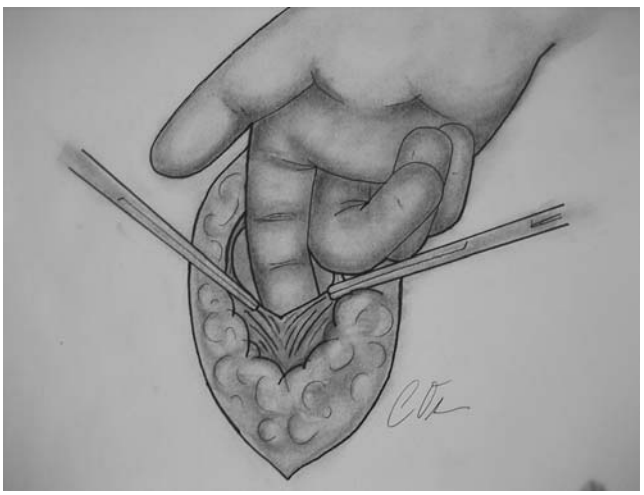

Figure 1 Upon opening the incision, hopefully, if one is using a vertical incision, it is possible to start higher up near the xiphoid. Once the fascia is opened, the easiest way to avoid making an enterotomy is to take one's index finger and, keeping it immediately under the peritoneum, push gently and get the bowel off the peritoneum, thereby allowing some room.

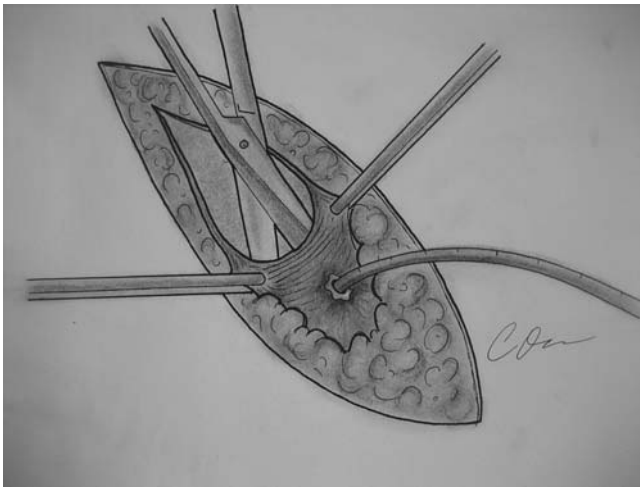

Figure 2 Once the wound is opened, the scissors may be placed underneath the area where the bowel has been removed and the fascia and peritoneum opened without making an enterotomy.

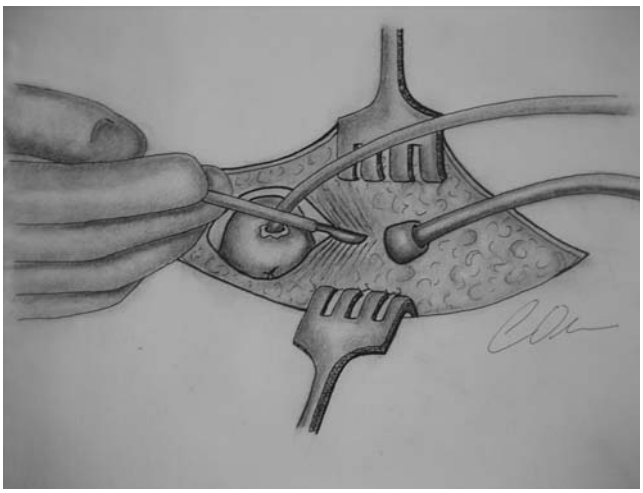

Figure 3 In this figure, the intubated fistula is shown and a knife with a 15 blade may be used to open up the fascia away from the bowel to avoid making an enterotomy.

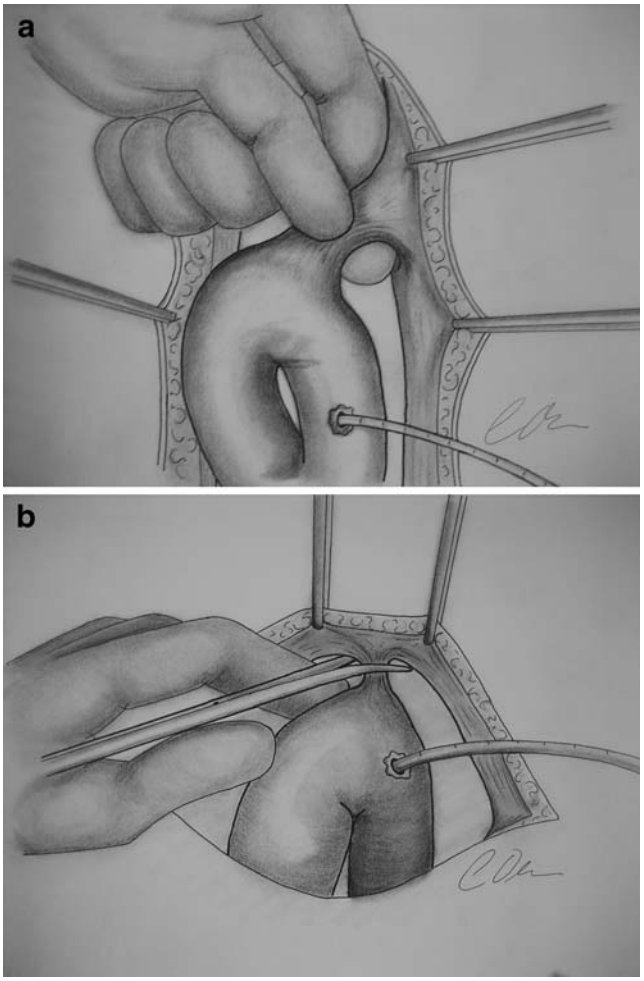

Figure 4 **a** Here, one sees the technique of delivering the bowel and the adhesions to the abdominal wall from the side rather than directly in front. A good trick to use is to take the adhesion from the abdominal wall to the bowel and compress it between the thumb and forefinger forcibly. If one does this, the adhesion becomes considerably shorter and simply has a very small area of adhesion to cut through. **b** The remainder of the adhesion has been compressed sufficiently between thumb and forefinger so that a large area of adhesion to the bowel wall and the abdominal is no longer present, and there is just a small adhesion to divide.

We usually put wound towels with the edge soaked in antibiotic solution on the abdominal wall immediately after dissecting all the bowel free from the abdominal wall, and we suture the edge to the peritoneum. This prevents bacterial contamination of the wound, perhaps preventing infection. In addition, one should have adequate exposure and utilize either Buchwalter or, our preference, a Thompson Farley retractor.

If there are serosal tears, some advocate not closing serosal tears. It is our practice to close the serosal tears with 4-O or 5-O prolene. The prolene will close the serosa and will not yield small micro abscesses and adhesions, which results with 4-O silk. Resection, which is almost always necessary, is followed by a hand-sewn two-layer interrupted permanent suture end-to-end anastomosis.[6] Of late, I have been using interrupted prolene on the outer layers and silk on the inner layer. Others may prefer a different anastomosis, but we do not use staples under these circumstances because the anastomosis may swell and the staple line can disrupt. The gastrostomy and feeding jeujunostomy should follow.

Closing the Abdominal Wall

Closing the abdominal wall with secure native abdominal wall is best in preventing the recurrence of the fistula. The use of mesh or biological closure materials may be more prone to infection and a tendency to increase fistulization.[7–9] If there is any omentum left, this should be placed between the anastomosis, the enterotomies, and the abdominal wall.

The abdominal wall should be mobilized in a wide fashion so there is no tension on the closure. A component separation or an Abrahamson type of closure is appropriate if necessary.

If wide mobilization of the abdominal wall is not possible, and a component separation is insufficient, arrangements should have been made for the plastic service to swing a musculo-skeletal flap to get a secure closure. If a flap is not necessary, we usually use a running non-absorbable monofilament suture as this has been shown to have the lowest rate of herniation and evisceration.[10] 3-O vicryl is used to close the subcutaneous tissue and 4-O monocryl as a subcuticular closure. We use an occasional 5-O nylon suture if there is concern about the skin edges sticking together, but usually use steri strips. A sterile dressing with xeroform is placed, after all layers have been closed.

Postoperative Care

In the post-operative period, J-tube feedings are begun on the first postoperative day, with 10 cc an hour of half strength Impact[11] which have been shown to result in a better outcome in a nation-wide study, which was designed and coordinated by the late Dr. Robert Bower of the University of Cincinnati. After gas is passed or a bowel movement takes place, one may start with clear liquids and then go to a soft diet. J-tube feedings should then be discontinued by day to see whether or not the patient can support themselves entirely by mouth. Total parenteral nutrition (TPN) should be cycled because TPN interferes with the patient's appetite and ability to eat. The same is true of enteral feeds, which should be cycled overnight.

Conclusions

These operations are the epitome of attention to detail; doing so will result in a good outcome and lack of

mortality. However, at all times, it is essential that one use a meticulous detail both in preparation of the patient as well as technique at the time of surgery. We have followed these techniques and approaches in the last fifty patients requiring operation for closure of gastrointestinal cutaneous fistula. There has been no mortality.[12]

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REVIEW ARTICLE

Role of Ischemic Preconditioning in Liver Surgery and Hepatic Transplantation

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Abstract
Introduction The purpose of this review is to summarize intraoperative surgical strategies available to decrease ischemia–reperfusion injury associated with liver resection and liver transplantation.
Material and method We conducted a critical review of the literature evaluating the potential applications of hepatic ischemic preconditioning (IPC) for hepatic resection surgery and liver transplantation. In addition, we provide a basic bench-to-bedside summary of the liver physiology and cell signaling mechanisms that account for the protective effects seen with hepatic IPC.

Keywords Liver · Ischemia–reperfusion injury · Hepatic transplantation · Preconditioning · Surgery · Ischemic preconditioning

Introduction

Ischemia–reperfusion injury (IRI) is a pathophysiologic process where hypoxic organ damage is accentuated following return of blood flow and oxygen delivery to the reperfused tissue. Transient episodes of hepatic IRI occur during liver transplantation, trauma, hypovolemic shock, and elective liver resection when inflow occlusion is used to minimize blood loss. The pathophysiology of liver IRI includes both direct cellular damage as the result of the ischemic insult as well as delayed dysfunction and damage resulting from activation of inflammatory pathways. A comprehensive review of the pathophysiology of hepatic IRI is provided.[1]

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Overall strategies to mitigate IRI can be broadly classified into biochemical, genetic, and surgical strategies. The purpose of this review is to summarize the mechanisms and clinical application of ischemic preconditioning (IPC), which is a surgical strategy to decrease organ IRI. IPC is a deliberate brief interruption of blood flow to an organ, followed by a brief reperfusion period, then a more prolonged period of ischemia. For the liver, IPC has been achieved by occluding the portal triad with a tourniquet or a vascular clamp, thus, interrupting the afferent blood flow from the hepatic artery and the portal vein. After a given period of time (typically 10 min), the occlusion device or clamp is removed, and the liver is reperfused for an interval (10 to 15 min), after which the portal triad is once more occluded either intermittently or continuously for the duration of the hepatic procedure. The brief initial ischemic period does not damage the liver macroscopically and induces signaling pathways that result in subsequent protection against a more prolonged IRI.[2–4]

Hence, based on the experimental evidence supporting IPC, several authors conducted a series of clinical trials examining IPC for both liver resection and transplantation. To date, their results have been controversial, particularly in the area of liver transplant. In this study, we reviewed the current scientific literature to assess the specific strategies for hepatic IPC and summarize the signaling mechanisms and clinical applications.

Background

IPC was initially identified in the kidney by Zager et al.[5,6] and subsequently in the myocardium by Murry et al.[7] in 1986 who published this novel strategy in a heart ischemia–reperfusion model. In the heart model, brief episodes of ischemia slow the rate of adenosine triphosphate (ATP) depletion during subsequent ischemic episodes.[8] Additionally, intermittent reperfusion may be beneficial to the myocardium by washing out catabolites that have accumulated during ischemia. Therefore, the authors proposed that multiple brief ischemic episodes might actually protect the heart from a subsequent sustained ischemic insult.[7]

This innovative application of IPC has been applied experimentally in animal models of brain (1994),[9] skeletal muscle (1995),[10] intestine (1996),[11] lung (1996),[12] kidney (1997),[13] spinal cord (1998),[14] retina (1998),[15] and liver[16–19] IRI. IPC was first demonstrated in the liver by Lloris-Carsi et al.[20] in 1993. Occlusion of the portal triad for 5 min, followed by 10 min of reperfusion improved survival and hepatic function after 90 min of ischemia in rats. These findings were reproduced in similar models,[21,22] modifying both the duration of total ischemia and time intervals of IPC. These findings are characterized by a decrease in the intensity of the hepatocellular injury, as evident by increase in cellular levels of ATP,[17] reduction of tumor necrosis factor (TNF)-α[23] and interleukin (IL)-6 levels,[24] decreased leukocyte–endothelial cell (EC) interaction,[25] and reduction in EC injury.[26] This is associated with an increase in global blood flow to the liver,[27] increase in hepatic microcirculation,[28] reduction in hepatocellular apoptosis, maintenance of energy metabolism,[29] increase in intracellular hepatic oxygenation,[30] and protection of extrahepatic organ damage as well.[31]

Ischemic Preconditioning as a Protective Mechanism

The beneficial effect of IPC has been described in clinical studies by Clavien et al.[32,33] at the Universities of Duke and Zurich, in 2000 and 2003, respectively. Their group evaluated the protective effect of IPC (10 min of ischemia and 10 min of reperfusion) before parenchymal transection of the liver during planned hepatic resection. They demonstrated a decrease of EC apoptosis in patients after partial hepatectomy. Furthermore, IPC was associated with significant beneficial effects in patients after hepatic resection in steatotic liver as evident by control lipid peroxidation, hepatic microcirculation failure, and neutrophil accumulation, reducing the subsequent hepatic injury. This finding has been confirmed by animal studies as well.[34,35]

The effects of IPC are not limited to warm ischemia but also have been reported with cold ischemia. Arai et al.[36,37] from the University of North Carolina demonstrated that IPC prior to liver storage for up to 30 h in UW solution significantly reduced Kupffer cells (KC) activation and EC injury. IPC also decreased the morphological changes that induce EC detachment, apparently by reducing the activity of MP.[38] Interestingly, IPC limited to only one lobe of the liver can protect the nonpreconditioned liver after transplantation. In a rat model in which lobar IPC was followed by whole-organ transplantation, the findings showed that IPC protects sinusoidal endothelial cells and suppresses Kupffer cell activation after storage and reperfusion.[39] The benefits in the preconditioned liver portion were extrapolated to the contralateral liver, yielding a higher survival of the graft. This phenomenon is termed "heterologous preconditioning,"[39] and might be useful to protect the remnant liver against IRI during partial hepatectomy or transplantation.

The protective effect of IPC against IRI can be separated into two distinct phases.[40] The first is known as "acute preconditioning" and confers protection from the moment reperfusion begins and is maintained for 1 to 2 h.[41] Acute preconditioning uses preexisting substances without de novo synthesis of proteins.[42] "Delayed preconditioning" refers to the subsequent period in which the initial effects of IPC present 24 h after reperfusion, lasting up to 3 days.

Delayed preconditioning relies on gene expression within the reperfused tissue to synthesize new proteins.[41] The release of these substances into the systemic circulation allows other distant organs to benefit,[43,44] a phenomenon known as "remote preconditioning."[39,43] Similar mechanisms are probably responsible for the effects seen in both remote and heterologous preconditioning.[38]

The precise mechanisms by which IPC confers protection to the liver against IRI are still unknown. Several mediators and pathways have been implicated, among which the most studied are preservation of ATP levels through activation of the adenosine-monophosphate-dependent kinase, induction of antioxidant systems,[19] regulation of TNF-α synthesis,[45] release of nitric oxide (NO), and increase in adenosine levels.[46] Because of its reproducibility, IPC is potentially applicable to multiple clinical conditions, and the search to identify and understand its mechanisms has become the focus of several researchers worldwide.

Molecular Basis and Cellular Mechanisms of Ischemic Preconditioning

Multiple mechanistic pathways are involved in IPC.[22] IPC protects the liver by acting fundamentally on adenosine and nitric oxide (NO), as well as intracellular kinases. Activation of these pathways initiate a cascade of events that progressively increase both the intensity and the extent of the protective effects of IPC. IPC suppresses reactive oxygen species (ROS) production by KC[34] and induces cellular resistance to ROS,[47] and IPC directly protected hepatocytes after warm IR.[48] It also decreased TNF-α synthesis,[49] which could partially explain the mechanism of remote preconditioning. IPC decreases hepatic P-selectin expression, reducing leukocyte adhesion, migration, and activation.[50] The decrease of adhesion molecule expression is probably a consequence of the reduction of TNF-α synthesis. IPC induces the production and release of adenosine, activating the adenosine A2 receptor, increasing NO production, and protecting EC.[51,52] Adenosine inhibits leukocyte adhesion, decreases the expression of adhesion molecules, inhibits leukocyte and platelet function,[53] and inhibits the production of ROS.[54] It is also has a potent vasodilating effect.[55] NO plays a central role in both acute and delayed preconditioning, acting as trigger in the former[56] and as a mediator in the latter.[57] In acute preconditioning, NO is synthesized entirely by endothelial nitric oxide synthase (eNOS) within hepatic EC.[58] In delayed preconditioning, NO production requires the synthesis of inducible nitric oxide synthase (iNOS).[59] NO also reduces oxygen and energy consumption by opening ATP-dependent K^+ channels and increasing cyclic guanosine monophosphate (cGMP0 concentrations.[60–62] NO exerts anti-inflammatory effects by inhibiting activation of stellate cells,[63] neutrophil adhesion,[64] and platelet aggregation.[65] NO activates intracellular kinases that increase iNOS transcription,[66] and it also modifies apoptosis by inhibiting caspase activity,[67] preventing release of cytochrome-c,[68] increasing cGMP, and promoting Bcl-2[69] and Hsp[67] expression. Some of these effects can be reproduced by the exogenous administration of NO donors.[55]

The decrease of ROS synthesis and neutrophil-mediated injury by IPC confers structural protection to microcirculation, and, in conjunction with NO production in IPC, hepatic perfusion is significantly improved.[70,71] Several intracellular kinases are activated by adenosine and other substances, including bradykinin.[61] Protein kinase C (PKC), p38-activated protein kinase (p38 mitogen-activated protein kinase (MAPK)), and protein kinase B (Akt/PKB)[72–74] play an important role in IPC, although the actual mechanisms are not completely understood.[75] PKC and p38 MAPK are important to the intracellular Na^+ homeostasis.[76] The adenosine A2 receptor activates Akt/PKB in hepatocytes,[77] conferring antiapoptotic effects[78] and stimulating NO production through eNOS synthesis.[79]

The conservation of energy observed in IPC results from a decrease in cellular metabolism.[28,80] Energy conservation prevents necrosis, and the availability of energetic substrate allows for a controlled cellular modulation regulated by apoptosis.[81]

Delayed preconditioning is caused by genetic modification and results from the proteins and substances expressed by the modified genes. One example is nuclear factor (NF)-κB;[82] its increased expression and activation by IPC produces synthesis of antioxidants,[83] Hsp,[84] and iNOS.[58]

Ischemia–Reperfusion Injury Mechanisms

Although a complete summary of IRI mechanisms is beyond the scope of this review, we will summarize the signaling pathways as they pertain to IPC. The main mechanisms involved in IRI can be summarized in cell activation and cytokine release, expression of adhesion molecules, and microcirculatory alterations with subsequent cellular death.[85]

During the first hours of reperfusion, KC are activated[86] and start to produce and release ROS[87] and the proinflammatory cytokines TNF-α and IL-1.[88] These cytokines promote β2-integrin/intercellular adhesion molecule (ICAM) expression by EC,[89] potentiating the second IR stage that involves activation, recruitment, and adhesion of neutrophils.[90,91] Among the adhesion molecules implicated in IRI are ICAM-1 from EC and the β2-integrin Mac-1 from neutrophils. Selectins promote EC and neutrophil interaction[92] through ICAM-1 and Mac-1.[93] Once adhered,

neutrophils leave the intravascular space and migrate to the interstitial space, where they regulate the intensity of the injury through phagocytosis and through the production and release of ROS and proteases.[94] The reduction of the sinusoidal lumen decreases blood flow, producing leukocyte accumulation, impairing reperfusion of liver parenchyma even after blood flow is reestablished. IRI increases ET-1, disrupting the balance between the vasoconstriction effects of ET-1[95] and the vasodilating actions of NO.[96,97] Stellate cells also respond to ET-1 and NO variations, affecting microcirculatory blood flow as well.[96–98]

EC and hepatocytes are the main targets of IRI. One of the mechanisms by which IR produces cell death is by apoptosis,[99] which requires caspase expression.[100,101,102] However, necrosis has been documented to play an important role in cell death after IRI. Because of these findings, cell death occurring via both pathways overlap, under the concept of necroapoptosis.[103]

IPC Applications within Liver Surgery

Based on our current understanding of the pathophysiology of IRI, several techniques have been adapted to reduce blood loss and counteract the effects of ischemia on hepatic parenchyma during liver resection surgery.[104,105]

It is well documented that one of the main problems associated with hepatic surgery is blood loss, with a clear relationship between the severity of the hemorrhage and postoperative complications.[51] The risk of major hemorrhage is inherent to liver surgery. It can occur during mobilization of the liver, dissection of the portal triad, or division of the hepatic parenchyma. Moreover, hemorrhage can also occur postoperatively and can compound the oxidative stress induced intraoperatively.[106] If the blood loss is substantial, the hypotension, body fluid distribution, and ischemia induced by hypovolemic shock significantly increases morbidity and mortality. Hemorrhage and hypovolemic shock increase the need for blood transfusions, and transfusions *per se* increase the risk of postoperative complications[107] and represent a greater risk of infections due to the immunosuppressive effects of allogenic blood.[108,109]

Occlusion of the portal triad (Pringle maneuver) is still employed almost a century after its original description to interrupt hepatic blood flow and decrease hemorrhage.[110] One technical variation is ipsilateral occlusion of the hepatic artery and portal vein instead of clamping the entire portal triad.[52,104,111] It is important to emphasize the differences between continuous and intermittent vascular occlusion of the liver. Continuous vascular occlusion (CVO) of the portal triad has proven effective in reducing hepatic hemorrhage, thus, decreasing the need of transfusions, but prolonged CVO results in greater IRI. To reduce the effects of continuous ischemia, hepatic blood flow can be reduced intermittently by clamping and unclamping the hepatic pedicle.[112] Intermittent vascular occlusion (IVO) allows for short periods of ischemia (from 15 to 30 min), followed by brief periods of reperfusion (from 5 to 10 min).[113] Based on this theory, experimental[113–115] and clinical[33,52] studies have demonstrated the efficacy of IVO to decrease IRI when compared to CVO, resulting in better hepatic function as evident by less elevation of liver enzymes and improved survival and allows for longer ischemic periods.[52,115] However, IVO is associated with increased blood loss during each reperfusion period.[32,113] Although the mechanisms by which IVO reduces the intensity of IRI are unclear, reduction in hepatocyte apoptosis is one major factor, particularly in procedures with prolonged ischemic periods (larger than 75 min).[116] Both CVO and IVO are protective against IRI by maintaining hepatic microcirculation and decreasing Kupffer cell activation for clinically relevant ischemic periods. Intermittent clamping appears superior for prolonged ischemia.[70] Many of the changes observed in IVO's cytoprotection against IRI are similar to those observed during ischemic preconditioning.[32]

Figure 1 presents a schematic description of IPC based on the different strategies of hepatic IR. The times have been adjusted according to trials that favor IPC and represent continuous ischemia and intermittent portal triad clamping.

Clinical Applications of IPC During Hepatic Resection

IPC should be applicable clinically during hepatic resection and transplantation. In humans, IPC was first performed on 24 patients undergoing elective hemihepatectomy and significantly reduced elevation in postoperative serum transaminases and decreased EC apoptosis.[32] The same group also conducted a randomized prospective study of IPC for hepatic resection. They evaluated IPC in 100 patients with similarly favorable results, particularly in patients with steatosis.[33] Several subsequent studies have reproduced the beneficial effects of IPC during hepatic resection surgery.[117–119] These studies have also demonstrated an improved hemodynamic stability in the post-reperfusion stage,[118] better postoperative evolution of cirrhotic patients,[119] and greater protection and tolerance to longer ischemic periods.[117] Another randomized study showed less postoperative hemorrhage, bile leak, and liver failure with use of IPC.[120] However, in spite of the encouraging findings in several randomized clinical studies, another group was not able to demonstrate any benefit to IPC during hepatectomy under vascular exclusion of the

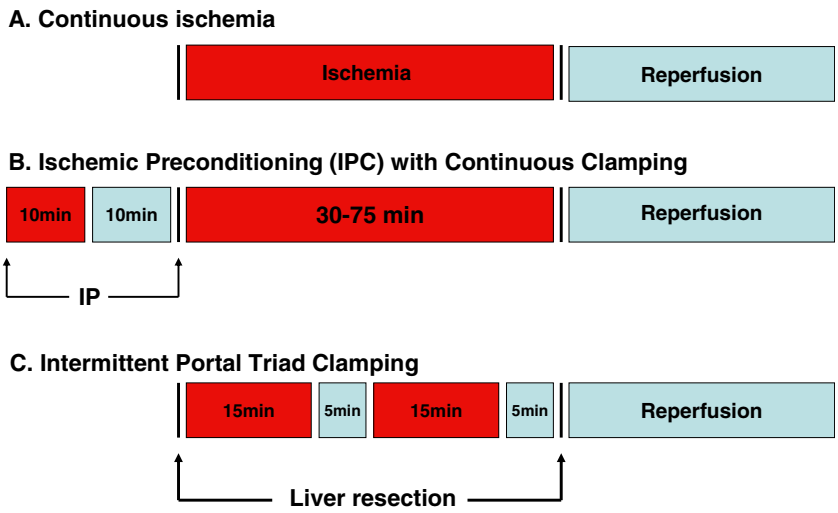

Figure 1 Surgical strategies for liver IPC.

liver with preservation of the caval flow.[121] In this study, IPC did not improve postoperative liver function or affect morbidity/mortality rates.

IPC and Liver Transplantation

It stands to reason that IPC could also be applied in the liver transplant setting which involves donor graft ischemia associated with the donor condition, followed by a prolonged cold storage period, and then warm ischemia during implantation and reperfusion. However, there is significant controversy regarding the use of IPC during donor hepatectomy. A recent review on this topic revealed lack of evidence to support or refute this practice.[122] Ischemic preconditioning for 10 min in deceased donors decrease serum transaminases but did not appear to have any long-term clinical impact on liver graft survival.[123] A prospective trial of IPC at time of deceased donor hepatectomy with donor clamping for 5 min showed that deceased donor livers can tolerate 5 min of ischemia, but application of IPC did not decrease graft injury posttransplant.[124] Recently, the same group published a randomized controlled trial using 10 min of IPC instead of the 5 min in their pilot study. Fifty donors were randomized to 10 min of IPC vs. 51 controls without IPC; however, no clinical benefit was demonstrated in the patients that were transplanted with the IPC-preconditioned donor grafts.[125] The authors suggest that use of other potential strategies including pharmacological therapy combined with IPC.[125] In contrast, another group performed a randomized prospective study with 60 liver transplant patients and showed a significant improvement in IRI and decreased apoptosis in the 10-min IPC group.[126]

IPC and Steatosis

Steatotic livers are particularly susceptible to IRI, and fatty livers have a greater risk of poor graft function or primary nonfunction after liver transplantation.[127] Use of IPC was especially beneficial in a subset of patients undergoing elective hepatic resection,[33] a finding well-documented in experimental models.[128,129] Recently, promising results were observed in steatotic livers using adiponectin, which activates peroxisome proliferator-activated receptor alpha (PPAR-α), a transcription factor that regulates inflammation in liver disease.[130] Improving the tolerance of steatotic livers to IRI with IPC and/or biochemical strategies could significantly increase the number of livers available for transplantation.

Conclusion

IR injury is important in both hepatic resection surgery and liver transplantation. IPC activates several signaling pathways leading to hepatic protection in animal models of liver surgery. However, the contradictory results with use of IPC during elective hepatic resection in randomized clinical trials from experienced groups underscores the complexity (or diversity) of the patient population undergoing hepatic resection surgery and the caution in routine clinical application. Further clinical trials are needed to identify the precise patient population that will benefit from IPC. Moreover, combined strategies of IPC with pharmacologic modulation may be useful, particularly with steatotic livers. Finally, further basic research into the complex signaling of IPC during hepatic surgery is warranted to better elucidate the mechanisms of protection.

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REVIEW ARTICLE

Laparoscopic Treatment of Stone Recurrence in a Gallbladder Remnant: Report of an Additional Case and Literature Review

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Abstract
Cholecystectomy is an effective treatment of gallstones. Nevertheless, recurrence of biliary symptoms following cholecystectomy, either laparotomic or laparoscopic, is quite common. Causes are either biliary or extrabiliary. Symptoms of biliary origin chiefly depend on bile duct residual stones or strictures. Rarely, they depend on stone recurrence in a gallbladder remnant. Diagnosis of gallstone recurrence in gallbladder remnant is difficult, mainly arising from ultrasonography, computed tomography, magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography.
Incomplete gallbladder removal may be either voluntary or inadvertent: in the first case, it is performed to remove gallstones without dissecting a difficult Calot's triangle or an excessively bleeding posterior wall of gallbladder caused by liver cirrhosis. Available data do not support the hypothesis that laparoscopic cholecystectomy entails an increased incidence of this condition, in spite of some opposite opinions. Treatment of lithiasis in gallbladder remnants is chiefly surgical. Although technically demanding, completion cholecystectomy can be safely performed in a laparoscopic way. We report a case of stone relapse in a gallbladder remnant, discovered 16 years following laparoscopic cholecystectomy and successfully treated by laparoscopic completion cholecystectomy. We furthermore review literature data in order to ascertain whether recent large diffusion of laparoscopic surgery causes an increase of such cases.

Keywords Gallstone · Cholecystectomy · Postcholecystectomy syndrome · Gallbladder remnant · Gallstone recurrence

Foreword

Cholecystectomy is a well-established effective operation which generally provides total relief of presurgical symptoms due to gallstones in up to 90% of patients. Nevertheless, after successful cholecystectomy, a small group of subjects continue to experience symptoms of serious and severe episodes of upper abdominal pain, similar to those experienced prior to surgery: this represents the so-called postcholecystectomy syndrome (PCS).[1,2] Incidence of persistent or recurrent gastrointestinal symptoms after cholecystectomy varies largely in different reports[3–5] ranging between 10% and 40%. Although psychological factors may play an important role in the onset of subjective symptoms in at least a subgroup of the PCS patients as a form of somatization,[6] the great majority of them continues to suffer from disorders of organic origin. This painful syndrome may depend on numerous extrabiliary as well as biliary disorders. Biliary strictures, retained stones in the common biliary duct, cystic duct stump, stenosis of Oddi's sphincter, are the main biliary causes of complaints.[7] A small amount of postcholecystectomy syndromes are related to a residual stone in a particularly long cystic duct[8] or to the relapse of lithiasis in a gallbladder remnant.[9] This latter condition, namely the so-called in former times gallbladder regeneration after cholecystectomy performed for lithiasis, is a consequence

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of either incomplete (unintentional or intentional) gallbladder removal or missed gallbladder duplication (or even triplication) with a retained gallbladder. A few cases concerning this condition are anecdotally reported in the last 50 years.[10–12] Incidence of incomplete gallbladder removal following laparotomic cholecystectomy appears very low,[1,2] although partial cholecystectomy was already described many years ago as an advisable technique in situations which would make the dissection of the Calot's triangle exceedingly difficult or separation of the posterior wall of gallbladder from the liver bed dangerous due to the difficult-to-control bleeding.[13–17]

In the laparoscopic era, data concerning the true incidence of unintentional incomplete gallbladder removal are uncertain, but they seem slightly larger than those previously reported with open cholecystectomy, according to the number of anecdotal reported cases.[18–28] Two main causes could be assumed to explain this: first, the tendency in laparoscopic cholecystectomy toward interrupting the cystic duct far away from his insertion on the common bile duct to avoid the risk of bile duct injury; second, the undiminished tendency toward delayed surgical treatment of acute cholecystitis, although clear evidence now exists in favor of early (within 72 h from onset of symptoms) cholecystectomy.[29–31] The hypothesis of delayed surgical treatment of cholecystitis, performed when adhesions and scars make gallbladder dissection exceedingly difficult, could be responsible for a greater number of inadvertent partial cholecystectomy.

Until recently, laparoscopic intentional partial cholecystectomy has been advocated as a safe and viable option in the emergency treatment of complex acute cholecystitis[32–35] and in patients with cirrhotic portal hypertension.[36] This nonconventional surgery allows removal of difficult gallbladder without dissection in Calot's triangle thus minimizing the risk of injury of bile duct and cystic artery and excessive bleeding that is difficult to control. Few data exist about incidence of recurrent or residual gallstones after intentional incomplete cholecystectomy which widely ranges between 0.0% and 16%.[37,38]

A small number of gallstone recurrence after cholecystectomy may arise from missed duplicated or accessory gallbladder.

Diagnosis of stone recurrence in a gallbladder remnant is not easy: it arises mainly from ultrasonography (US), computed tomography (CT), magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), and endoscopic ultrasonography (EUS).

In case of recurrence of lithiasis in a gallbladder remnant, surgical treatment is advised.

Due to the anecdotal character of the published reports concerning this condition, uncertainty still exists on what would be the best treatment of stone recurrence after partial cholecystectomy. Laparoscopic approach is generally regarded as particularly difficult due to the effects of the scar formed in consequence of the previous surgery around the Calot's triangle, so it is deemed advisable only if performed in institutions with advanced laparoscopic facilities.[37] Nevertheless, an increasing number of reports concerning this kind of approach are currently published.[4,39] Quite recently, laparoscopic approach was proposed even to treat Mirizzi's syndrome "type I" caused by retained stone in a gallbladder remnant.[40]

Our aim is to report a further case of stone recurrence in a gallbladder remnant, successfully treated by laparoscopic removal, and to review the previously reported cases. On this basis, we attempt to speculate whether laparoscopic cholecystectomy, particularly when performed to treat gallbladder cholecystitis, could produce an increased incidence of such cases. Finally, we consider the best treatment strategy in case of gallstone relapse in a gallbladder remnant however produced.

Literature Review Criteria

PubMed and EMBASE searches were performed limited to English, Italian, Spanish, and French language reports. Our choice bell on the following terms (alone or in some combination): "gallbladder," "gallstone," "cholecystectomy," "partial," "incomplete," "subtotal," "postcholecystectomy syndrome," "gallbladder regeneration," gallbladder pseudoregeneration," Mirizzi syndrome," "gallbladder double," "gallbladder duplicated," "gallbladder triple," "cholecystitis," "accessory gallbladder." Available bibliographic data from the reports found were also considered.

Case Report

A 52-year-old male patient was referred to our institution because of repeated abdominal severe pain episodes localized in the upper right abdomen. In 1992, the patient underwent laparoscopic cholecystectomy for lithiasis elsewhere. Operation and recovery were reported uneventful.

At the hospital admission, routine blood tests were all within normal range, and liver function test values were normal. US abdominal scanning showed a "pseudogallbladder" close to the common bile duct, containing a large stone. MRCP showed a gallbladder remnant the dimensions of which approximately ranged from 4 to 5 cm in diameter (Fig. 1). It was impossible to ascertain by means of radiological images whether it would be a gallbladder remnant due to previous partial gallbladder removal or a residual missed gallbladder of a pair. Due to the repeated

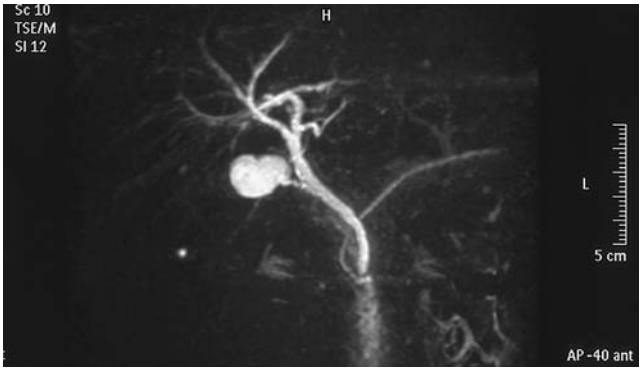

Figure 1 T2-weighted MRCP with MIP reconstruction showing fluid distension of residual cystic duct and gallbladder remnant with filling defect sustained by biliary stones.

painful episodes and the radiological findings, laparoscopic intervention was eventually proposed and informed consent was obtained. After a single intravenous dose of amoxicillin–clavulanic acid (2.000+200 mg) shortly before surgery, the operation was performed under general anesthesia through four trocars (the first of them was inserted in an open way) in the usual manner. Numerous fibrotic adhesions between the liver, transverse colon, duodenum, and great omentum were carefully removed to free the gallbladder remnant and to expose the Calot's triangle. Finally, gallbladder remnant containing stone could be firmly grasped and cystic artery and cystic duct were separately isolated and interrupted between clips. Intraoperative cholangiography was not performed. Ultimately, gallbladder remnant was successfully removed in a retrograde way. Close to the neofundus, two firmly adhering retained clips were seen, indicating a previous incomplete gallbladder removal (instead of a missed duplicated gallbladder). Residual gallbladder's content consisted of a mixed-type biliary stone 15 mm in diameter (Fig. 2). A subhepatic drain was inserted through one of the trocars and left for 24 h. Recovery was uneventful and discharge was possible in 48 h. An outpatient clinical control was made 7 days later and no complications were seen. Eight months after operation, the patient fared well.

Discussion

Postcholecystectomy syndrome is a well-known long-term complication of both laparotomic and laparoscopic gallbladder removal. The main causes are both biliary and nonbiliary; retained stones in the bile ducts are quite common; biliary strictures, cystic duct stump neuroma, and stenosis of Oddi's sphincter are rare causes; even more rarely painful symptoms, similar to those felt before the cholecystectomy, depend on lithiasis recurrence in a gallbladder remnant.[2,26] Reporting on 285 laparotomic intervention for postcholecystectomy syndrome, Glenn et al.[1] could not find any case of gallbladder remnant, although in a previous report he described 35 patients operated on for postcholecystectomy syndrome, in eight of whom he could find a gallbladder remnant.[41] Similarly, Tondelli and Gyr,[42] in their extensive review of the literature pertaining to postcholecystectomy syndrome in prelaparoscopic era, could not find any definite case of lithiasis in gallbladder remnant, although he cited a few reported cases of stone recurrence (or residual) in the cystic duct remnant after cholecystectomy. Conversely, Moody[43] included gallbladder remnant among the main causes of postcholecystectomy syndromes; he cited Bodvall's previous experience concerning 26 cases of gallbladder remnant as a cause of postcholecystectomy syndrome observed in a total of 103 cases operated on, equal to 25%.[44] Furthermore, he could find a little less than a thousand such cases in the past literature from the beginning of the century. Before him, other such cases have been anecdotally reported.[45–48] Bordley and White[49] reported 17 out of 340 (6.4%) reoperations on extrahepatic bile ducts performed to treat cystic duct or gallbladder remnant, without mention of stone recurrence in them; unfortunately, in their large report, he did not distinguish between the two pathologies, so that data concerning the exact number of reoperations performed to remove a gallbladder remnant are lacking. Deziel[50], in his extensive review, defined "unusual," among the late biliary complications of laparotomic cholecystectomy, a retained gallbladder remnant containing calculi. Zhou could find residual stones in cystic duct remnant or in inflammatory "small gallbladder" in only four out of 386 (1.04%) patients complaining for postcholecystectomy syndrome (whether laparoscopic or laparotomic).[51]

Liu et al.[52] quite recently reported about 149 biliary reoperations on patients previously submitted to cholecystectomy (only some of them laparoscopic) in a range of

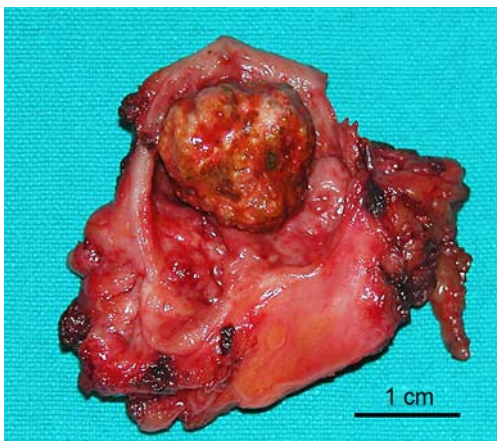

Figure 2 The open surgical specimen showing one mixed-type biliary stone inside.

time varying from 2 months to 18 years: he accounted for residual cholecystitis with or without stones in 28 (18.8%) of them. No explanation of such a notable incidence was attempted by the authors.

Recent progress in radiological imaging has greatly improved diagnostic accuracy in detecting the causes of postcholecystectomy syndrome.[8,53] US, CT, ERCP, and MRCP—these latter having comparable high sensitivity, specificity, and accuracy—are all effectively used to achieve diagnosis of gallbladder remnant with or without stones in patients complaining of symptoms consistent with postcholecystectomy syndrome. Nevertheless, diagnosis of residual gallbladder with gallstones remains difficult. When CT, ERCP, and MRCP all fail to reveal the presence of residual gallbladder, EUS could be an extremely valuable method to visualize small gallbladder remnant with stones.[25] The case we were dealing with was diagnosed trough US and MRCP, the former giving rise only to a suspect of lithiasis recurrence after incomplete gallbladder removal, the second being conclusive.

Differentiating between grossly dilated cystic stump and true gallbladder remnant is impossible even on the basis of histological findings,[54] so we agree to adopt Bodval's definition of gallbladder remnant "as a wider part of the free end of the remnant cystic duct, giving the impression of a diminutive gallbladder."[44] Applying this definition, our personal case of postcholecystectomy syndrome described as due to cystic duct remnant containing stones, as well as those reported by Shaw and others,[55] could be actually considered as gallbladder remnant with relapsed or residual stones.

Stone recurrence in a gallbladder remnant after cholecystectomy, either laparotomic or laparoscopic, may arise alternatively from three different conditions: inadvertent incomplete gallbladder removal, incorrectly performed subtotal intentional cholecystectomy (fundectomy alone), or ultimately the existence of a duplicated or even triplicated gallbladder inadvertently missed at the intervention (or probably voluntarily missed because seemingly healthy).

Incomplete gallbladder removal during cholecystectomy may be both voluntary and inadvertent. Subtotal cholecystectomy in case of difficult gallbladder depending on portal hypertension or cholecystitis is a well-established technique.[13] Since the second half of the 1990s, it was advocated also to treat Mirizzi's syndrome "type I"[56] (according to Csendes et al.[57] classification), although the laparoscopic indication in such cases is still not well defined.

Intentional laparoscopic subtotal cholecystectomy is reported since the early 1990s.[58–62] In most cases, such a procedure allows us to accomplish laparoscopically an intervention that otherwise would have been performed in a laparotomic way. This choice seems advisable, assuming that, in a number of cases of converted laparotomic cholecystectomy, partial gallbladder removal is however performed.[63]

Some criticism has been brought to consider the laparoscopic partial cholecystectomy as an alternative to conversion to open surgery when inflammation precludes safe laparoscopic intervention,[64,65] although no meaningful direct comparisons of subtotal laparoscopic cholecystectomy versus conversion to open cholecystectomy, as far as we know, are reported. Indications to laparoscopic or laparotomic subtotal cholecystectomy are exactly the same.

If correctly performed according to the Bornmann and Terblanche[13] rules, intervention would not leave any portion of gallbladder or Hartmann pouch connected to the biliary tree, so virtually it could not produce a relapse of gallbladder stones. Nevertheless, in case that a blind pouch is formed by approximation of Hartman's pouch, residual or recurrent stone is a possibility.[16] Palanivelu et al.[66] performed partial cholecystectomy in 206 out of 265 cirrhotic Child A and B patients complaining of gallstones symptoms: three of them (1.1%) were found to have stone in the gallbladder remnant on routine follow-up within the first year after operation.

Although rarely, subtotal cholecystectomy received some criticism because of the actual risk of relapse of gallstones[67] but precise data about incidence of this complication are lacking. In none of the 56 patients submitted to partial cholecystectomy by Chowbey et al.[62] was relapse or residual stones reported. Instead, Beldi and Glättli[38] found residual stones in six out of 46 patients submitted to partial cholecystectomy, after a median interval of 19 months (range 6–54 months) following surgery.

The main question is whether a larger diffusion of laparoscopic subtotal cholecystectomy to treat "difficult" gallbladder in order to reduce the number of laparotomic conversions can later produce an increased number of cases of residual or recurrent stones in gallbladder remnant. Up-to-date available data do not sustain this hypothesis but surgeons must be aware of this possibility.

The first case of inadvertent subtotal laparoscopic cholecystectomy was reported by Blackard and Baron[19] in 1995. It concerned a patient laparoscopically treated for biliary stones that showed an "anatomy...quite unclear," a large cystic duct, and the suggestion of an anatomic anomaly. Six days after discharge, the patient developed a biliary peritonitis caused by the leak of gallbladder remnant close to the site of a surgical clip. The placement of an endoprosthesis (i.e., a biliary stent) in the attempt to close the leak was ineffective. The patient could rapidly recover only after an open completion cholecystectomy.

Following this report, a few others were published.[18,20,21,23–25,27,28,68]

Recent reported cases are dealing mainly with the first condition considered that is inadvertent incomplete gallbladder removal.

Uncertainty still exists whether laparoscopic cholecystectomy entails more risk of inadvertently incomplete removal of the gallbladder, as claimed by Shaw et al.[55] and Witson and Wolpert,[26] due to the attempt to interrupt the cystic duct as near to the infundibulum as possible to avoid incidental lesion of common bile duct. Existing data do not actually support this thesis.

As far as an intentional partial cholecystectomy is concerned, rigorous accomplishment of laparoscopic (or laparotomic) gallbladder removal according to the established rules by the Bornman and Terblanche could minimize but not entirely rule out the risk of gallstone recurrence. As a matter of fact, if a "fundectomy" is performed instead of a true subtotal cholecystectomy, gallstones relapse is more than a possibility. This seems to be the case, at least in few instances, and certainly this was the cause of stone recurrence in the patient we present here, considering the presence of clips applied on gallbladder remnant neofundus.

As far as duplication of gallbladder is concerned, this anomaly can be accountable for incomplete gallbladder removal as assumed by Cohen et al.[20] in his anecdotal report. Duplicate gallbladder could be missed at laparoscopic exploration of peritoneal cavity, mainly if cholecystectomy is performed to treat acute cholecystitis, due to the effects of inflammation on biliary anatomy. Gallbladder duplication is a very rare condition, occurring approximately at a rate of 1:3–4,000 in autopsy series.[69]

Preoperative diagnosis of twin gallbladder is very rare,[70] in no more than half cases in large reported series.[71] In fact, ultrasonography, which is ordinarily sufficient for preoperative investigation of gallbladder diseases, may fail to detect a second gallbladder due to the insensitivity of the test itself or misinterpretation of the findings.[72,73] Moreover, even the laparoscopic exploration of peritoneal cavity may produce the same disappointing result,[74] so that single-staged successful laparoscopic removals of double gallbladders are rarely reported.[75,76] Either gallbladder may be stone-diseased[77] thus causing missing or, potentially, intentional sparing of one gallbladder in the course of laparotomic as well laparoscopic cholecystectomy, with subsequent possible complications or relapse of lithiasis[78] and the need of a second operation to remove the missed gallbladder.

Time interval between incomplete gallbladder removal (in any way accomplished) and biliary symptoms recurrence varies largely from a few days to many years: noteworthy in our case, the onset of symptoms occurred 16 years later after the incomplete cholecystectomy.

In case of stone recurrence in gallbladder remnant (as in an enlarged cystic duct remnant), surgery is largely advocated, although extracorporeal shockwave lithotripsy is also reported.[79] Successful interventions using ERCP alone are rarely cited.[80] Laparoscopic approach is generally regarded as particularly difficult due to the effects of the scar around the Calot's triangle formed in consequence of the previous surgery, so it is deemed advisable only if performed in institution with advanced laparoscopic facilities.[37]

To our knowledge, the first reported case of successful laparoscopic gallbladder remnant removal is attributed to Gurel et al.[81] Later on, an increasing number of reports concerning this kind of approach is published.[4,28,37,39,82,83] Three out of 206 cirrhotic patients operated on by Palanivelu et al.[66] with modified subtotal cholecystectomy later developed gallbladder remnant stone recurrence: all three underwent successful laparoscopic removal of gallbladder remnant.

Although some previously reported contrary opinions, actual laparoscopic approach to biliary tract reoperation appears to be a minimally invasive, safe, feasible, and effective procedure when done by expert laparoscopic surgeons.[83]

In our opinion, in case of laparoscopic completion cholecystectomy, preoperative MRCP imaging could render unnecessary intraoperative cholangiography or ultrasonography to prevent bile duct inadvertent lesions or common bile duct stone missing, according to data previously reported.[84] In our single experience, gallbladder remnant removal could be laparoscopically safely performed on the basis of MRCP imaging alone.

Conclusions

After successful cholecystectomy for gallstone disease, either laparotomic or laparoscopic, a number of patients ranging between 10% and 40% show symptoms resembling those previously experienced or however related to the biliary tract. The causes are both extrabiliary and biliary; among these, the main causes are common bile duct stones or biliary strictures. Only a small amount of patients suffering for postcholecystectomy syndrome shows lithiasis relapse in a gallbladder remnant. Residual gallbladder may arise from inadvertent or voluntary incomplete gallbladder removal or from a missed duplicated or accessory gallbladder.

Although partial cholecystectomy is a proven safe and viable option in the emergency treatment of complex acute cholecystitis, if correctly performed, it does not seem to produce increased incidence of gallstone relapse. Until now, no data exist indicating an increased incidence of such complication in laparoscopic era, although a number of anecdotal reports, concerning gallstone recurrence after incomplete cholecystectomy, have been recently published.

Among the causes of postcholecystectomy syndrome, residual or recurrent stones in a gallbladder remnant must be certainly considered.

Diagnosis of lithiasis in gallbladder remnant is difficult; it may arise from US, CT, and ERCP, MRCP and EUS being the best methods, although no sufficient data exist about sensitivity and specificity of each method due to the paucity of reported cases in literature.

Treatment is chiefly surgical, although, in case of Mirizzi syndrome depending on a stone recurrence in gallbladder remnant, extracorporeal shockwave lithotripsy and ERCP could represent a viable alternative. Completion cholecystectomy can be safely performed trough laparoscopic approach, on condition that it is performed by experienced laparoscopic surgeons.

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GI IMAGE

Low Common Bile Duct Bifurcation Incidentally Discovered During Pancreaticoduodenectomy

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Abstract
Introduction Bile duct injury due to failure to recognize anatomical variations can have considerable consequences.
Discussion We report an incidental discovery of a low common bile duct bifurcation below the level of the cystic duct, incidentally discovered during pancreaticoduodenectomy.

Keywords Bile duct injury · Anatomical variations · Pancreaticoduodenectomy

An 80-year-old male patient developed obstructive jaundice due to biliary obstruction subsequently decompressed by endoscopic stent. A computed tomography scan of the abdomen revealed a resectable pancreatic head mass. The patient was consented and brought to the operating room for pancreaticoduodenectomy.

Staging laparoscopy revealed no extrapancreatic disease. After mobilization of the right colon, a wide Kocher

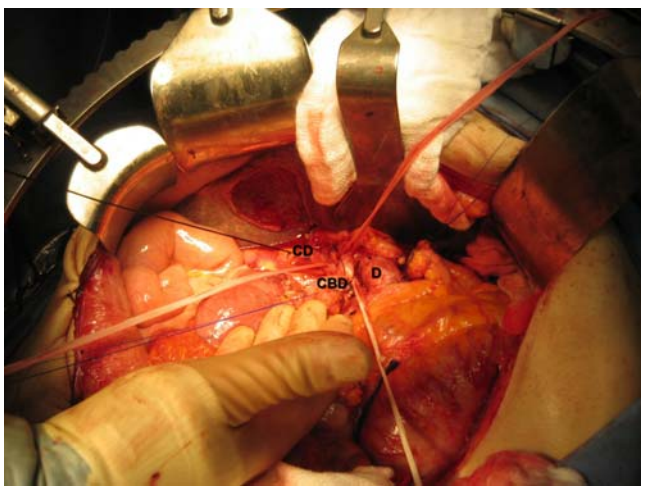

Figure 1 After mobilization of the duodenum (*D*) by Kocher maneuver, a dilated common bile duct (*CBD*) was identified. The gall bladder is removed and the cystic duct (*CD*) is ligated.

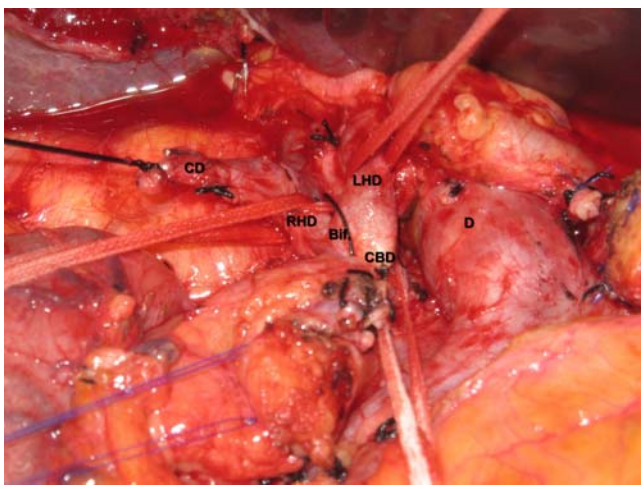

Figure 2 The duodenum (*D*) is completely mobilized. Careful evaluation of the biliary duct reveals a low bifurcation (*Bif*) of the common bile duct (*CBD*) below the level of the cystic duct (*CD*). The CD arise from the right hepatic duct (*RHD*). If unrecognized, a left hepatic duct (*LHD*) injury can occur.

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maneuver was undertaken, with full mobilization of the second part of the duodenum exposing a significantly dilated biliary duct measuring about 1.5 cm (Fig. 1). A careful evaluation of the biliary duct revealed a low bifurcation of the common bile duct well below the origin of the cystic duct (Fig. 2) arising from the right hepatic duct. Circumferential control of the biliary duct was achieved. A cholecystectomy was performed with fundus first technique and the cystic duct was identified and divided between two silk ties. The common bile duct below the bifurcation and away from the tumor was divided and the procedure was completed as planned with a choledochojejunostomy using a running 4/0 absorbable monofilament suture, an end to side pancreatojejunostomy, and a Roux en Y gastrojejunostomy.

Pathological evaluation of the resected specimen revealed a T3N1 pancreatic adenocarcinoma invading the common bile duct, but with negative margins. If undiagnosed, a division of the right hepatic duct mistakenly taken for the common bile duct would have resulted in a major biliary injury of the left hepatic duct with considerable consequences. We hope that this report will stress on the importance of definitive identification of biliary anatomy before proceeding with biliary division and reconstruction.