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2009 SSAT POSTER PRESENTATION

Applicability and Feasibility of Incorporating Minimally Invasive Esophagectomy at a High Volume Center

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Introduction

Esophageal resection remains an integral part of treatment for esophageal cancer. Traditionally, the two open operative techniques which incorporate a cervical esophago-gastric anastomosis have been transhiatal esophagectomy (THE) and transthoracic esophagectomy, with en bloc lymphadenectomy (TTE). The clinical decision as to which of these two procedures would best serve the patient often rests on the following notion: the goal of THE is to reduce early postoperative morbidity and mortality by avoiding a thoracotomy, whereas the goal of TTE is to increase long-term survival by employing wide excision and extensive node dissection in the mediastinum and abdomen.[1,2] En bloc esophagectomy has been shown to increase survival by decreasing locoregional disease recurrence related to micrometastatic disease.[3] Transhiatal esophagectomy is often associated with increased locoregional failure rates in the absence of extended lymphadenectomy and has the potential to cause significantly more bleeding than other approaches because of the blunt mediastinal dissection.[1,4] Despite these concerns, THE is a valid option for patients with or at risk for respiratory disorders, as it significantly decreases perioperative pulmonary complications.[1,4]

Esophageal resections are associated with a morbidity and mortality of up to 70% and 14%, respectively.[4,5] Minimally invasive esophagectomy (MIE) has been proposed as a method of esophageal resection for malignant esophageal diseases. Some of the proposed benefits of MIE as compared with open techniques include decreased time to recovery and shorter hospital stays, benefits that are deemed important especially in patients with poor prognosis who may spend a significant proportion of their survival recovering from the procedure itself. Other benefits may include decreased blood loss, fewer wound and pulmonary complications, and less postoperative pain.[1,6] Additionally, superior laparoscopic visualization may enhance mediastinal dissection.[1] The procedure is not met with universal acceptance amongst surgeons, however, because of a steep learning curve, longer operative duration, perceived inadequate oncological integrity, and increased risk of gastric conduit ischemia with a minimally invasive technique.[6]

Minimally invasive esophagectomies have been incorporated into our operative armamentarium since 2005. The aim of this study is to assess a high volume center's experience with MIE and to compare the morbidity and mortality associated with different techniques.

Materials and Methods

Data Collection

Patients undergoing esophageal resection at Creighton University Medical Center were entered into a prospectively maintained database. After approval from the Institutional

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Review Board, the database was queried to identify patients who underwent esophagectomy for malignant disease. Data regarding patient characteristics and co-morbidities, tumor staging, neoadjuvant therapy, preoperative work-up, operative findings, postoperative course, and complications was collected into an Excel database (Microsoft Office Excel ® 2003) and analyzed. Patients undergoing emergent procedures, those with Ivor Lewis esophagectomy (intra-thoracic anastomosis), or those with colonic or jejunal interposition were excluded, as were patients not undergoing lymph node dissection (primary diagnosis of high-grade dysplasia).

Pre-treatment Staging

The staging work-up was individualized and affected whether the patient was seen before or after initiation of neoadjuvant treatment. All patients had CT scan and positron emission tomography scan at diagnosis. In patients who underwent neoadjuvant treatment, a post-treatment CT scan was done 3–4 weeks after completion of the therapy. Endoscopic ultrasound was performed selectively (usually avoided in patients with bulky disease in whom the decision to give neoadjuvant treatment had already been made). In some instances, patients receiving neoadjuvant therapy also underwent pre-treatment staging laparoscopy and laparoscopic ultrasound with or without a feeding jejunostomy tube placement.

Neoadjuvant Regimen

The decision to undergo neoadjuvant therapy followed by surgery versus primary surgery was usually made in consultation with the referring oncologist (most patients were seen after an oncology consult), and the regimen used varied extensively. However, some generalizations regarding treatment strategy can be made: most patients with node-positive disease underwent neoadjuvant therapy whereas those with node-negative disease (specially T1 or T2, N0, M0) underwent primary resection; the chemotherapy regimen usually consisted of a platinum-based agent with 5-fluorouracil; the radiotherapy dose ranged from 40–60 Gy.

Surgical Technique

Transhiatal Esophagectomy

The surgical technique for THE has been described previously by Orringer et al..[7] Following extensive upper abdominal lymphadenectomy, the greater curvature of the stomach and the distal esophagus is mobilized, preserving the right gastroepiploic artery. The hiatal dissection is performed, and the periesophageal tissues contained within the mediastinal pleura on either side of the distal esophagus are mobilized en bloc with the esophagus. An oblique incision is made paralleling the anterior border of the left sternocleidomastoid. After identifying the esophagus at the level of the thoracic inlet, blunt dissection is carried in the paravertebral plane. The esophagus is then mobilized and divided at the level of the thoracic inlet, and the thoracic esophagus is delivered through the diaphragmatic hiatus. Partial gastrectomy is performed to create a long, narrow gastric tube which is then pulled into the neck and anastomosed to the cervical esophagus. The hiatus is partially closed, and the gastric tube is secured to the hiatus to prevent herniation. A pyloromyotomy is performed to prevent post-vagotomy delayed gastric emptying, and a jejunal feeding tube is placed.

Open Transthoracic Esophagectomy

Details of this technique have been described elsewhere.[8] A right posterolateral thoracotomy is performed. The esophagus is mobilized en bloc, including the azygous vein, thoracic duct, and surrounding lymph node-bearing mediastinal tissues. Dissection is continued over the aorta onto the left pleura. Anteriorly, the dissection is carried on the pericardium to connect with the posterior dissection. The dissection then proceeds superiorly to the carina to include the lymphatic tissue and inferiorly down to the esophageal hiatus. After repositioning the patient, the distal esophagus and stomach are mobilized via a midline laparotomy, as in the transhiatal procedure. Additionally, a celiac axis lymphadenectomy is performed. The cervical part of the procedure is performed in a similar fashion to transhiatal esophagectomy. Finally, a pyloromyotomy and feeding jejunostomy are performed.

Minimally Invasive Esophagectomy

Our technique for minimally invasive esophagectomy was adopted from that which was described by Kent et al. in 2006.[9] Five ports are placed for the thoracoscopic part of the procedure in the left lateral decubitus position. The azygous vein is divided. Lymphadenectomy is performed in continuty with the esophagus. The mediastinal dissection of the esophagus is carried inferiorly to the hiatus and superiorly to the carina. If identified, the thoracic duct is divided between clips. The patient is repositioned, and laparoscopic ports are placed in the abdomen. The dissection is carried over the greater curvature, while preserving the right gastroepiploic artery. Celiac axis lymphadenectomy is performed. The dissection is carried into the mediastinum to meet the dissection that was previously done through the chest. A gastric conduit is fashioned with a generous partial proximal gastrectomy. An

oblique incision is made in the left neck anterior to the sternocleidomastoid muscle. The esophagus is dissected to the level of the thoracic inlet, and using laparoscopic guidance, the specimen is pulled into the neck. A cervical anastomosis is created. Finally, pyloromyotomy is performed, and a feeding jejunostomy is placed.

Postoperative Care

All patients receive a preoperative epidural and are admitted to the intensive care unit (ICU) postoperatively. Great attention is paid to perioperative fluid management and pulmonary toilet. Tube feeds are started on postoperative day 3. Patients are closely monitored for any signs of complications which, if identified, are aggressively managed. The nasogastric tube is removed after return of gastrointestinal function. Contrast swallow study is performed routinely with gastrograffin followed by thin barium, usually on postoperative day 7. Cervical anastomotic leaks are managed conservatively.

Results

Demographics

One hundred and thirty-three total esophagectomies were performed at Creighton University Medical Center between 2003 and 2008 by a single surgeon (SKM), with more than 30 per year in the last 3 years. Eighty consecutive patients who underwent esophagectomy with gastric pull-up and cervical esophago-gastric anastomosis met inclusion criteria. Of these, 22 were open TTE, 33 open THE, and 25 were MIE. The average overall age of the patients undergoing esophagectomy for malignant disease was 62.2 years. The mean age of patients in the TTE group was significantly less than that in the THE group (58.9 versus 65.7 years). Ten (12.5%) of the patients were female. Esophagectomy was performed for adenocarcinoma in 69 of the 80 patients and for squamous cell carcinoma in the remaining 11 patients. There was a significantly lower prevalence of diabetes mellitus in the TTE group (4.5% versus 30.3% in THE and 28.0% in MIE). The cohorts were similar with respect to other patient variables and co-morbidities. The average number of co-morbidities per patient was compared for each of the three groups. Of note, the patients in the TTE group had an average of 0.73 co-morbidities per patient while the patients in the THE and MIE groups had an average of 1.12 co-morbidities per patient (Table 1).

Preoperative and Intraoperative Variables

Of the 80 patients undergoing esophagectomy, 53 received neoadjuvant chemo or chemoradiation therapy. The proportion of patients receiving neoadjuvant therapy was 72.7%, 60.6%, and 68.0% in the TTE, THE, and MIE groups ($p>0.05$), respectively. The majority of patients were stage II or III prior to treatment or resection. The anastomosis in most cases was stapled (85% of the patients in the series; Table 1).

Intraoperatively, blood loss was greatest in the TTE and least in the MIE group, but this difference did not achieve statistical significance. However, fewer patients in the MIE group required blood products than in the TTE or THE groups ($p<0.001$), and of those patients requiring blood products, fewer units were required in the MIE group. Estimated blood loss (EBL) in the series ranged from

Table 1 Preoperative Patient Characteristics

	Open TTE (n=22)	Open THE (n=33)	MIE (n=25)	p value
Age	58.9	65.7	62.0	0.003[a]
Females (%)	1 (4.5)	4 (12.1)	5 (20.0)	NS
Squamous cell carcinoma (%)	2 (9.1)	4 (12.1)	5 (20.0)	NS
Adenocarcinoma (%)	20 (90.9)	29 (87.9)	20 (80.0)	NS
Neoadjuvant (%)	16 (72.7)	20 (60.6)	17 (68.0)	NS
Diabetes mellitus (%)	1 (4.5)	10 (30.3)	7 (28.0)	0.019[a]/0.033[b]
Hypertension (%)	10 (45.5)	17 (51.5)	14 (56.0)	NS
Coronary artery disease (%)	3 (13.6)	7 (21.2)	5 (20.0)	NS
COPD (%)	2 (9.1)	3 (9.1)	2 (8.0)	NS
Other cancer (%)	2 (9.1)	3 (9.1)	6 (24.0)	NS
Smoking (%)	2 (9.1)	9 (27.3)	8 (32.0)	NS

NS nonsignificant, *COPD* chronic obstructive pulmonary disease

[a] Significant difference between TTE and THE

[b] Significant difference between TTE and MIE

200 cc to 4,500 cc, with the number of blood products administered ranging from 0 to 10 units of packed red cells. Average operative duration was shortest in the THE group and longest in the TTE group. Overall operative duration in the series ranged from 210 to 620 min, the shortest being in the THE group and the longest being in the MIE group. More lymph nodes were harvested from patients undergoing TTE than THE, with an average of 19.5 nodes harvested in the TTE group as compared with 13.2 nodes in the THE group ($p<0.01$). The average number of lymph nodes harvested in the MIE group was 17.2, which was also significantly greater than in the THE group (Table 2).

Postoperative Variables

The mean stay in the ICU was 8.3 days overall (the shortest stay being in the THE group and longest in the MIE group). The mean hospital stay was between 17 and 19 days and did not differ significantly among the three cohorts. Thirty-day mortality was not significantly different between the three groups and ranged from 0% in the TTE group to 4% in the MIE group. Overall operative and 30-day mortality was 2/80 (2.5%; Tables 2 and 3).

Postoperatively, five patients had an anastomotic leak, all from the THE group (overall leak rate of 6.25%). There was no significant difference in the incidence of other postoperative complications between the cohorts (Table 3). The rate of complications in this series is as follows: wound dehiscence 2.5%, chyle leak 12.5%, pneumonia 7.5%, pleural effusion 8.8%, ARDS 1.3%, and arrhythmia 33.8%.

Median survival for the MIE group is 22.8 months (mean=23.8, standard deviation 11.8) and 29.7 months for TTE (mean=24.6, standard deviation 19.9). The median survival for THE has not yet been reached. Median time to follow-up is 28.7 and 49.0 months for MIE and TTE groups, respectively. However, statistical analysis of survival for these cohorts is limited because the stage-specific groups are small within each type of surgery.

MIE Learning Curve

To determine whether our early experience with MIE (a plausible learning curve) affected outcomes, MIE patients from 2005 through 2006 were excluded (initial eight consecutive patients). The variables previously discussed were re-analyzed. No difference was noted except a decrease in EBL in the MIE group with our increasing experience. Mean EBL in the MIE group without the initial eight patients was 484 cc (standard deviation=290 cc), which is significantly less than the TTE and THE groups ($p<0.05$).

Discussion

Minimally invasive techniques have revolutionized general surgery, affording decreased perioperative morbidity and pain and allowing earlier return to work. Common surgical procedures such as appendectomy and cholecystectomy have served as models for the transition to more advanced and complex procedures. Systematic review and meta-analysis shows clear superiority of laparoscopic cholecystectomy with decreased hospital stay and convalescence period along with fewer complications compared with a traditional open procedure.[10] Minimally invasive techniques have been applied to esophageal surgery since the 1990s and currently, laparoscopic fundoplication for gastroesophageal reflux disease and Heller myotomy for achalasia are considered standard of care. Prospective data comparing laparoscopic to conventional Nissen fundoplication has

Table 2 Operative Outcomes

	Open TTE (n=22)	Open THE (n=33)	MIE (n=25)	p value
Stapled anastomosis (%)	18 (81.8)	28 (84.8)	22 (88.0)	NS
Duration (min) (range)	501 (420–600)	324 (210–540)	424 (330–620)	<0.001[d]
Blood loss (cc)	916	800	661	NS
Blood products given (units)	2.8	2.3	1	0.033[b]/0.005[c]
Patients requiring blood products (%)	17 (77.3)	24 (71.9)	7 (28)	0.001[b]/<0.001[c]
Nodes harvested (range)	19.5 (11–39)	13.2 (6–25)	17.2 (3–42)	0.004[a]/0.05[b]
Hospital stay (days)	17	18	19	NS
Intensive care unit stay (days)	8	7	10	NS

NS nonsignificant

[a] Significant difference between TTE and THE

[b] Significant difference between THE and MIE

[c] Significant difference between MIE and TTE

[d] Significant difference between all three groups

Table 3 Postoperative Complications

	Open TTE (n=22)	Open THE (n=33)	MIE (n=25)	p value
Anastomotic leak (%)	0 (0)	5 (15.2)	0 (0)	0.042[a]
Wound dehiscence (%)	0 (0)	2 (6.1)	0 (0)	NS
Chyle leak (%)	3 (13.6)	5 (15.2)	2 (8.0)	NS
Pneumonia (%)	2 (9.1)	3 (9.1)	1 (4.0)	NS
Reintubation (%)	3 (13.6)	1 (3.0)	1 (4.0)	NS
Pleural effusion (%)	1 (4.5)	4 (12.1)	2 (8.0)	NS
ARDS (%)	0 (0)	0 (0)	1 (4.0)	NS
Arrhythmia (%)	7 (31.8)	12 (36.4)	8 (32.0)	NS
Bleed (%)	2 (9.1)	1 (3.0)	0 (0)	NS
RLN injury (%)	0 (0)	1 (3.0)	1 (4.0)	NS
Mortality (%)	0 (0)	1 (3.0)	1 (4.0)	NS

NS nonsignificant, *RLN* recurrent laryngeal nerve injury

[a] Significant difference between THE and MIE

revealed a significantly decreased incidence of incisional hernias, equal effectiveness, and decreased incidence of postoperative pulmonary and wound complications.[11,12] The application of laparoscopic approaches to the treatment of malignant esophageal diseases has been more sedate, not only because of increased technical complexity but also because doubts have been raised about the oncological adequacy of the extent of resection with laparoscopic techniques. However, with respect to esophagectomy, the advantages of decreased blood loss, minimal postoperative pain, and shortened hospital stay and recovery time have been reported as with other laparoscopic procedures, providing the impetus of utilizing minimally invasive esophagectomies with increased frequency and feasibility.

Esophageal resection is a technically demanding procedure, both open and laparoscopically. Improved outcomes at high volume centers performing greater than 20 cases per year have been reported.[13–15] The learning curve for minimally invasive esophageal resection is steep and suspected to be more than 50 procedures.[16] Irrespective of the approach, significant morbidity secondary to complications related to esophagectomy, such as anastomotic complications, chyle leak, and graft ischemia, does exist, and the purported benefits of MIE, such as decreased time to recovery, may be negated by these complications. To date, no randomized trials have been performed to compare minimally invasive esophagectomy to open esophagectomy. The limited data in the current literature has been unable to prove a significant decrease in morbidity with the utilization of a minimally invasive approach.[16] However, some trends in favor of MIE have emerged as a suspected result of smaller incisions and superior laparoscopic visualization of the mediastinum, especially if the procedure is done transhiatally.[1] In a study by Smithers et al., MIE had significantly decreased total blood loss and transfusion requirements, as well as shorter intensive care unit and hospital stay when compared with open esophagectomy. As in our study, there was no significant difference in lymph node harvest, complication rate, or overall survival rates.[17] In a meta-analysis to evaluate outcomes of MIE versus open esophagectomy, significantly fewer anastomotic leaks were reported, and nonsignificant trends toward decreased pulmonary complications, length of hospital stay, blood loss, and operative mortality were observed.[18] However, almost all minimally invasive esophagectomy series are from high volume centers, which could be a confounding factor on the results. It is well known that high volume centers have decreased procedure-related morbidity and mortality. Plausibly, the improved outcomes with MIE may due to a center effect, rather than a benefit of the procedure itself.

Our experience with minimally invasive esophagectomy is similar to previously described studies at high volume centers. We have shown a trend toward decreased blood loss in MIE and significantly decreased requirement for blood products intraoperatively. MIE resulted in equivalent number of lymph nodes harvested to open transthoracic procedures and significantly more than open transhiatal procedures. Morbidity and mortality rates following resection were similar with MIE compared with open techniques; this could be due to a small patient population. However, it is more likely that the high rates of morbidity and mortality observed in esophageal resections are predominantly due to extensive surgery involving both thoracic and abdominal cavities, such that added morbidity due to the conventional open incision is insignificant and as such, a minimally invasive approach would not be expected to significantly alter perioperative outcomes. A similar concern exists with the transition from open to laparoscopic technique for other complex procedures, particularly pancreaticoduodenectomy. Universal acceptance of laparoscopic Whipple procedures has not been achieved despite the initial description of the technique over 15 years ago. This is because, although feasible, few series have shown any short- or long-term benefit of a minimally invasive approach.[19]

Although MIE did not have significantly reduced morbidity or mortality as compared with the open procedures in our series, it is noteworthy that the patients in the MIE group did have the same number of co-morbidities on average as those in the THE group. Traditionally, THE is the procedure of choice in patients with significant co-morbidities who are believed to be at greater risk of perioperative and postoperative morbidity associated with a thoracotomy. This finding suggests that MIE, with a laparoscopic thoracoscopic approach, is an acceptable surgical option in patients with significant co-morbidities in place of THE.

All anastamotic leaks occurred in the THE group and significance was noted when compared to MIE. Most likely, this is a type I error due to small sample size. However, it is conceivable that patients with THE, given the blunt mediastinal dissection, have a relatively compromised gastric conduit near the thoracic inlet, perhaps as a result of venous congestion. Furthermore, other variables such as experience and technique (hand sewn vs stapled anastomosis) could be factors and were unequally distributed across the series.

This study has the inherent limitations of retrospective review; however, all data was prospectively collected. A randomized controlled trial to compare open techniques with minimally invasive techniques would be ideal, but as of yet, has not been described. Additionally, technique utilization was not uniform in our study. Initially in our experience, all procedures were open, and the decision was made to perform TTE for younger/healthier patients and THE for those with increased age and co-morbidity. MIE was slowly incorporated approximately 2 years into the study period with progressively increasing frequency. The decision as to which technique was employed was made based on "surgeon perception", and no single co-morbidity was used to aid in procedure selection.

Finally, the setting of our center is unique in that most of our patients present for surgery from rural locations and are discharged only once we are completely comfortable with the patient's ability to manage tube feeds, etc., so hospital stays may be increased in comparison with other reports.

Conclusion

In centers with laparoscopic expertise, MIE can be safely incorporated without prolonged operative duration, oncological compromise, or risk of increased morbidity or mortality. Additionally, even early in experience, MIE confers the benefit of decreased blood loss and decreased requirement for transfusion of blood products.

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ORIGINAL ARTICLE

Expression of C1q Complement Component in Barrett's Esophagus and Esophageal Adenocarcinoma

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Abstract
Aims C1q, an element of the first component of complement, is known to be expressed by interdigitating and follicular dendritic cells in the spleen, where it has been suggested that C1q is involved in capturing immune complexes. The present study investigated whether C1q is expressed in Barrett's esophagus and esophageal adenocarcinoma and, if so, whether its expression is associated with dendritic cells.
Material and Methods Endoscopic biopsy or operative surgical specimens were obtained from 15 patients with Barrett's esophagus, 13 patients with esophageal adenocarcinoma and 12 patients whose biopsy specimens did not show the presence of specialized intestinal metaplasia or adenocarcinoma. Barrett's esophagus was diagnosed by the presence of a macroscopic area of columnar-lined esophagus as well as microscopic intestinal metaplasia with goblet cells. Immunohistochemistry utilizing anti-C1q and markers for dendritic cells and macrophages was performed on sections of tissue samples embedded in paraffin. Double immunostaining with C1q/CD83 and C1q/CD68 was used to analyze the possible co-localization of C1q with dendritic cells and macrophages. The expression of C1q by dendritic cells and macrophages was also examined in in vitro studies using reverse transcriptase polymerase chain reaction (RT-PCR) and Western blotting.
Results In all specimens studied, C1q expression was detected as being distributed irregularly throughout the lamina propria. A computerized quantitative analysis showed that C1q expression was significantly higher in tissue specimens without specialized intestinal-type metaplasia than in Barrett's esophagus specimens and specimens with adenocarcinoma. Double immunostaining revealed that dendritic cells and macrophages expressed C1q in all analyzed esophageal specimens. The expression of C1q by dendritic cells and macrophages was also demonstrated in in vitro studies using RT-PCR and Western blotting.
Conclusion The findings suggest that reduced levels of the expression of C1q by dendritic cells and macrophages in the esophagus may play a role in the formation of immune responses associated with the formation of specialized intestinal metaplasia and the development of adenocarcinoma.

Keywords Barrett's esophagus · Intestinal metaplasia · Adenocarcinoma, C1q · Dendritic cells · Inflammation

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Introduction

It is known that premalignant conditions develop in the presence of chronic inflammation and that immune mechanisms are critically involved in the development of cancer in these precancerous tissues.[1–3] A variety of inflammatory and immune cells populate the Barrett's esophagus mucosa, including T- and B-lymphocytes, mast cells and macrophages.[4–7] We recently reported that antigen-presenting

dendritic cells are present in Barrett's esophagus, with a significant increase in their spatial density in adenocarcinoma compared to benign Barrett's esophagus.[8] Because dendritic cells are powerful initiators and regulators of immune reactions,[9–11] we hypothesized that dendritic cells may play a role in the pathogenesis of Barrett's esophagus and adenocarcinoma.[8] In that study[8] we noted the formation of focal aggregations of dendritic cells as well as the occurrence of dendritic cells and T cells contacting each other in Barrett's esophagus and adenocarcinoma. Clustering of dendritic cells with each other is known to occur in a number of autoimmune diseases, and has not been described in any disease in which autoimmune mechanisms are not involved.[9,12,13] However, no studies have yet been carried out which evaluate the possible role of recruitment of autoimmune mechanisms in the formation of Barrett's esophagus and the development of adenocarcinoma.

In recent years, it has become clear that dendritic cells interact at several levels with the complement system, an ancient and well conserved part of the innate immune system,[14,15] and that complement component C1q plays an important role in the functioning of dendritic cells.[15,16] C1q, an element of the first component of complement,[14,15] is known to be expressed by interdigitating dendritic cells and follicular dendritic cells in the spleen, where it has been suggested that C1q is involved in capturing immune complexes.[17] A deficiency in C1q has also been linked to the development of such autoimmune diseases as systemic lupus erythematosus (SLE).[18–20]

The present study was undertaken in order to investigate whether C1q is expressed in Barrett's esophagus and esophageal adenocarcinoma and if so, what are its cellular sources.

Materials and Methods

Tissue Specimens and Routine Histology

Endoscopic biopsy or operative surgical specimens were obtained from 15 patients with Barrett's esophagus, 13 patients with esophageal adenocarcinoma, and 12 patients whose biopsy specimens did not show the presence of specialized intestinal metaplasia or adenocarcinoma. Barrett's esophagus was diagnosed by the presence of a macroscopic area of columnar-lined esophagus as well as microscopic intestinal metaplasia with goblet cells. Materials were collected in accordance with the principles outlined in the Declaration of Helsinki after approval by the Institutional Review Board of St. Vincent's Hospital, Sydney, and informed consent was obtained from each patient. Tissue specimens were processed by standard formalin fixation and paraffin embedding. Paraffin sections cut at 5–7 μm thickness were stained with Mayer's haematoxylin and eosin.

Single and Double Immunostaining Procedures and Quantitative Analysis

Single immunostainings for C1q (Sigma; cat no C 3900;1:400 dilution) and dendritic cell markers, including CD83 (Immunotech; cat no IM-2069; 1:50 dilution)[21] and DC-SIGN (Santa Cruz; cat no sc-65892; 1:50 dilution),[22] T cells (Dako; cat no A0452; 1:100 dilution), B cells (Beckman-Coulter; cat no 1925; 1:50 dilution) and macrophages (Dako; cat no M0876; 1:50 dilution) were performed. For single immunostaining, after elimination of endogenous peroxidase activity by 3% H_2O_2, sections were preincubated with normal non-immune serum and then tested by avidin–biotin complex (ABC) using a standard ABC immunoperoxidase method as detailed previously.[8] Briefly, after washing in tris-phosphate buffered saline (TPBS), pH 7.6, the sections were incubated with a biotin-labeled secondary antibody, followed by a treatment with avidin–biotin complex (ELITE ABC, VECTOR PK61000). After washing in TPBS, brown staining was produced by 5 min treatment with 3,3′-diaminobenzidine (DAB). All the incubations were completed at room temperature. Archival lymph node sections were used for positive controls. For negative controls, the first antibodies were omitted or the sections were treated with an immunoglobulin fraction of non-immune serum as a substitute for the primary antibody. None of the negative control sections showed positive immune staining. Counterstaining was performed with Mayer's haematoxylin.

A computerized quantitative analysis of C1q expression was carried out at ×400 magnification using the Image-Pro Plus image analysis program (Media Cybernetics, Bethesda, MD). C1q expression was measured in each section in at least seven randomly selected microscopic fields. A statistical comparison of expression, measured in pixels per standard microscopic field (0.04 mm^2), was performed by *t* test using Prism® 4 (GraphPad Software, San Diego, CA).

Double immunostaining with C1q/CD83 and C1q/CD68 was used to analyze the possible co-localization of C1q with dendritic cells and macrophages, using previously reported methods.[8] In brief, after visualization of C1q with the ABC substrate kit, sections were washed with 0.1 M glycine-hydrochloric acid buffer, pH 2.2, and then incubated with anti-CD83 or anti-CD68 antibody. After rinsing in TPBS, the sections were incubated with biotinylated secondary antibody and then with alkaline phosphatase-conjugated streptavidin (Dako) or with avidin–biotin complex (Dako). A combination of the peroxidase-anti-peroxidase and alkaline phosphatase-anti-alkaline phospha-

tase techniques, with antigen visualization with DAB or Fast Red, was also used. Controls were the same as for single immunostaining. Counterstaining was performed with Mayer's haematoxylin.

Real-time PCR

To examine C1q expression in dendritic cells at the transcriptional level, monocytes were isolated from peripheral blood mononuclear cells as previously described.[23–25] Monocytes were isolated by adhesion to culture plates and cultured for 6 days. Dendritic cells were generated in the presence of IL-4 and GM-CSF (20 ng/ml). Macrophages were cultured with M-CSF (20 ng/ml). RNA was isolated from monocytes, macrophages and dendritic cells using the NucleoSpin RNA II kit (Macherey-Nagel). cDNA was synthesized using the Advantage reverse transcriptase (RT)-for-polymerase chain reaction (PCR) Kit (Becton Dickinson) and quantitative real-time PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA), using the SYBR Green PCR master mix. Primers specific for the three human C1q chains (C1qA, C1qB and C1qC) and, as a control, GAPDH are listed below: C1qA (5′-CTTCCTCATCTTCCCATCT-3′/5′-GTT CAGCAGACACAGACA-3′); C1qB (5′-AGGCGTCTGA CACAGTATG-3′/5′-CCTGGAAGCCCTTTCTCT-3′); C1qC (5′-ACCTGCAGTTCCTTCTCC-3′/5′-TTC TCCCTTCTGCCCTTT-3′); and GAPDH (5′-CGGAGTCA ACGGATTTGGTCG-3′/5′-TCTCGCTCCTGGAAGA TGGTGAT-3′). In all experiments, data were normalized to values obtained with the GAPDH primers.

Western Blotting

Cultured macrophages and dendritic cells were harvested at days 2, 4, and 6. After washing with cold PBS, the cells were lysed on ice using a lysis buffer (Biosource, Camarillo, CA) supplemented with a Protease Inhibitors Cocktail (Sigma). The lysates were subjected to SDS-PAGE (12.5% (*w*/*v*)) and Western blotting using a goat anti-C1q antibody (Sigma). To detect β-actin, the blots were first stripped by incubating for 20 min at 50°C in 0.1 M 2-mecaptoethanol, 2% SDS and 62.5 mM Tris-HCl (pH 6.8). The blots were then blocked and incubated with a monoclonal antibody specific for β-actin (Sigma). Signals were visualized using the Immuno-Star chemiluminescent substrate (Bio-Rad).

Results

Immunohistochemical examination demonstrated that all sections prepared from esophageal specimens, including specimens without metaplasia (non-Barrett's esophagus), specimens with intestinal-type metaplasia (Barrett's esophagus) and specimens with adenocarcinoma, contained cells specifically stained with antibodies against C1q. Staining of the tissue sections with anti-C1q was irregular with areas manifesting intense staining bordering on areas with no staining (Fig. 1a–d). The intensity of anti-C1q antibody binding with the cells varied and was usually stronger in cells which formed clusters than in isolated cells (Fig. 1a–d). It was apparent that not only cells, but the extracellular matrix also was specifically stained with anti-C1q antibody (Fig. 1a–d). The extracellular matrix was usually diffusely stained but, in practically all areas, C1q extracellular staining was associated with areas that also contained C1q+ cells (Fig. 1a–d). Diffuse extracellular binding of C1q antibody was detected in all specimens. A computerized quantitative analysis showed that there was no significant difference between the mean of C1q expression in Barrett's tissue specimens and adenocarcinoma specimens (1,144+139 vs 1,199+175; t=0.2457), but C1q expression was significantly higher in the matrix surrounding cardiac glands in esophageal specimens without specialized intestinal metaplasia or adenocarcinoma, compared to that in either Barrett's tissue specimens or adenocarcinoma (1,756+228 vs 1,144+139 and 1,756+228 vs 1,199+175, accordingly) (Fig. 2).

Examination of tissue sections prepared from esophageal specimens without metaplasia (non-Barrett's esophagus), specimens with intestinal metaplasia (Barrett's esophagus) and specimens with adenocarcinoma and stained with cell-type-specific antibodies showed the presence of dendritic cells, T cells and macrophages in all specimens. The numbers and patterns of their distribution were consistent with those previously reported by us.[8] Consistent with our previous report,[8] dendritic cells were often seen to form clusters with each other and were most frequently observed in areas enriched by CD3+ cells.

Double immunostaining utilizing a CD83/C1q antibody combination identified that dendritic cells expressed C1q (Fig. 3a–d). In the extracellular matrix, which surrounded C1q+ dendritic cells, the presence of C1q was also evident (Fig. 3a–d). The intensity of C1q expression varied markedly among C1q+ dendritic cells and in some dendritic cells, the content of C1q was low or undetectable. Double immunostaining utilizing a CD83/CD68 antibody combination revealed an association of C1q with macrophages.

In vitro study showed the ability of dendritic cells to produce all three C1q chains, i.e., C1qA, C1qB and C1qC. As shown in Fig. 4a, both types of cells, including dendritic cells and macrophages, expressed mRNA for the three C1q chains although the two cell types also expressed different levels of C1q mRNA. In vitro, macrophages expressed C1q mRNA at a level approximately tenfold higher than

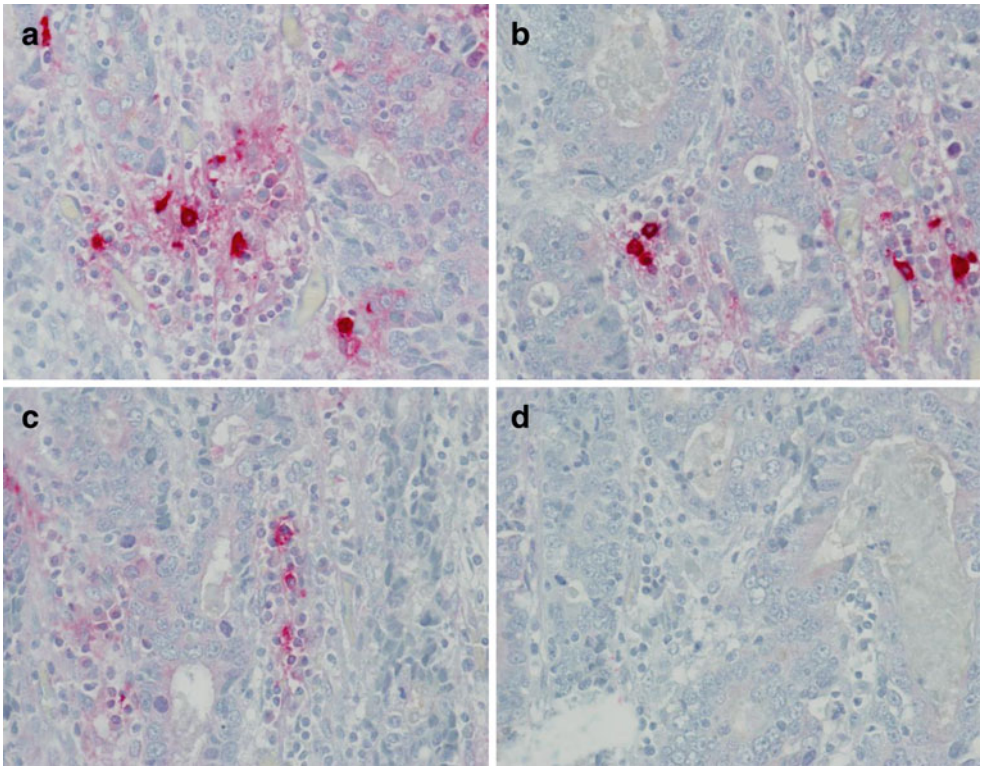

Fig. 1 Typical patterns of the distribution of C1q in esophageal tissue specimens (**a–d**). C1q antigen was visualized using a Fast red substrate kit (rose reaction product). Counterstaining with Mayer's haematoxylin. Magnification: ×600 (**a–d**).

dendritic cells with all three chains. In contrast, monocytes expressed little C1q mRNA.

A similar pattern of C1q expression was also observed at the protein level. C1q was barely detectable in the lysate of monocytes (Fig. 4b). However, it was detected in macrophages and dendritic cells 2 days after monocytes were cultured. C1q levels were further increased by days 4 and 6. Although macrophages showed a level of C1q mRNA approximately tenfold higher than dendritic cells, this was not proportionally reflected at the protein level. However, the macrophage lysates did contain higher levels of C1q as compared to that of dendritic cells. Nonetheless, these results were consistent with the detection of C1q in vivo in both dendritic cells and macrophages in esophageal tissue specimens.

Discussion

This is the first report to show that C1q is expressed in the normal and Barrett's esophagus. It was found that dendritic cells and macrophages were the main sources of C1q.

C1q is the recognition protein of the classical pathway of the complement system.[26,27] Contrary to most complement proteins which are produced by the liver, C1q is mainly produced by myeloid cells.[15,16] It has been shown that dendritic cells are an important source of C1q.[14–16] Immature dendritic cells produce large amounts of C1q and are known to induce tolerogenic responses.[14–16] DC activation has been reported to lead to the dendritic cell maturation associated with a reduced capacity to produce C1q and the induction of immunogenic responses.[14–16] The present study demonstrated the expression of C1q by dendritic cells; the levels of C1q expression were significantly higher in esophageal specimens without specialized intestinal metaplasia or adenocarcinoma, compared to that in either Barrett's tissue specimens or adenocarcinoma.

C1q deficiency is the strongest susceptibility factor for the development of SLE, a systemic autoimmune syndrome with unknown etiology.[14–16] Certain strains of C1q-deficient mice exhibit an autoimmune phenotype resembling human SLE.[28,29] The complex role of C1q and other

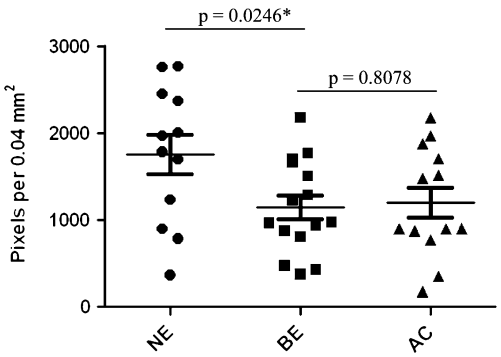

Fig. 2 C1q expression in esophageal tissue specimens without metaplasia (non-Barrett's esophagus (*NE*)), specimens with intestinal-type metaplasia (Barrett's esophagus (*BE*)) and specimens with adenocarcinoma (*AC*), evaluated as a number of pixels per standard field using a computerized quantitative analysis (see "Materials and Methods").

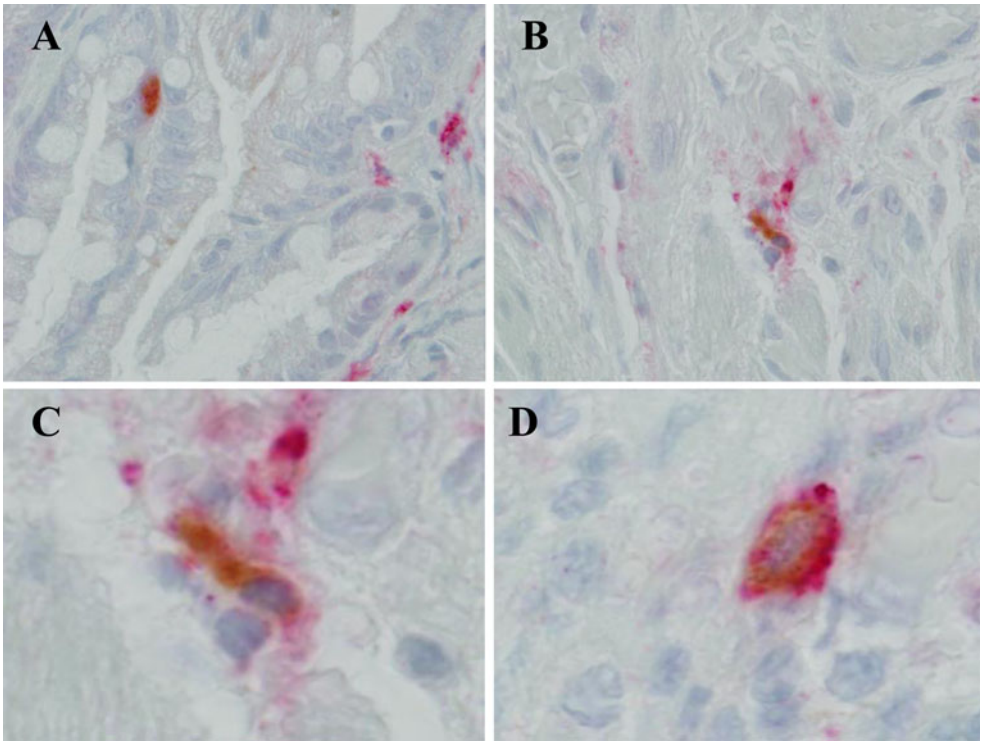

Fig. 3 Double immunostaining demonstrating the expression of C1q by dendritic cells (**a**–**d**). CD83 antigen was visualized using ABC immunoperoxidase reaction (brown reaction product) while C1q was visualized using a Fast red substrate kit (rose reaction product). Counterstaining with Mayer's haematoxylin; **c** is a detail of **b**. Magnifications: ×600 (**a**, **b**) and ×1,000 (**c**, **d**).

complement proteins in the pathogenesis of SLE is explained by two hypotheses.[28,29] The reduced clearance of apoptotic cells in C1q−/− mice has led to the waste disposal hypothesis in which C1q, working as an opsonin, facilitates the safe clearance of apoptotic cells, which are considered the main source of autoantigens.[28,29] Alternatively, the complement components might be pivotal in the removal of self-reactive lymphocytes.[15,16] Patients affected by SLE are characterized by a hyperactivity of cells of the immune system.[30] A hyperactive state has been recently demonstrated in dendritic cells, which have been suggested to play a pathogenic role in SLE.[15,16] Dendritic cells continuously circulate from the bloodstream throughout the periphery, towards the draining lymph nodes, and can be activated by several danger signals derived from either exogenous or endogenous agents.[9–12] SLE-specific endogenous activators of dendritic cells have been described in patients' sera such as nucleosomes, autoantibody-DNA

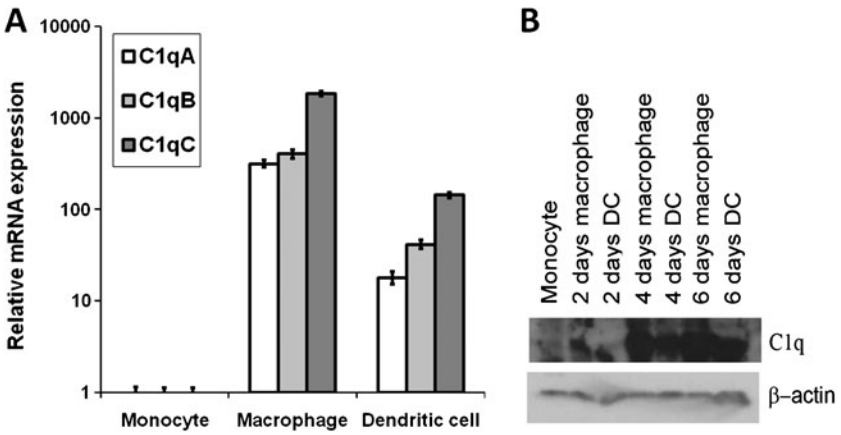

Fig. 4 C1q expression by cultured macrophages and dendritic cells. RNA was isolated from monocytes as well as macrophages and dendritic cells. cDNA was synthesized using these RNA (1 μg/each) which was then subjected to quantitative real-time PCR on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA), using the SYBR Green PCR master mix. In each experiment, the mRNA of GAPDH was also determined which was used to normalize across the different experiments. Monocytes express little C1q mRNA and the levels of mRNA in macrophages and dendritic cells were presented as folds compared with monocytes.

complexes, defensins, antibody-chromatin complexes and nucleic acid.[14–16,28–30] These endogenous dendritic cell activators are capable of triggering the production of pivotal cytokines for the development of autoimmunity such as the type I IFN.[31,32]

Consistent with our earlier report,[8] focal aggregations of dendritic cells as well as dendritic cells contacting each other were identified in Barrett's tissue specimens and adenocarcinoma specimens. The clustering of dendritic cells with each other is known to occur in a number of autoimmune diseases.[9–12] It is tempting, therefore, to speculate that the presence of aggregates of dendritic cells observed in esophageal tissues might represent an ongoing autoimmune reaction or that autoimmune mechanisms are at least involved. The issue of whether the clustering of dendritic cells might relate to the reduced levels of C1q expression in Barrett's esophagus and esophageal adenocarcinoma requires further investigation. Notably, the quantitative analysis provided by the present study showed that there was no significant difference between the mean of C1q expression in Barrett's tissue specimens and adenocarcinoma specimens.

It is interesting to note that in the present study, dendritic cells were frequently found in areas where C1q was observed to be bind to the extracellular matrix of the connective tissue surrounding glands in the lamina propria. The complement protein C1q is known to have multiple immune functions including acting as a chemoattractant for neutrophils, eosinophils, mast cells, and dendritic cells,[14–16] and this might explain the co-localizaton of extracellular C1q and dendritic cells in esophageal tissues. It has been shown that C1q functions as a chemotactic factor for immature dendritic cells, and migration is mediated through the ligation of both gC1qR and cC1qR/CR, present on the surface of dendritic cells.[14–16] The ability of dendritic cells to produce all three of the C1q chains (C1qA, C1qB, and C1qC), has been confirmed in this study.

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ORIGINAL ARTICLE

Medical Comorbidities Should Not Deter the Application of Laparoscopic Fundoplication

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Abstract
Introduction Laparoscopic Nissen fundoplication offers significant improvement in gastroesophageal reflux disease (GERD) symptom severity and frequency. This study was undertaken to determine the impact of preoperative medical comorbidities on the outcome and satisfaction of patients undergoing fundoplication for GERD.
Methods Prior to fundoplication, patients underwent esophageal motility testing and 24-h pH monitoring. Before and after fundoplication, the frequency and severity of reflux symptoms were scored using a Likert scale. Medical comorbidities were classified by organ systems, and patients were assigned points corresponding to the number of medical comorbidities they had. In addition, all patients were assigned Charlson comorbidity index (CCI) scores according to the medical comorbidities they had. A medical comorbidity was defined as a preexisting medical condition, not related to GERD, for which the patient was receiving treatment. Analyses were then conducted to determine the impact of medical comorbidities as well as CCI score on overall outcome, symptom improvement, and satisfaction.
Results Six hundred and ninety-six patients underwent fundoplication: 538 patients had no medical comorbidities and 158 patients had one or more medical comorbidities. Preoperatively, there were no differences in symptom severity and frequency scores between patients with or without medical comorbidities. Postoperatively, all patients had improvement in their symptom severity and frequency scores. There were no differences in postoperative symptom scores between the patients with medical comorbidities and those without. The majority of patients were satisfied with their overall outcome; there was no relationship between the number of medical comorbidities and satisfaction scores. These findings were mirrored when patients' CCI scores were compared with satisfaction, overall outcome, and symptom improvement.
Conclusion These results promote further application of laparoscopic Nissen fundoplication, even for patients with medical comorbidities.

Keywords GERD · Comorbidities · Fundoplication · Nissen

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Introduction

Gastroesophageal reflux disease (GERD) is the most common upper gastrointestinal disorder in the Western world and affects 20% of the United States adult population on a weekly basis.[10] GERD is a huge public health concern with over ten billion dollars spent annually on anti-acid medications.[1] Laparoscopic fundoplication has proven to provide excellent palliation of symptoms with high patient satisfaction and significant decrease in the need for prolonged anti-acid medication use.[2] Furthermore, the sequelae of severe GERD such as Barrett's metaplasia, esophageal dysplasia and adenocarcinoma, and peptic stricture can be prevented or halted by anti-reflux surgery.

In 2006 the number of Americans over age 65 exceeded 37 million with the U.S. Census Bureau projecting a logarithmic rise through the year 2030.[10] This aging population poses a challenge to surgeons with the number and severity of preoperative medical comorbidities that these patients have. Frequently, patients with multiple medical comorbidities are denied the option of elective anti-reflux surgery secondary to concerns of increased intraoperative or postoperative complications. It is also possible that patients with preoperative medical comorbidities are more likely to have poor symptomatic relief and be dissatisfied with their outcome after operative intervention.

This study was undertaken to determine the impact of preoperative medical comorbidities on the outcome and satisfaction of patients undergoing fundoplication for GERD. Our hypotheses were that patients' overall outcome and satisfaction would not be adversely affected by their preoperative medical comorbidities and that laparoscopic fundoplication would improve symptom severity and frequency scores of GERD in all patients.

Methods

Since 1992, patients undergoing laparoscopic fundoplications have been prospectively followed and assimilated into a comprehensive database. Among these patients, we selected 696 who were available for long-term follow-up. Before fundoplication, patients underwent esophageal motility testing by stationary water perfusion esophageal manometry or barium-laden food bolus esophagram in a 15° head-down position and 24 to 48 h ambulatory pH monitoring using commercially available instrumentation. Patient data collection and study design were conducted in concordance with a protocol approved by the Institutional Review Board of the University Of South Florida College Of Medicine.

Preoperative and postoperative measurements of patients' severity and frequency of symptoms as well as overall satisfaction were scored using a Likert scale.[1] Medical comorbidities were classified by organ systems and patients were stratified by the number of comorbidities they had preoperatively (Fig. 1). In addition, each patient was assigned a Charlson comorbidity index (CCI) score using one of several, readily available, online calculators.[3] A medical comorbidity was defined as a preexisting medical condition, not related to GERD, for which the patient was receiving treatment.

The technique used at our institution of a laparoscopic Nissen fundoplication has been described in detail previously.[4] In summary, a five-port technique was used with the patients supine. After widely opening the gastrohepatic omentum, dissection along the right crus was carried into the mediastinum as needed to deliver approximately 6–8 cm of intraabdominal esophagus. If present, any hiatal hernia was reduced and a posterior curoplasty was undertaken to close the esophageal hiatal defect. At this point the posterior fundus was brought behind the esophagus to construct the fundoplication. A 52 F to 60 F bougie was placed per os into the stomach. A three suture technique was used to construct the fundoplication, followed by a lateral gastropexy to tack the posterior fundus to the right crus to remove tension and prevent twisting of the lower esophagus. Trocar sites were closed under laparoscopic visualization using absorbable monofilament suture. The majority of patients began a liquid diet when awake and were discharged home within 24 h with instructions to advance to a soft mechanical diet over the next week.

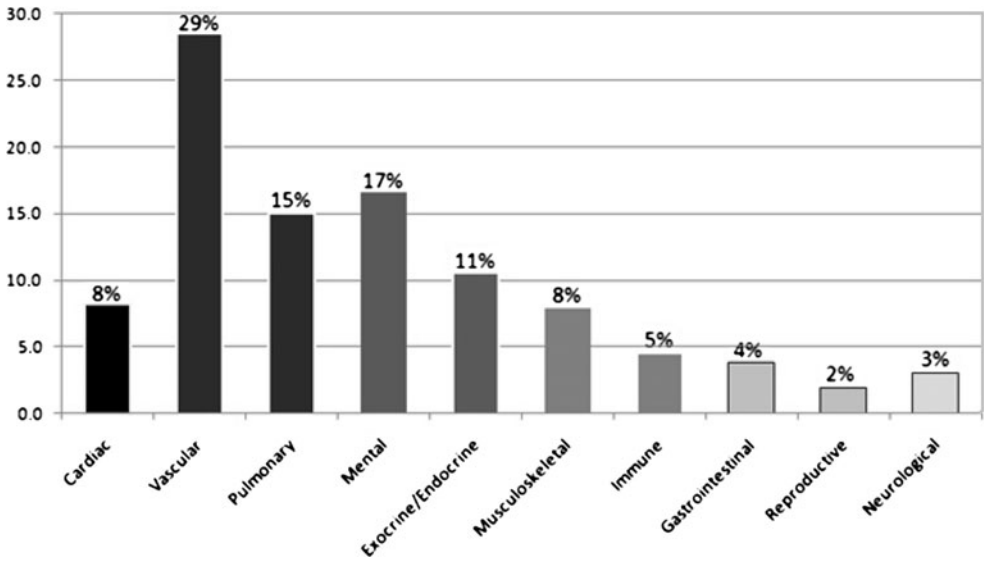

Fig. 1 Distribution of patient comorbidities by organ system.

Table 1 Demographic Data

	Preoperative medical comorbidities	No preoperative medical comorbidities	*p* value
Number of patients	158 (23%)	538 (77%)	
Age	58 years (58± 13.1 years)	53 years (53± 15.4 years)	0.0006
BMI	26 kg/m^2 (26± 5.0 kg/m^2)	27 kg/m^2 (27± 4.5 kg/m^2)	0.51
Redo operations	24 (15%)	73 (14%)	0.42
Length of stay	2 days (3±4.2 days)	1 day (2± 2.9 days)	0.009

For follow-up, patients were seen in clinic at 2 weeks after surgery, 6 months after surgery, and annually thereafter. At the time of each contact, patients again graded the frequency and severity of their symptoms via our symptom frequency and severity questionnaire. For preoperative and postoperative score comparisons, we used the patients' preoperative questionnaires and their postoperative questionnaire, at the time of last follow-up. For uniformity, we also compared preoperative symptom frequency and severity scores to postoperative scores obtained 3 years from the date of surgery, for all patients. Patients' scores were obtained by unbiased medical assistant staff that were trained in our questionnaires and the interpretation of our Likert scales.

Data are maintained on an Excel (Microsoft) spreadsheet and analyzed by Wilcoxon matched pairs test or Mann–Whitney *U* test, when appropriate. For statistical calculations, GraphPad InStat version 3.06 (GraphPad Software, San Diego, CA, USA) was utilized. For each patient, preoperative and postoperative symptom scores were compared using Wilcoxon matched pairs test. To compare symptom scores of patients with and without medical comorbidities, Mann–Whitney *U* test was utilized. For all other comparisons, Spearman nonparametric correlation was used. Where appropriate, data are presented as median or mean±standard deviation.

Results

Of the 696 total patients, 44% were male with median age of 53 years. Median length of follow-up was 6.5 years. The patients were separated by the presence or absence of medical comorbidities, 538 (77%) were without preoperative medical comorbidities and 158 (23%) had one or more medical comorbidity (Table 1). Of the patients with preoperative medical comorbidities, 24 (15%) were reoperative, or underwent "redo" fundoplications. Similarly, of the patients without medical comorbidities, 73 (14%) were "redo" fundoplications. Seventy-five percent of the "redo" fundoplications were referred after having their initial fundoplication undertaken at outside institutions. The median duration of symptoms prior to fundoplication was 10 years (13±10.6 years). The median length of stay for patients with medical comorbidities was 2 days (3± 4.2 days) and for those patients without medical comorbidities was 1 day (2±2.9 days; p=0.009).

Preoperatively there were no differences in symptom severity and frequency scores between patients with or without medical comorbidities (Figs. 2 and 3). Postoperative symptom severity and frequency scores were significantly improved for all symptoms in patients, regardless of the presence or absence of preoperative

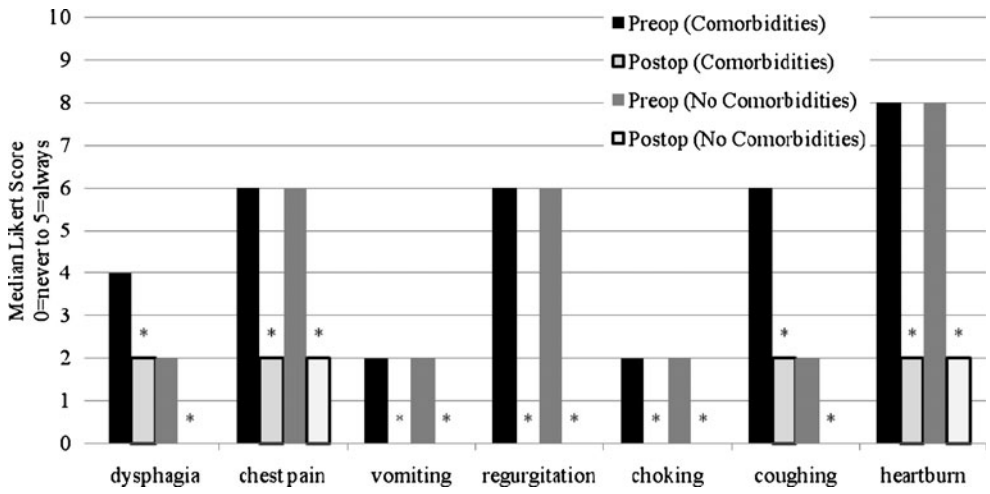

Fig. 2 Symptom frequency scores.

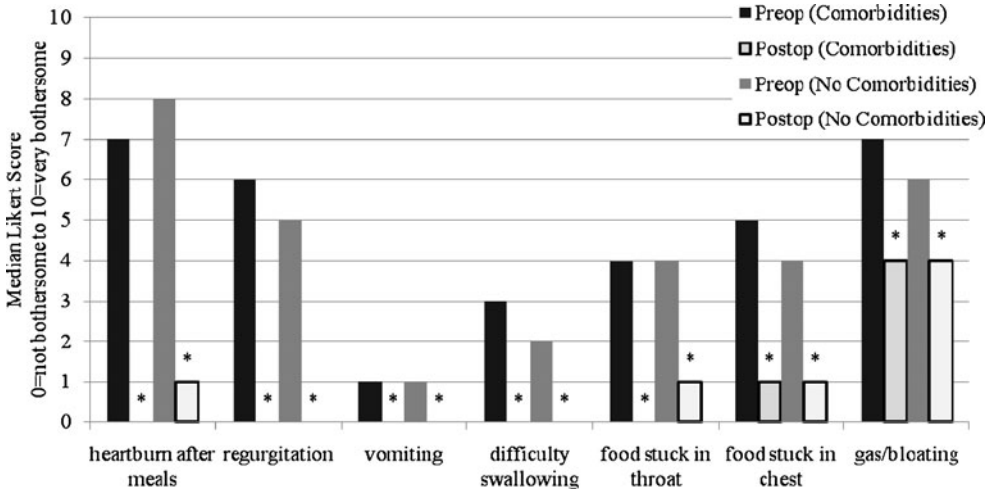

Fig. 3 Symptom severity scores.

medical comorbidities ($p<0.0001$; Figs. 2 and 3). Similarly, there were no differences in postoperative symptom scores between the patients with preoperative medical comorbidities and those without (Figs. 2 and 3). Also no difference was noted between patients with preoperative medical comorbidities and those without, when comparing preoperative and 3-year postoperative symptom severity and frequency scores ($p<0.03$).

Eighty-seven patients had a CCI score greater than zero (3.3±1.5). As CCI score increased, patients' postoperative symptom scores such as heartburn, chest pain, nausea, and the sensation of food stuck in the throat or chest, decreased. Additionally, the margin of change in symptom scores between the preoperative and postoperative period increased with increasing CCI score. CCI score did not positively correlate with poor symptom resolution. Furthermore, CCI score did not correlate with patients' perceived symptom resolution, overall experience or likelihood of undergoing the operation again, $p=0.471$, $p=0.238$, and $p=0.467$, respectively. Preoperative medical comorbidities did not impact the severity or frequency of GERD symptoms nor their improvement after fundoplication.

Eighty-eight percent of patients were "satisfied" or "very satisfied" with their outcome and perioperative experience (Fig. 4). By regression analysis, neither the number of preoperative medical comorbidities nor CCI score affected patient satisfaction.

Discussion

Gastroesophageal reflux disease affects a huge number of Americans and is a ten billion dollar burden on the health care system annually.[13, 14] The clinical manifestations of GERD have a significant impact on patients' quality of life with a direct correlation to several pathologic processes of the distal esophagus such as Barrett's metaplasia, esophageal dysplasia and adenocarcinoma, and peptic strictures. Laparoscopic

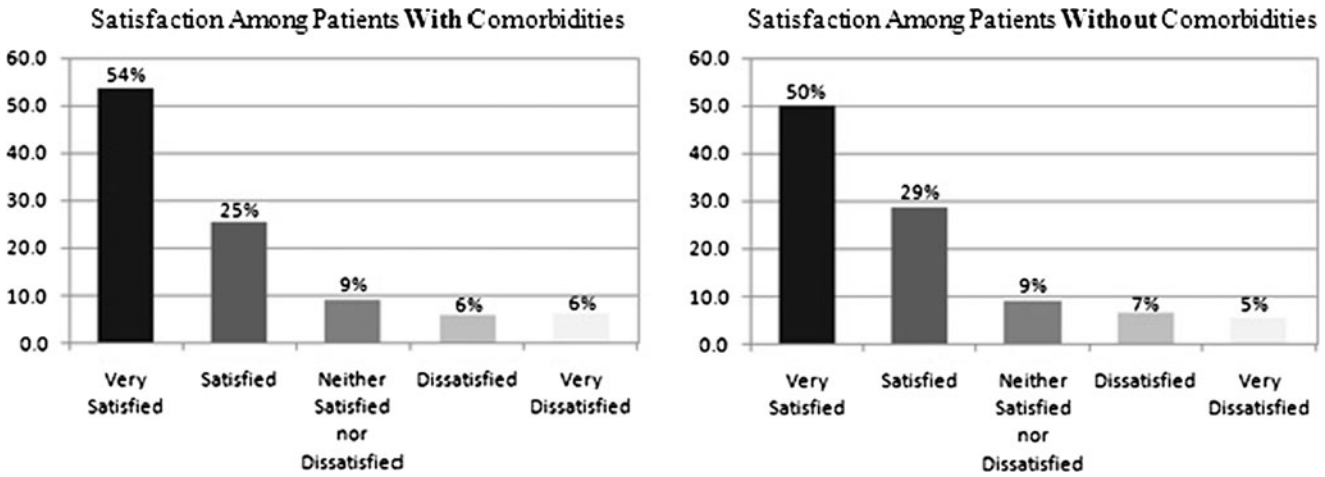

Fig. 4 Satisfaction among patients with (*left*) and without (*right*) comorbidities.

Nissen fundoplication has proven to be the operation of choice for GERD with excellent results.[5–7, 11, 12] However, the number of preoperative medical comorbidities for patients with GERD is increasing due to an aging patient population.[4] This unique study documents the application of laparoscopic fundoplication to patients who have one or more medical comorbidities and focuses on overall outcome, improvements in symptom frequency and severity, and overall patient satisfaction. This study clearly documents that selected patients with medical comorbidities and GERD can undergo laparoscopic anti-reflux surgery with encouraging results that mirror the outcomes of patients without medical comorbidities and further encourages application of laparoscopic anti-reflux surgery.

The patient population in this study is similar to the general population of patients with GERD with more patients being women and most patients of middle age. All patients did have excessive gastroesophageal acid reflux, though over a broad range, and all had frequent and severe symptoms. All had failed medical therapy for GERD and had waited, on average, almost 11 years before undergoing definitive treatment. Lastly, all patients had demonstrated acceptable esophageal motility to undergo Nissen fundoplication.[16, 18, 19]

The efficacy and durability of laparoscopic fundoplication was evident in this study. When the 158 patients with preoperative medical comorbidities underwent laparoscopic fundoplication, notable improvements were noted between pre- and postoperative frequency and severity of symptoms. When the postoperative symptom scores of the patients with preoperative medical comorbidities were compared to the scores of the patients without medical comorbidities, there were no differences. In fact, the patients with the highest CCI scores experienced the greatest improvement of symptoms from the preoperative period to the postoperative period.

To account for disparities in length of postoperative follow-up among patients, a separate comparison was performed between preoperative symptom severity and frequency scores and scores obtained 3 years postoperative (our median length of follow-up was 6.5 years however the longest period of time for which all 696 patients had completed a follow-up questionnaire was 3 years). Again, no difference was noted in symptom severity and frequency regardless of the presence or absence of medical comorbidities. In a prior publication at our institution, we have shown that early salutary outcomes predict long-term salutary outcomes.[1, 15]

Most patients had an uneventful hospital course, and although inadvertent intraoperative events or postoperative complications did occur, they were uncommon, generally minor, and of no apparent long-term consequence.

Furthermore, no difference was noted when comparing patient satisfaction between patients with and without preoperative medical comorbidities. Nearly 90% of patients with or without preoperative medical comorbidities reported that they were "very satisfied" or "satisfied" with their overall outcome and perioperative experience. This held true regardless of which medical comorbidity stratification system we used, organ systems based or CCI score. Our observations advocate for laparoscopic Nissen fundoplication regardless of the presence or absence of preoperative medical comorbidities, as all patients benefit from significant improvement of symptoms.

A comprehensive review of the pertinent literature documents that this study is "one-of-a-kind" because of the classification of preoperative medical comorbidities by multiple (e.g., ten) organ systems as well as CCI score and involves the greatest number of patients of any study looking at the impact of preoperative medical comorbidities on outcome after anti-reflux surgery. Several studies have looked at preoperative predictors of poor outcome after laparoscopic fundoplication.[4, 8, 9, 17, 20] Some of these predictors include morbid obesity (BMI >35 kg/m^2), history of preoperative psychiatric illness, atypical symptomatology, no/poor response to proton pump inhibitors, prior abdominal surgery, age greater than 50 years, presence of a large (greater than 3 cm) hiatal hernia, tobacco use, and esophageal dysmotility.[5, 8, 9] Of these predictors the two that are very prevalent in the general population are obesity and psychiatric illness. When looking at our large database of patients, we found no difference in outcome or satisfaction for patients with psychiatric illnesses, which included major depressive disorder. The median BMI in our patient population was (27±4.5 kg/m^2), and given the significant improvements in symptom severity and frequency scores as well as high level of patient satisfaction that we observed, a role for preoperative weight loss counseling prior to elective laparoscopic fundoplication should be proposed.

As previously stated, laparoscopic Nissen fundoplication dramatically improved the frequency and severity of all symptoms queried. The postoperative improvements in symptom frequency and severity scores were similar among patients, regardless of the presence, type, or number of preoperative medical comorbidities. Patients with GERD and other medical comorbidities who were previously denied definitive therapy for fear of perioperative complications or poor outcome should not be forced into lifelong anti-reflux medication use. Laparoscopic Nissen fundoplication should continue to be applied in all patients suffering from GERD with expectations of successful long-term relief of symptoms.

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ORIGINAL ARTICLE

SOX2 in Gastric Carcinoma, but not Hath1, is Related to Patients' Clinicopathological Features and Prognosis

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Abstract
Background SOX2 and Hath1 are transcription factors that are critical for the control of terminal cell differentiation in the gastrointestinal mucosa. This study investigated the correlations between SOX2 and Hath1 expression in gastric carcinoma and patients' clinicopathological features and prognosis.
Methods Hath1 and SOX2 were detected by immunohistochemistry in gastric carcinoma (n=50). Probability of survival of patients after surgery was estimated by the Kaplan–Meier method and compared using Log-rank test.
Results Hath1 and SOX2 were inversely expressed in gastric carcinoma. Patients with strong SOX2 expression (++ to +++) showed lower incidences of lymph node metastasis (p=0.007), deeper invasion (p=0.010), and III–IV clinical stages (p=0.011) compared to patients with low SOX2 expression (− to +). There was no significant difference in SOX2 and Hath1 expression in the cancerous tissues of the patients with and without *Helicobacter pylori* infection (p>0.05). The patients with strong expression of SOX2 in their cancerous tissues (++ to +++) had a better prognosis than those with low expression of SOX2 (− to +; p=0.005). There was no correlation between Hath1 expression level and prognosis (p=0.676).
Conclusions SOX2 and Hath1 are inversely expressed in gastric carcinoma. SOX2 provides a survival advantage to patients of gastric carcinoma and appears to be associated with metastasis and clinical stages.

Keywords Gastric carcinoma · SOX2 · Hath1 · Prognosis

Introduction

Patients with gastric cancer have a particularly poor prognosis because of distant metastasis is present in approximately one third of gastric cancer patients at the time of diagnosis.[1] The prognosis is generally judged according to the International Union against Cancer stage, which is mostly based on results of endoscopic ultrasonography, computed tomography, and positron emission tomography. Histological differentiation, the most widely used is Lauren classification system, also can provide many helpful information to estimate patients survival. Molecular and genetic markers of tumor are a new generation of technique to predict prognosis individually. Therefore, it is critical to find the biological factors which have a prognostic significance.

SOX2 is a member of the SRY-related high-mobility-group box proteins, which is comprised of more than 20 families. SOX2 plays a key role in the development of the embryo in its early stages, particularly with the differentiation of the nervous system and the growth of the lens.[2,3] Recently, there has been evidence that SOX2 expression changes during the development of the gastrointestinal tract and during tumorigenesis in the stomach. SOX2 is expressed in the fundus and pylorus mucosa of the stomach and controls the expression of MUC5AC, which is a main signal of gastric epithelial cells.[4,5] SOX2 is frequently

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downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis.[6] Hath1, which was first found in Drosophila, plays a key role in the development of the nervous system.[7] It is a basic helix-loop-helix transcription factor that plays a critical role in the terminal cell differentiation of the intestinal epithelium, the dorsal interneuron of the spinal cord, granule cells in the cerebellum, and inner hair cells in the auditory system.[8–11] Hath1 can upregulate gastric mucin gene expression in gastric cells, suggesting that Hath1 may be involved in gastric carcinogenesis.[2,12]

Although a growing numbers of studies have demonstrated the regulation role of SOX2 and Hath1 in the development of gastrointestinal system and their involvement in the tumorigenesis of stomach,[13–15] the clinicopathological significance of Hath1 and SOX2 expression in the gastric carcinoma is unclear, especially from the perspective of clinical epidemiology.

Materials and Methods

Patients and Clinicopathological Information

Fifty consecutive patients who had undergone surgical resections of gastric carcinoma at Southwest Hospital, Chongqing, People's Republic of China from August 2004 to December 2004, were selected for the present study. Inclusion criteria were: (1) diagnosis of gastric carcinoma pathologically; (2) ages between 20 and 80 years; (3) without distant metastasis; (4) completely clinical and pathological data. Exclusion criteria were: (1) with other malignancies; (2) chronic renal failure; (3) history of cardiac failure; (4) infection; (5) other chronic diseases. The histologically normal mucosa (n=10), peri-cancerous mucosa with intestinal metaplasia (IM; at least 5 cm adjacent to the carcinoma; n=11) and gastric carcinoma tissue (n=50) were available from 50 consecutive patients after surgical resection. Age, gender, tumor location, tumor size, histological proliferation, clinical stage, and pathological data, such as depth of invasion and presence of lymph node metastasis, of all 50 patients were obtained from hospital records. Clinical stages were based on the international standard TNM method (according to the World Health Organization). The cohort consisted of 35 men (mean age, 57.8 years; range, 29–79 years) and 15 women (mean age, 51.2 years; range, 30–70 years). Formalin-fixed, paraffin wax embedded sections of histologically corroborated gastric carcinomas (n=50) were assessed. The sections were independently examined and diagnosed by two pathologists. The study protocol was approved by the Ethics Committee of the Southwest Hospital, Third Military Medical University, according to the ethical guidelines of the 1975 Declaration of Helsinki. The Lauren type of gastric carcinoma was determined according to literature.[16]

Immunohistochemistry

Tissue sections were dewaxed, rehydrated, incubated with 3% hydrogen peroxide in methanol for 30 min to abolish endogenous activities, and then washed with 1X phosphate-buffered saline (PBS), pH7.4. After incubation with non-immune horse serum for 30 min at room temperature, the sections were washed and incubated with anti-SOX2 or anti-Hath1 polyclonal antibodies (Chemicon, Temecula, CA) overnight at 4°C. Biotin-labeled goat anti-rabbit antibodies (1:100 dilution; Vector Laboratories, Burlingame, CA) were used as secondary antibodies. After incubation for 30 min, sections were treated with streptavidin-biotin peroxidase (Vector Laboratories) for 30 min, and the reaction products were visualized with 3,3′-diaminobenzidine tetrahydrochloride (Zymed Laboratories, San Francisco, CA) and H_2O_2. Sections were lightly counterstained with hematoxylin. Negative controls included substitution of the primary antibodies with PBS or unrelated antibodies.

To obtain a more precise relationship between expression of SOX2 or Hath1 and prognostic indicators, a semiquantitative analysis was performed using SOX2 or Hath1 expressing cells in the total tumor-bearing population. We reviewed five to ten fields of each cancerous tissue at high magnification (×400) and counted the number of cells in each field expressing SOX2 or Hath1. The analysis was graded as "+++" if 50–100% of cancerous cells stained positive for SOX2 or Hath1, "++" if 10–50% cancerous cells stained positive, and "+" if less than 10% of cancerous cells were positive according to the scoring system used for the immunohistochemical staining.[17] Patients were then categorized into the low expression group (− to +) and the strong expression group (+ to +++).

Diagnosis of *Helicobacter pylori* Infection

Histological examination with Warthin–Starry and Giemsa staining techniques as well as the rapid urea test were used to identify *H. pylori* infection in all cases. Tissues were designated as positive for *H. pylori* infection if more than two of the methods described above gave positive results.

Follow-Up Study

Follow-up data for all 50 patients were obtained by reviewing hospital records or by direct communication with the patients or their relatives after surgical resection. The follow-up period was defined as the date of surgical resection of the tumor to the date of death or last follow-up.

Statistical Analysis

The Chi-squared test was used to analyze the data. Differences were considered as statistically significant if p values were <0.05 and highly significant if p values were <0.01. The probabilities of survival after surgical resection of the tumor were estimated using the Kaplan–Meier method and compared using the Log-rank test.

Results

SOX2 Expression in Normal Gastric Mucosa, Intestinal Metaplasia, and Gastric Carcinoma

The expression of SOX2 was assessed by immunohistochemistry. In all 10 sections of normal gastric mucosa studied, abundant amounts of SOX2 were detected (100%; Fig. 1a). In addition, immunoreactivity for SOX2 was found in the nuclei of positive cells, which were mainly distributed at the neck zone of the gastric glandular epithelium. Fig. 1b showed the typical immunohistochemical results of SOX2 expression in the gastric mucosa undergoing IM. The nuclear staining of SOX2 in IM was weaker than in normal gastric mucosa, and the positive staining cells in intestinal metaplasia mainly belonged to the columnar cells, with no staining of SOX2 observed in goblet cells. Among 11 cases with IM, seven cases showed SOX2 expression (63.6%). In all 50 samples of gastric carcinoma, 35 cases (70.0%) were positive for SOX2 expression. Among them, 19 cases weakly expressed SOX2 (+), eight cases were positive (++), and eight cases strongly expressed SOX2 (+++; Fig. 1c). SOX2 was strictly localized to the cytoplasm of the cancerous cells and showed a granular pattern, its expression pattern in cancerous cells of gastric carcinoma was different from that in normal gastric mucosa and IM. It indicated that SOX2 expressed in the cytoplasm of the cancerous cells cannot translocate at the nucleus of cancerous cells as in the normal cells and cells of benign lesions, such as IM. The exact mechanism of its abnormal location was still unclear. Although the positive rates of SOX2 in normal gastric mucosa, intestinal metaplasia, and gastric carcinoma were not statistically different ($p>0.05$), there was a trend that SOX2 expression in the intestinal metaplasia and gastric carcinoma was decreased compared with normal gastric mucosa.

Hath1 Expression in Normal Gastric Mucosa, Intestinal Metaplasia, and Gastric Carcinoma

There was very low expression of Hath1 in normal gastric mucosa, with a scattered distribution at the basal antral glands and its positive rate in normal gastric mucosa ($n=10$) was 100% (Fig. 2a); however, Hath1 was strongly expressed in areas of IM (Fig. 2b). Among the 11 cases of IM, all of them were positive for Hath1 expression (100%). Hath1 was granularly located at the nucleus of metaplastic tissues in both the columnar cells and the goblet cells. In all 50 samples of gastric carcinoma, 46 cases (92.0%) were positive for Hath1 expression. Among them, 10 cases weakly expressed Hath1 and belonged to mild expression (+), 15 cases were positive (++), and 21 cases strongly expressed Hath1 (+++; Fig. 1c). Hath1 was strictly distributed in the nucleus and cytoplasm of positively stained cancerous cells and displayed a homogeneous pattern. Its cytoplasmic location in the cancerous cells was also different from that in normal gastric mucosa and IM. It indicated that Hath1 expressed partially in the cytoplasm of the cancerous cells did not translocate at the nucleus of cancerous cells as in the normal cells and the cells in intestinal metaplasia. The exact mechanism of its abnormal location was unclear. Although the positive rates of Hath1 in normal gastric mucosa, intestinal metaplasia, and gastric

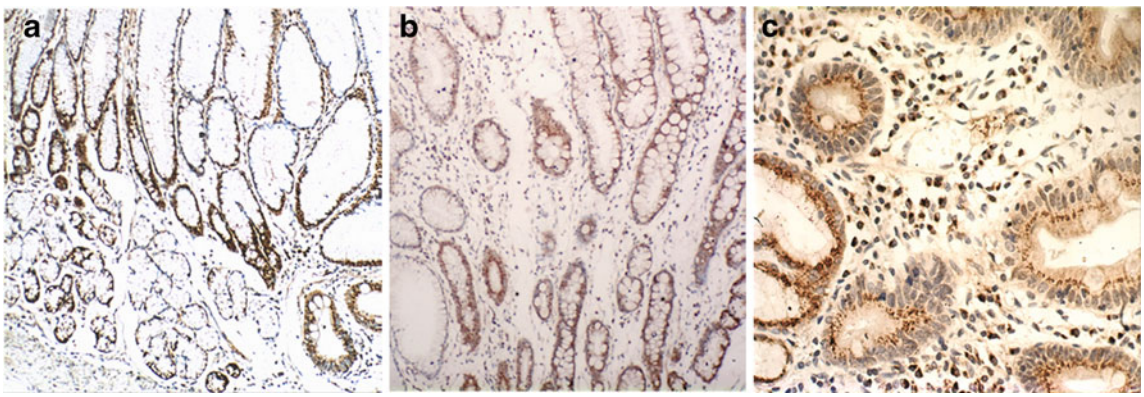

Fig. 1 Distribution of the SOX2 transcription factor by immunohistochemistry in sections of normal gastric mucosa (**a**), intestinal metaplasia (**b**), and gastric carcinomas (**c**). SOX2 is located in the nuclei of positively stained cells of normal gastric mucosa and intestinal metaplasia but located at the cytoplasm of cancerous cells (original magnification, **a, b** ×100; **c** ×400).

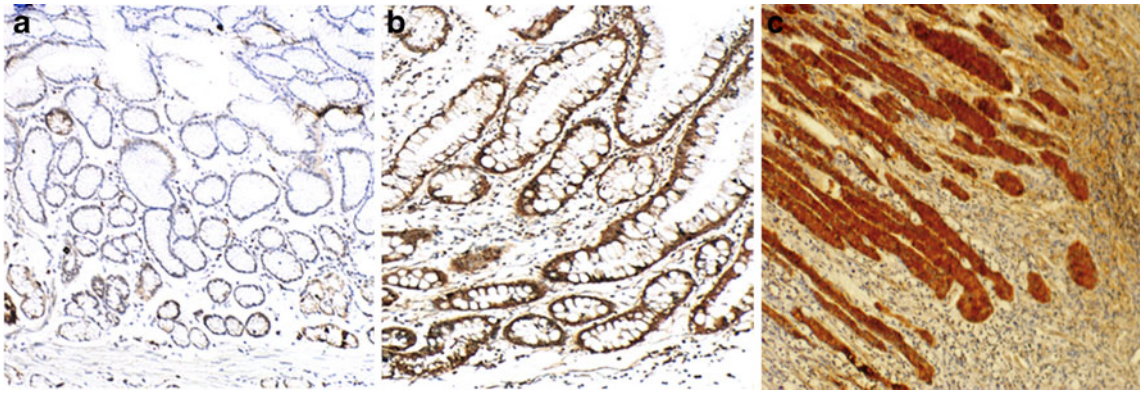

Fig. 2 Immunohistochemical localization of Hath1 in normal gastric mucosa (**a**), intestinal metaplasia (**b**), and gastric carcinomas (**c**). Hath1 was located in the nuclei of positively stained cells of normal gastric mucosa and intestinal metaplasia, but located in the nucleus and cytoplasm of cancerous cells (original magnification, **a** ×100; **b** ×400; **c** ×200).

carcinoma were not statistically different ($p>0.05$), there was a trend that the intensity of Hath1 expression in the intestinal metaplasia and gastric carcinoma was increased compared with normal gastric mucosa.

Relationship of Hath1 and SOX2 Expression in Gastric Carcinoma

Of 50 cases of gastric carcinoma, 14 cases had negative or mild Hath1 expression (− to +) and 34 cases had negative or mild SOX2 expression (− to +), 36 cases had strong Hath1 expression (++ to +++), and 16 cases had strong SOX2 expression (++ to +++). Comparing with the weak expression of Hath1 and strong expression of SOX2 in the normal gastric mucosa, in the cancerous tissues of gastric carcinoma, aberrant expression of Hath1 and SOX2 were obvious. Based on the semiquantitative analysis of Hath1 and SOX2 in the cancerous tissues, there was an obvious trend, along with the elevated expression of Hath1 in the cancerous tissues compared with low expression of Hath1 in normal gastric mucosa, SOX2 expression in gastric carcinoma was decreasing compared with strong expression of SOX2 in normal gastric mucosa, so Hath1 and SOX2 in the cancerous tissues of gastric carcinoma were inversely expressed.

Clinicopathological Significances of Hath1 and SOX2 in Gastric Carcinoma

As shown in Table 1, Hath1 immunoreactivity levels did not correlate with respect to patient gender, tumor location, tumor size, grade of differentiation, lymph node metastasis, depth of invasion, clinical stages, or Lauren's types ($p>0.05$). When we analyzed the relationship between the expression of SOX2 in the cancerous tissues and patients' clinicopathological features, there was no significant correlation between SOX2 immunoreactivity and patient gender, tumor location, tumor size, grade of differentiation ($p>0.05$); however, the patients with low SOX2 expression (− to +) in their cancerous tissues had more lymph nodes with metastasis compared to those with strong SOX2 expression (++ to +++) in their cancerous tissues ($p=0.007$), the patients with low SOX2 expression (− to +) in their cancerous tissues had deeper invasion of gastric wall compared with those with strong SOX2 expression (++ to +++) in their cancerous tissues ($p=0.010$). SOX2 expression was also significantly correlated with different clinical stages (I–II vs. III–IV; $p=0.011$). In patients with stage III–IV cancers, the expression of SOX2 was significantly decreased. There was no significant difference of SOX2 expression level among the three Lauren types ($p=0.412$). There was no significant difference of SOX2 and Hath1 expression in the cancerous tissues between the patients with *H. pylori* infection and patients without *H. pylori* infection ($p=0.194$ and $p=0.082$).

Survival Analysis

To examine the correlation between aberrant expression of Hath1 or SOX2 in cancerous tissues and patient prognosis, the patients were divided into strong expression (++ to +++) group and weak expression (− to +) group according to the level of SOX2 or Hath1 expression in the cancerous tissues, respectively. The Kaplan–Meier survival analysis showed that there was a very significant difference between the patients with strong SOX2 expression and those with weak SOX2 expression in their cancerous tissues (median survival 30 months and 4-year survival 45% versus median survival 14 months and 4-year survival 8%, log rank test $p=0.005$, Fig. 3a). In other words, 34 patients with strong SOX2 expression (++ to +++) in their cancerous tissues had a significant survival advantage than those patients ($n=16$) with weak SOX2 expression in their cancerous tissues. The

Table 1 Relationship between Hath1 and SOX2 Expression in Cancerous Tissues and Clinicopathological Features

Variables		Number	Hath1		Number	SOX2	
			− to +	++ to +++		− to +	++ to +++
Gender	Male	35	12	23	35	23	12
	Female	15	2	13	15	11	4
p Value			>0.05			>0.05	
Tumor location	Fundus	10	4	6	10	4	6
	Body	15	4	11	15	12	3
	Antrum	25	6	19	25	18	7
p Value			>0.05			>0.05	
Tumor size	<5 cm	29	8	21	29	21	8
	>5 cm	21	6	15	21	13	8
p Value			>0.05			>0.05	
Differentiation	Well/moderate	19	5	14	19	12	7
	Poor	31	9	22	31	22	9
p Value			>0.05			>0.05	
Depth of invasion	T_1	5	2	3	4	1	3
	T_2	25	6	19	24	13	11
	T_3	14	3	11	14	12	2
	T_4	6	3	3	8	8	0
p Value			>0.05			0.010	
Lymph node metastasis	N_0	16	3	13	16	15	1
	N_1	15	7	8	25	12	13
	N_2 and N_3	19	4	15	9	7	2
p Value			>0.05			0.007	
Clinical stages	I-II	31	10	21	31	17	14
	III-IV	19	4	15	19	17	2
p Value			>0.05			0.011	
H. pylori infection	(−)	31	6	25	31	19	12
	(+)	19	8	11	19	15	4
p Value			0.082			0.194	
Types of carcinoma	Intestinal	21	8	13	21	10	2
	Diffuse	20	4	16	20	18	11
	Mixed	9	2	7	9	6	3
p Value			>0.05			>0.05	

result of the patients' prognosis was consistent with the observation of the clinicopathological features between the patients with strong SOX2 expression (++ to +++) in their cancerous tissues and the patients with weak SOX2 expression (− to +) in their cancerous tissues. SOX2 effect on prognosis might be mediated through its affection on lymph node metastases, invasion of gastric wall, and clinical stages of gastric cancer patients. However, the Kaplan–Meier survival analysis showed that there was no significant difference between the patients with strong Hath1 expression in their cancerous tissues and those with weak Hath1 expression in their cancerous tissues (median survival 14 months and 4-year survival 23% versus median survival 26 months and 4-year survival 16%, log rank test $p=0.676$, Fig. 3b). That is, Hath1 expression level in their cancerous tissues was not related to the prognosis of patients with gastric carcinoma treated by surgical resections.

Discussion

SOX2 is an important transcription factor during the normal process of gastric epithelial cell differentiation and forced expression of SOX2 can induce the expression of endogenous MUC5AC. MUC5AC and MUC6 are mucins mainly expressed in the gastric mucosa, which has been reported

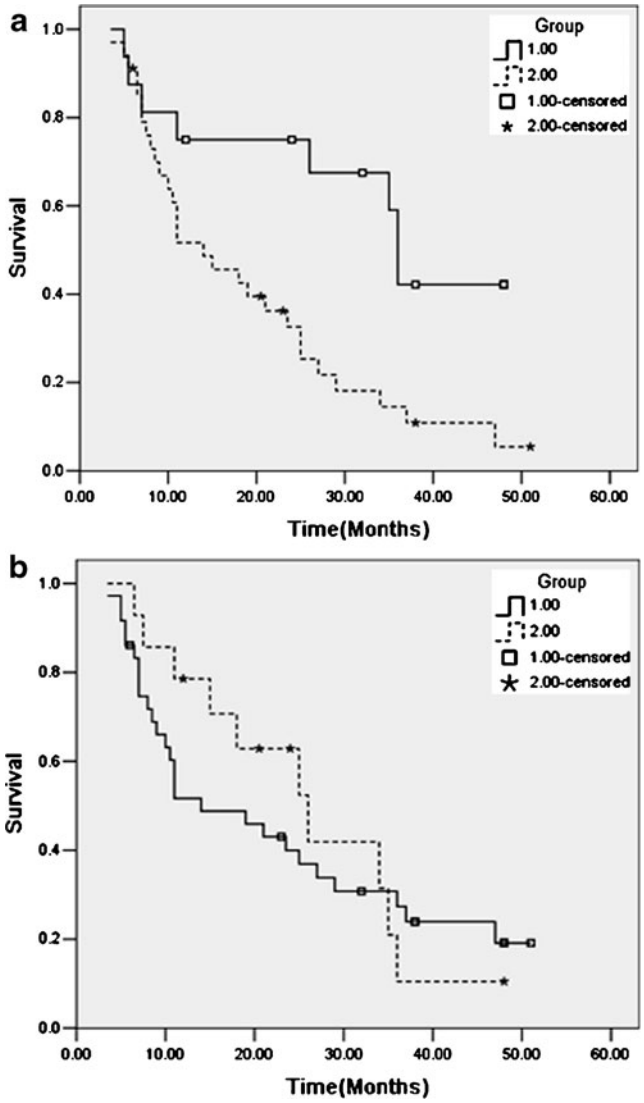

Fig. 3 Kaplan–Meier survival curves for the 50 gastric cancer patients according to SOX2 (**a**) and Hath1 (**b**) expression level. Patients with high SOX2 expression (++ to +++; *continuous line*) had a significantly better prognosis than those with low SOX2 expression (− to +; *dotted line*; $p=0.005$, log rank test). Patients with high Hath1 expression (++ to +++; *continuous line*) had no significant difference in prognosis comparing with those with low Hath1 expression (− to +; *dotted line*; $p=0.676$, log rank test). The *x*-axis represents survival time after surgery, and the *y*-axis represents cumulative overall survival.

by our group and by others.[17,18] Hath1 is another transcription factor that is important during the normal process of intestinal epithelial cell differentiation. The balance of Hath1 and SOX2 expression might be important in maintaining the normal differentiation and maturation of gastric or intestinal epithelium. If these factors are interfered with, the epithelial cells of the stomach and intestine may unavoidably become abnormal. The down-regulation of SOX2 and upregulation of Hath1 may generate homotropic effects, which finally lead to intestinal metaplasia and gastric carcinoma.

One notable finding in our study is the clinical significance identified for Hath1 and SOX2 in gastric cancer. This is supported, in part, by our examination of Hath1 and SOX2 expression in gastric cancer samples. Through initial analysis of immunohistochemical experiments, we found that the two important transcription factors that are necessary for the maturation and differentiation of the epithelium cells of gastric and intestinal mucosa, Hath1 and SOX2, are inversely expressed in the cancerous tissues of gastric carcinoma. Hath1 was detected in the cancerous tissues of 46 out of 50 patients with gastric carcinoma (92%), and SOX2 was detected in the cancerous tissues of 35 out of 50 patients with gastric carcinoma (70%), compared with the low expression of Hath1 and strong expression of SOX2 in the normal gastric mucosa. The cytoplasmic location of SOX2 and Hath1 in the cancerous cells of gastric cancer may be a significant feature and causes the dysfunction of these two transcription factors in the cancerous cells. The expression level of SOX2 in the cancerous tissues was related to lymph node metastases ($p=0.007$), depth of invasion ($p=0.010$), and clinical stages ($p=0.011$), but the expression level of Hath1 in the cancerous tissues was not related to the clinicopathological features ($p>0.05$), including lymph node metastases, depth of invasion, and clinical stages. There was no significant difference of SOX2 and Hath1 expression in the cancerous tissues of patients with *H. pylori* infection and patients without *H. pylori* infection ($p>0.05$), this kind of data need to be confirmed by in vitro study. The present data indicate that SOX2, but not Hath1, is related to the clinicopathological features of gastric cancer patients.

The most important finding in the study is that the expression of SOX2 in the cancerous tissues of gastric carcinoma and analysis of its expression level could provide a primary indication for patients' prognoses. If gastric cancer patients have a high level expression of SOX2 (++ to +++) in their cancerous tissues, their prognosis will be better, while the patients with low expression of SOX2 in their cancerous tissues are likely to have a worse prognosis. But there is no correlation between Hath1 expression level and patients prognosis. The results of our study suggest that a raised SOX2 level in the cancerous tissues of gastric carcinoma is an indicator for survival in patients with gastric carcinoma after surgical resection. There is a number of articles that showed an aberrant expression of Hath1 or SOX2 to be indicative for survival in lung adenocarcinoma and esophageal squamous cell carcinoma.[19,20] Westerman et al. found that patients with ATOH1-expressing lung adenocarcinomas have a worse prognosis.[19] Oct3/4 and SOX2 are significantly

associated with an unfavorable clinical outcome in human esophageal squamous cell carcinoma.[20] To our knowledge, this is the first report analyzing patients' prognoses using SOX2 in cancerous tissues of gastric cancer.

However, an important and potential limitation of this study is that it is the small number of patients included in the study, the data could provide limited statistical power and this may lead to casual inferences. Our data need to be confirmed in additional studies with more patients and in multiple centers. But a significant difference in survival is so remarked between the patients with high level expression of SOX2 (++ to +++) in their cancerous tissues and the patients with low level expression of SOX2 (− to +) in their cancerous tissues that we think the difference is worth reporting.

Conclusion

In summary, our findings suggested that Hath1 and SOX2 transcription factors were inversely expressed in the cancerous tissues of gastric cancer patient, and the detection of SOX2 as well as further comparison of their expression patterns might provide a primary indication for the prognoses of patients with gastric carcinoma.

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ORIGINAL ARTICLE

PinX1 Inhibits Telomerase Activity in Gastric Cancer Cells Through Mad1/c-Myc Pathway

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Abstract
Introduction The aim of this study was to investigate the role of Mad1/c-Myc in telomerase regulation in gastric cancer cells in order to gain insight into telomerase activity and to evaluate PinX1 as a putative inhibitor of gastric cancer.
Methods PinX1 and PinX1siRNA eukaryotic expression vectors were constructed by recombinant technology and transfected into gastric carcinoma cells using Lipofectamine™ 2000. Telomerase activity was measured by the telomeric repeat amplification protocol. Apoptosis of gastric cancer cells was analyzed by flow cytometry and transmission electron microscopy. Reverse transcription-polymerase chain reaction and Western blotting were used to assess the expression levels of PinX1 and Mad1/c-Myc.
Results We found that PinX1-negative gastric cancer cells showed significantly higher telomerase activity than did the PinX1-postive cells. PinX1-transfection reduced telomerase activity in PinX1-negative gastric cancer cells and exhibited an upregulation of Mad1 and downregulation of c-Myc expression. Pinx1 RNAi treatment led to downregulation of Mad1 and upregulation of c-Myc.
Conclusion Suppression of telomerase activity mediated by PinX1 is involved in the Mad1/c-Myc pathway.

Keywords Cell growth · Gastric carcinoma cell · PinX1 gene · Telomerase · Molecular mechanism

Introduction

Human telomerase is a ribonucleoprotein composed of human telomerase RNA component (hTR), human telomerase catalytic subunit (hTERT), and a telomerase-associated protein (TEP).[1–3] There is a strong correlation between the presence of hTERT mRNA and telomerase activity, and it is recognized that hTERT is the rate-limiting determinant of the enzymatic activity of human telomerase.[4–6] It is widely accepted that hTERT expression levels may be used as a surrogate marker for telomerase activity.[7,8] High levels of telomerase activity have been detected in most cancers including those arising from gastrointestinal epithelium.[9–12] We have previously demonstrated that telomerase activity is present in a majority of gastric cancer (GC) and colorectal cancer[11,12] types, and antisense human telomerase reverse transcriptase could partially reverse in vitro malignant phenotypes of gastric carcinoma cell lines.[13] All these studies suggest that activation of telomerase is a common and a critical event for transformation of gastric cancer cells, and it may be a critical step in the development of gastric cancers.

The transcriptional regulation of hTERT expression has been extensively analyzed in cancer cells.[14–17] A novel Pin2/TRF1-binding protein, PinX1, appears to be involved in negatively regulating telomerase function.[18] In humans, hPinX1 directly binds to and inhibits the enzymatic activity of hTERT. Ectopic overexpression of hPinX1 results in a decrease of both the telomerase activity and tumorigenicity

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of cancer cells, while suppression of hPinX1 expression leads to an increase in both telomerase activity and cancer cell tumorigenicity.[19] These observations lead to the suggestion that hPinX1 may be a putative tumor inhibitor in human cells. It has been found that loss of heterozygosity (LOH) and histone hypoacetylation of the PinX1 gene are associated with reduced expression in gastric carcinomas.[20] It has also been found that genetic instability of PinX1 plays an important role in the reduced expression of PinX1 in gastric carcinoma, and an increased expression of PinX1 could possibly contribute towards a better prognosis.[21] These results indicate that the downregulation of PinX1, due to LOH, contributes to activation of telomerase in stomach carcinogenesis.

The proto-oncogene c-Myc, a positive regulator of telomerase, is one of the major determinants of hTERT expression as it directly binds the hTERT promoter to induce transcription.[22] The ability of c-Myc to activate telomerase may contribute to its ability to promote tumor formation.[23] It was found that anti-c-myc siRNA decreased expression of c-Myc and hTERT, and the downregulation of c-Myc and hTERT inhibited cell growth and suppressed telomerase activity in colon cancer Colo 320 cells.[24] Mad1 is a transcriptional repressor that suppresses c-Myc-mediated transactivation by competing for the ubiquitous binding partner Max from binding to c-Myc.[25] The decreased expression of Mad1 in normal cells could reduce its antagonistic activity toward c-Myc and, in turn, activate genes involved in tumorigenesis, including hTERT.[25,26] Depletion of Mad1 by siRNA increased the association of c-Myc with the hTERT promoter, presumably through an increase of c-Myc/Max binding to the promoter.[25]

Although much is known regarding the role of PinX1 in regulating telomerase function, less is known about the role of Mad1/c-Myc pathway in telomerase regulation by PinX1 in gastric cancer. The aim of this study was to investigate the roles of Mad1/c-Myc in telomerase regulation in gastric cancer cells in order to gain insight into the activity of telomerase and to evaluate PinX1 as a putative inhibitor of gastric cancer.

Materials and Methods

Cell culture The gastric cancer cell lines BGC-823 and SGC-7901 were maintained in our laboratory, and MKN28 was obtained from the Fourth Military Medical University. Cells were cultured in RPMI-1640 (Gibco, USA) medium supplemented with 10% fetal calf serum, 100 units/mL penicillin, and 100 g/mL streptomycin. They were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Construction of PinX1-expressing plasmid and RNAi plasmids Construction of the PinX1 expression plasmid was described previously.[20] For RNAi design, we selected three pieces of interference sequence targeting PinX1 by using an on-line design tool. The oligonucleotides used to generate the three different PinX1 siRNA fragments were as follows: interference1 sense 5′-GATCCGTAATATGATCTG TGGCTCCTCAAGACGGGAGCCACAGATCATATTA TTTTTTA-3′ and antisense 3′-GCATTATACTAGACACC GAGGAGTTCTGCCCTCGG TGTCTAGTATAATAAA AAATTCGA-5′; interference2 sense 5′-GATCCGTCTGAG AGCCACGATTGAGTCAAGACGCTCAATCGTGGCT CTCAGATTTTTTA-3′ and antisense 3′-GCAGACTCTC GGTGCTAACTCAGTTCTGCGAG TTAGCACCGAGAG TCTAAAAAATTCGA-5′; interference3 sense 5′-GATCC GTTACGTTCCACCTGCGT CTTCAAGACGAGACG CAGGTGGAACGTAATTTTTTA-3′ and antisense 3′-GC AATG CAAGGTGGACGCAGAAGTTCTGCTCTGCG TCCACCTTGCATTAAAAAATTCGA -5′. In addition, an independent fragment to human genomic DNA was designed, synthesized, annealed, and cloned into the vector pRNAT-U6.1/Neo (6,380 bp, GenScript) using the restriction sites of BamH I and Hind III. The PinX1-expressing plasmid, individual RNAi plasmids, independent sequence plasmid, or control plasmid were transfected into gastric cancer lines with Lipofectamine™ 2000 (Invitrogen, USA) according to the manufacturer's protocol.

Telomerase activity assays Telomerase activity was measured by telomeric repeat amplification protocol (TRAP) as described previously.[9] Cellular extracts were prepared with 1× CHAPS lysis buffer. Telomerase activity was measured with the TRAPeze telomerase detection kit (Chemicon, USA) according to the instruction of the manufacturer.

Reverse transcription-PCR Total RNA was extracted with Trizol reagent (Invitrogen). The reverse transcription-polymerase chain reaction (RT-PCR) was performed using the RT-PCR system (Takara, China) in a 20-μL reaction mixture that contained 1 μg total RNA. The PCR of PINX1 and β-actin was carried out in a total volume of 25 μL (12.5 ul 2× Master Mix, 1 μL each primer, 8.5 μL double-distilled water, and 2 μL of template). The PCR cycles, primers, and size of amplified product have been presented in Table 1. Amplified products were visualized on 1.5% agarose gel with ethidium bromide.

MTT assays Tumor cell viability was determined by using 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. Gastric cancer cells were plated in 96-well plates at 15×10^3 cell density per well. After the indicated treatments and time points, the cells were incubated for 2–3 h with 0.5 mg/ml of MTT and lysed with dimethyl

Table 1 The Polymerase Chain Reaction Primers and Amplified Products

	PCR primers (5′→3′)	PCR cycles	Size of amplified products (bp)
PinX1 sense PinX1 antisense	GGGTGGTCTAAAGGAAAGGGTT CGCCCTCGGGAGTCTTCTTAC	95°C for 4 min, 30 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 30 s, and 72°C for 5 min	364
β-actin sense β-actin antisense	GCTCCAACCGACTGCTGTCA GTCCTGTGGCATCCACGAAAC	Same as above	500
c-myc sense c-myc antisense	CCAGGAGTGTATGTGGAGCG CTTGAGGACCAGTGGGCTGT	95°C for 6 min, 30 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, and 72°C for 8 min	505
Mad1 sense Mad1 antisense	GAAAAGCCGTTCACCAAATCGA CACTGAAGTTTACGTGCAAGGGAGT	95°C for 6 min, 35 cycles of 94°C for 30 s, 68°C for 30 s, 72°C for 30 s, and 72°C for 8 min	432

sulfoxide (DMSO). Absorbance rates were measured at 490 nm in a microplate reader (Bio-Rad, Hercules, CA, USA). Cell viability percentage was calculated by using the following formula: $(\text{absorbance of treated cells}/\text{non-treated cells}) \times 100$.

Analysis of cell cycle and apoptosis The cell lines mentioned above were washed with PBS and harvested by 0.25% pancreatin. The counted cells (2×10^6) were washed in PBS and centrifuged at 1,000 rpm for 5 min. Seventy percent alcohol (1.5 mL) was used to fix the pellet for 24 h at 4°C. After centrifugation, cells were resuspended in 1 mL PBS and incubated for 30 min with 1 mg/mL RNase at 37°C. Then cells were stained with 50 μg/mL propidium iodide (PI) at 4°C for 30 min before analysis by flow cytometry (BD Corporation, USA). All the experiments were repeated twice. Apoptosis was detected by using the Annexin-V-FITC Apoptosis Detection Kit (China). Apoptotic cells were confirmed under transmission electron microscopy.

Western blotting Lysates from all cell lines were prepared as described previously.[18] Protein samples (10 μg) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, USA). The membrane was blocked in 10% milk for 4 h at room temperature and incubated with a monoclonal anti-PinX1 (Abnova, Taiwan), anti-c-Myc, or anti-Mad1 antibody (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) for 1 h at room temperature, followed by incubation with a horseradish peroxidase-conjugated rabbit anti-mouse IgG secondary antibody (Amersham Biosciences, Buckinghamshire, England) for 1 h at room temperature. The signal was visualized using Kodak-X-Omat LS film. All the experiments were repeated at least two times with similar results.

Statistics Unless otherwise stated, all experiments were run in triplicate, and the results have been depicted as mean ± SD. Statistical analysis was performed using Student's *t* test. The difference was considered statistically significant when the *P* value was less than 0.05. All statistical analyses were conducted with SPSS 13.0 software.

Results

PINX1 and Telomerase Activity in Gastric Cancer Cells

Endogenous PINX1 expression in gastric cancer MKN28, SGC-7901, and BGC823 cells was detected with RT-PCR and Western blot methods. PinX1 expression was negative in MKN28 cells, weakly positive in SGC7901 cells, and strongly positive in BGC823 (data not shown). Therefore, MKN28 cells and BGC823 cells were chosen for subsequent experimentation. MKN28 cells without PinX1 expression showed significantly higher telomerase activities than did the BGC823 cells with strong expression of PinX1.

PinX1 Transfection Increases PinX1 Expression

Western blotting and RT-PCR analysis indicated that PinX1-transfected MKN28 cells expressed PinX1 at higher levels, while no expression was detected in control cells or untransfected MKN28 cells (Fig. 1a, b). Two RNAi vectors were identified that were able to specifically suppress the expression of PinX1 in BGC823 cells (Fig. 1c, d).

PinX1-RNAi rate analysis showed that among the three interference sequences (interference 1–3), interference 2 showed the highest interference rate (61%), so it was used to investigate the effects of PinX1-RNAi on gastric cancer cells and its corresponding mechanisms (data not shown).

PinX1 Transfection Inhibited MKN28 Cell Growth

To evaluate the effects of PinX1 transfection on MKN28 cells, cell growth curves obtained by the MTT assay were

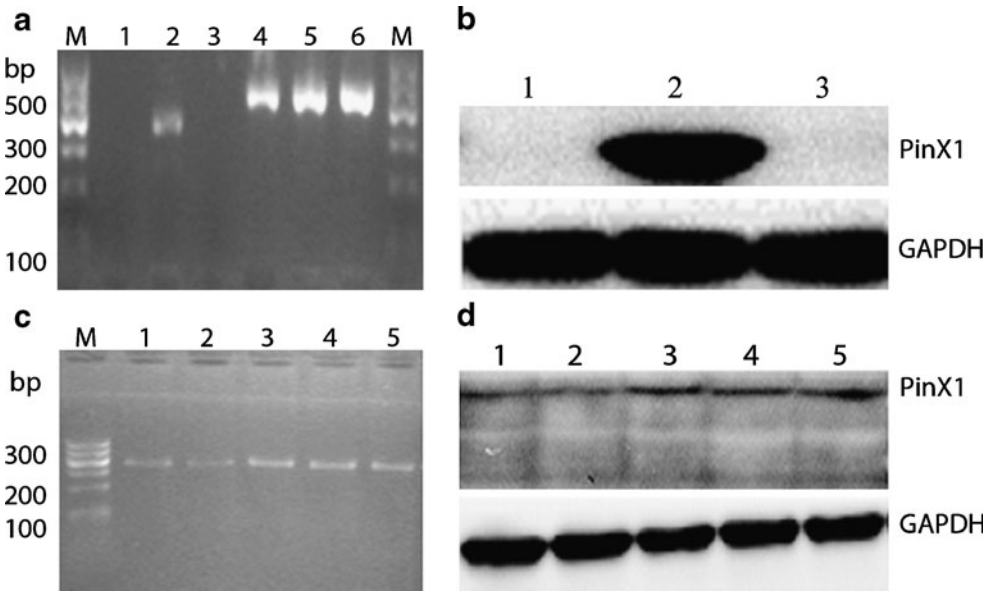

Fig. 1 PinX1 expression before and after gene transfection. **a** PINX1 mRNA expression in MKN28 cells. *Lane M*, marker; *lane 1*, untransfected MKN28 cells; *lane 2*, PinX1-transfected MKN28 cells; *lane 3*, control cells; *lanes 4–6*, propidium iodide (PI) at 4°C for 30 min before analysis by flow cytometry (BD Corporation), β-actin expression in related gastric carcinoma cells. **b** PinX1 protein level in MKN28 cells. *Lane 1*, untransfected cells; *lane 2*, PinX1-transfected cells; *lane 3*, control cells. **c** PINX1 mRNA expression in BGC823 cells. *Lane M*, marker; *lane 1*, interference 1 cells; *lane 2*, interference 2 cells; *lane 3*, interference 3 cells; *lane 4*, control cells; *lane 5*, BGC823 cells. **d** PinX1 protein expression in BGC823 cells. *Lane 1*, interference 1 cells; *lane 2*, interference 2 cells; *lane 3*, interference 3 cells; *lane 4*, control cells; *lane 5*, BGC823 cells.

plotted. As shown in Fig. 2a, the growth rate was much lower in PinX1-transfected cells than in control and untransfected cells.

We also examined the cell cycle changes in PinX1-transfected MKN28 cells with flow cytometry. The cell cycle distributions of MKN28 cells have been shown in Table 2. Flow cytometric analysis displayed an arrest at the G0/G1 phase and a significantly decreasing proliferation index (PI) in PinX1-transfected MKN28 cells as compared with control and untranfected cells (15.24±0.61 vs 34.08±0.80 or 31.28±0.53, $P<0.05$).

PinX1 Transfection Promoted Apoptosis in MKN28 Cells

Cellular apoptosis was examined by the Annexin-V/PI method in MKN28 cells. Annexin-V binds to those cells that express phosphatidylserine on the outer layer of the cell membrane, a characteristic feature of cells entering apoptosis. Apoptosis was then quantified by flow cytometry. Our results showed that PinX1 transfection was able to promote the apoptosis of MKN28 cells effectively as compared with control and untranfected cells. The apoptosis rates (percent) were 11.41±2.85 in transfected cells, 2.40±1.31 in control

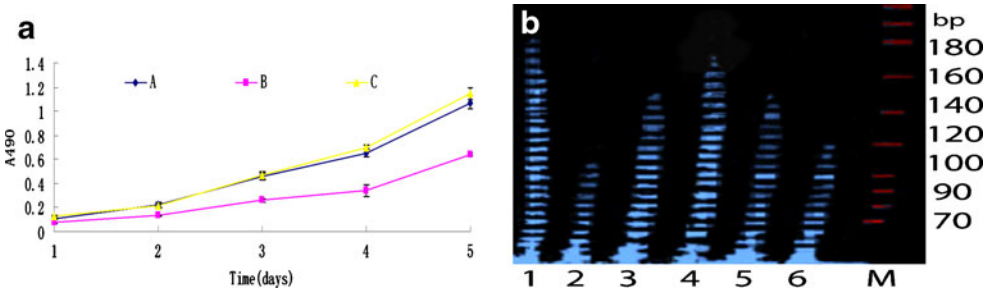

Fig. 2 Cell growth curves (**a**) and telomerase activity (**b**) after PinX1 transfection. **a** PinX1 transfection inhibited the growth of MKN28 cells. *Line A*, MKN28 cells transfected with empty vectors; *line B*, PinX1-transfected MKN28 cells; *line C*, MKN28 cells. The data showed that PinX1-transfected MKN28 cells grew much slower than control or untranfected cells ($P<0.05$). **b** PinX1-transfected MKN28 cells showed reduced telomerase activity, and PinX1-RNAi displayed robust telomerase activity. *Lane 1*, MKN28 cells; *lane 2*, PinX1-transfected MKN28 cells; *lane 3*, control MKN28 cells; *lane 4*, PinX1-RNAi cells; *lane 5*, untransfected BGC823 cells; *lane 6*, control cells; *lane M*, marker.

Table 2 Effect of PinX1 Transfection on Cell Cycle of Gastric Carcinoma MKN28 Cells

	G_0/G_1	*S*	G_2/M	PI
PinX1-untransfected cells	68.74±6.96	8.65±6.82	22.64±1.17	31.28±0.53
PinX1-transfected cells	84.76±3.92	4.44±3.68	10.80±2.48	15.24±0.61
Control cells	65.91±3.00	6.06±6.43	28.02±5.80	34.08±0.80

cells, and 0.85±0.10 in untranfected cells ($P<0.05$; Fig. 3a–c).

We also examined apoptosis of PinX1-transfected MKN28 cells under transmission electron microscopy. Apoptotic cells are characterized morphologically by cell shrinkage, chromatin condensation, and cellular fragmentation into apoptotic bodies (Fig. 3d–f).

Telomerase Activity in PinX1-Transfected Gastric Cells

We examined telomerase activity in PinX1-transfected MKN28 cells and PinX1-RNAi BGC823 cells and found that PinX1-transfected MKN28 cells showed reduced telomerase activity, while PinX1-RNAi transfected cells displayed robust telomerase activity. In contrast, there was no significant change observed by TRAP-assay in the telomerase activity in either the control and untransfected cells (Fig. 2b).

Expressions of Mad1/c-Myc

We observed the expression of c-Myc and Mad1 in gastric cancer cells using RT-PCR and Western blotting in response to PinX1 and PinX1-RNAi transfection. As expected, PinX1-transfected MKN28 cells exhibited upregulation of Mad1 and downregulation of c-Myc expression, whereas RNAi led to downregulation of Mad1 and upregulation of c-Myc in BGC823 cells (Fig. 4).

Discussion

The process of malignant transformation from normal human somatic cells to immortal tumor cells involves complex regulatory mechanisms. The transformation process is characterized by the acquisition of mutant alleles of

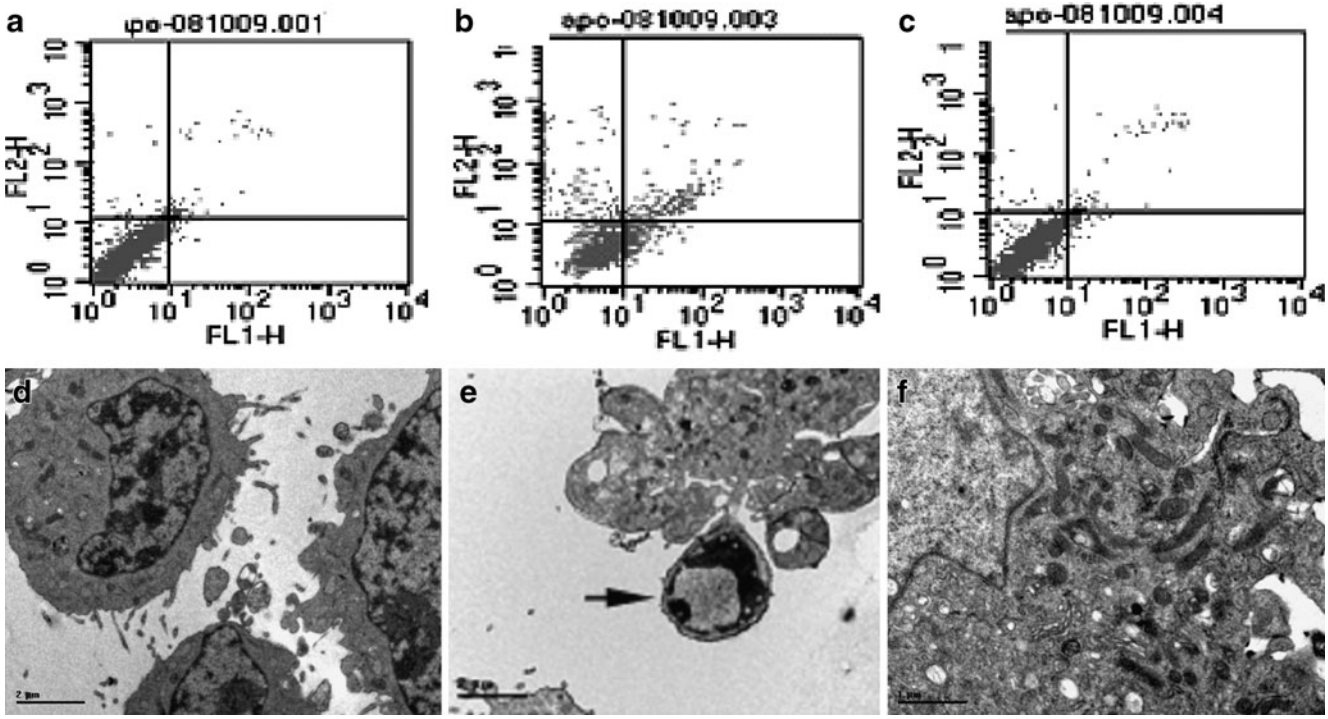

Fig. 3 Apoptosis induced by PinX1 transfection in MKN28 cells. **a–c** Apoptosis in MKN28 cells before and after PinX1 transfection: (**a**) MKN28 cells, (**b**) PinX1-transfected MKN28 cells, and (**c**) control MKN28 cells. **d–f** Morphological changes observed under transmission electron microscopy: (**d**) MKN28 cells; (**e**) PinX1-transfected MKN28 cells, *arrow* denotes an apoptotic body; and (**f**) control cells.

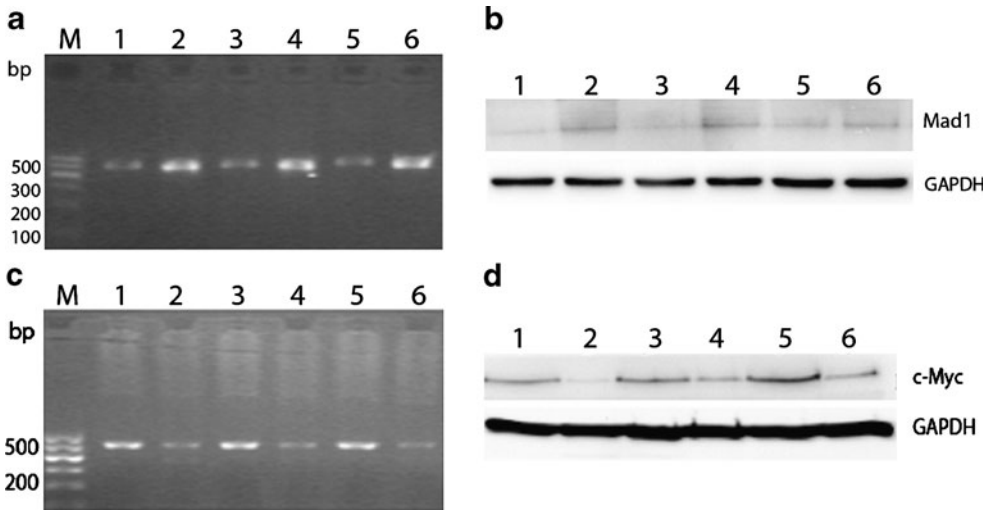

Fig. 4 Expressions of Mad1/c-Myc in PinX1-transfected gastric carcinoma cells. PinX1-transfected MKN28 showed upregulation of Mad1 and downregulation of c-Myc expression, whereas RNAi led to downregulation of Mad1 and upregulation of c-Myc in BGC823 cells. Mad1 mRNA (**a**) and protein (**b**) levels in gastric carcinoma cells. c-Myc mRNA (**c**) and protein (**d**) levels in gastric carcinoma cells. *Lane 1*, untransfected MKN28 cells; *lane 2*, PinX1-transfected MKN28 cells; *lane 3*, control MKN28 cells; *lane 4*, BGC823 cells; *lane 5*, PinX1 RNAi cells; *lane 6*, control cells; *lane M*: marker.

oncogenes and/or tumor suppressor genes and other genetic changes that directly or indirectly control cell proliferation.[27] Besides alterations in oncogenes and tumor suppressor genes, immortalization and transformation are associated with telomerase activation.[28] The nucleolar protein PinX1 has been proposed to be a putative tumor suppressor due to its binding to and inhibition of the catalytic activity of telomerase.

The study presented herein shows that PinX1 is a potent telomerase inhibitor, as its expression was associated with decreased telomerase activity in gastric cancer cell lines and an enhancement of the same in response to RNAi-mediated knockdown. This finding was further supported by the significantly decreased cell growth of the PinX1-transfected cells. Apoptosis was promoted effectively and progressed at a higher rate in cells with PinX1 expression. TRAP-assay demonstrated that the telomerase activity in PinX1-transfected MKN28 cells was reduced in contrast to no significant change of telomerase activity in control and untransfected cells by PinX1. There was an upregulation of Mad1 in PinX1-transfected MKN28 cells and a downregulation of c-Myc expression, whereas RNAi led to downregulation of Mad1 and upregulation of c-Myc in BGC823 cells.

A major mechanism regulating telomerase activity is the transcriptional control of hTERT.[29] PinX1 inhibits telomerase activity via direct binding to hTERT, effectively blocking the RNA template from binding. The hTERT gene is transcriptionally controlled by a variety of signaling pathways that promote or suppress carcinogenic processes in humans.[30,31] The hTERT promoter contains functional Myc binding sites, and modulation of the c-Myc activity directly affects the hTERT promoter activity in normal and tumor cells.[32,33] Both hTERT and c-Myc are expressed in normal and transformed proliferating cells while they are downregulated in quiescent and terminally differentiated cells. They can induce immortalization when constitutively expressed in transfected cells[34]. By using mouse models, Flores et al. found that mTERT was transcriptionally activated by c-Myc in the skin and that this results in c-Myc-induced telomerase activation.[35] These results indicate that hTERT is a target of c-Myc activity and identify a pathway linking cell proliferation and chromosome integrity in normal and neoplastic cells. Consistent with the reported association between c-Myc overexpression and induction of telomerase activity, we found that PinX1 transfection downregulated c-Myc expression, whereas PinX1 RNAi led to upregulation of c-Myc in gastric cancer cells. Our results suggest that c-Myc may be involved in the inhibition of telomerase mediated by PinX1 in gastric cancer cells. The expression of Mad1 suppresses c-Myc-mediated cell proliferation and transformation. The loss of MAD1 protein expression may be related to tumor recurrence after surgical resection.[36] Previous studies have shown that depletion of Mad1 by siRNA increased the association of c-Myc with the hTERT promoter, presumably through an increase of c-Myc/Max binding to the promoter.[25,26] However, the mechanism that regulates the turnover of Mad1 protein is poorly understood. In this study, we have shown that PinX1 transfection upregulated Mad1 expression, whereas PinX1 RNAi downregulated Mad1 expression, suggesting that PinX1-mediated inhibition of telomerase activity may involve an upregulation of Mad1 expression.

In conclusion, this study revealed that PinX1 transfection inhibits gastric cancer MKN28 cell growth by inducing apoptosis. PinX1 inhibits telomerase activity and regulates telomerase-mediated telomere length maintenance in gastric cancer cells. Our results further suggest that this positive role of PinX1 on telomerase activity regulation seems to be functionally linked to mediating upregulation of Mad1expression and downregulation of c-Myc expression.

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ORIGINAL ARTICLE

Comparative Study between Surgical and Non-surgical Treatment of Anismus in Patients with Symptoms of Obstructed Defecation: A Prospective Randomized Study

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Abstract
Purpose This study came to compare the results of biofeedback retraining biofeedback (BFB), botulinum toxin botulinum type A (BTX-A) injection and partial division of puborectalis (PDPR) in the treatment of anismus patients.
Patients and Methods Consecutive patients treated for anismus fulfilled Rome II criteria for functional constipation at our institution were evaluated for inclusion. Participants were randomly allocated to receive BFB, BTX-A injection, and PDPR. All patients underwent anorectal manometry, balloon expulsion test, defecography, and electromyography activity of the anal sphincter. Follow up was conducted weekly in the first month then monthly for about 1 year. Study variables included clinical improvement, patient satisfaction, and objective improvement.
Results Sixty patients with anismus were randomized and completed the study. The groups differed significantly regarding clinical improvement at 1 month (50% for BFB, 75%BTX-A injection, and 95% for PDPR, $P=0.006$) and differences persisted at 1 year (30% for BFB, 35%BTX-A injection, and 70% for PDPR, $P=0.02$). Constipation score of the patients significantly improved postPDPR and BTX-A injection. Manometric relaxation was achieved significantly in the three groups.
Conclusion Biofeedback retraining has a limited therapeutic effect, BTX-A injection seems to be successful for temporary treatment but PDPR is found to be an effective with lower morbidity in contrast to its higher success rate in treating anismus.

Keywords Obstructed defecation · Chronic constipation · Puborectalis · Pelvic floor

Introduction

Constipation is a common medical problem which has various etiologies among which are outlet obstruction and slow transit.[1] Anismus is believed to be a behavioral disorder in which a failure of relaxation or a paradoxical contraction of the puborectalis muscle occurs during attempted defection.[2] However, neither the cause nor the pathophysiology of the condition has been well defined. The symptoms in patients with anismus may include severe, prolonged straining, inability to initiate defection, feeling of incomplete evacuation, need for manual disimpaction, laxative or enema abuse, and rectal pain. Diagnosis of anismus has been made by anal manometry,[3] balloon expulsion test,[4] electromyography (EMG) of the external anal sphincter and puborectalis muscle,[5] colon transit time, [6] and defecography.[7] Unfortunately, no single test has been conclusive in determining the presence of anismus.[8]

Anismus is not simple to diagnose; also, it has proved to be difficult to treat. The initial phase of management usually involves a high-residue diet to try to elicit rectal voiding. The next step is to use increasing doses of laxatives and enemas. Neither procedure is effective in solving the problem. Several surgical techniques have been described for dividing the puborectalis muscle in patients

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with constipation who have paradoxical contraction. Wasserman [9] and Wallace & Madden [10] reported very good results after partial division of puborectalis muscle. However, the results of subsequent studies for anismus patients managed by division of the puborectalis muscle in the posterior midline or laterally have been disappointing.[11–13] These disappointing results pushed several investigators to say that surgery appears to have no role in the therapeutic approach of anismus patients and it should be considered as a last resort for treating these patients [14–16]

Biofeedback (BFB) retraining has been used for treating anismus patients. Previous studies of biofeedback therapy in anismus have yielded conflicting results, with efficacy rates that ranged from 31% to 89% [17–19]

A recently described non-surgical alternative is the injection of Clostridium botulinum type A (BTX-A) neurotoxin directly into the puborectalis muscle.[20] BTX-A is a potent neurotoxin that causes paralysis of muscles by presynaptic inhibition of acetylcholine release.[21] The results of BTX-A injection for treating anismus is also conflicting. So up till now, there is no definitive line for the treatment of anismus.[22]

The aim of this study was to compare the results of biofeedback retraining, BTX-A injection and partial division of puborectalis (PDPR) in treating patients suffering from anismus.

Patients and Methods

Patients

Consecutive patients who were treated for outlet obstruction due to anismus at the Colorectal Surgery Unit of Mansoura University Hospital, Mansoura, Egypt, during the period from September 2006 through January 2010 were eligible for the study. All patients fulfilled Rome II criteria for functional constipation.[23] All patients were unresponsive to laxatives or enema use. Pregnant patients, patients with sphincteric defect, any patient proved to have colonic inertia by colon transit time and any patient with previous history of pelvic surgery, e.g., mesh rectopexy, Duhamel operation were excluded. The protocol was approved by local ethical committee.

Informed consent was obtained from all patients to be included in the study, after explanation of the nature of the disease and possible treatment. The study was approved by the local ethics committee.

Study Procedures

Diagnosis of anismus was based on the determination of intestinal transit time, anorectal manometry, balloon expulsion test, defecography, and EMG activity of the EAS.

Ano-rectal manometry was performed using a standard low compliance water perfusion system and eight-channel catheters with pressure transducer connected to 5.5 mm manometric probe with spirally located ports at 0.5-cm interval. The protocol of performance is the stations pull through technique with recording the functional length of the anal canal, mean maximum resting pressure, and mean squeeze pressure. Rectoanal inhibitory reflex was assessed to exclude Hirschsprung's disease. Pressures were recorded using a computerized recording device (Sandhill Bioview programs, USA).[17,19] An immediate decrease in the resting pressure to baseline rectal pressure was considered as full manometric relaxation.[19,24]

Evacuation proctography With the patient in the left lateral position, the rectum was filled with 120 ml of barium paste, then the patient was seated upright on a specially designed commode and asked to empty the rectum as rapidly and completely as possible. Plain X-rays were taken under fluoroscopic control with the patient at rest, with voluntary anal contraction, and during defecation.[19,25,26]

Surface electromyography All patients were investigated with EMG of the external anal sphincter using surface electrodes 1 cm lateral to the anal verge at 3 and 9 o'clock. The patient was carefully instructed and then requested to squeeze and strain while electromyographic activity was recorded.[19,27]

Balloon expulsion test The balloon expulsion test was done by using a rubber balloon that was inserted into the rectum and inflated with 60 ml saline .The patient was asked to expel the balloon into a toilet.[19,28]

Anismus was defined as non-relaxing anal sphincter on manometric straining in an attempt to defecate, inability to expel water filled rectal balloon, and non-relaxing puborectalis on proctography accompanied by prolonged evacuation time or inability to expel the barium paste in the presence of normal perineal descent.[17–28] Only patients fulfilling all theses criteria were included in the study.

The patients are then randomized into three groups. The randomization was achieved through computer-generated schedule and its results were sealed into 60 envelopes. The envelopes were drawn and opened by a nurse not otherwise engaged in the study.

Group I patients (BFB group; 20 patients) All were subjected to biofeedback therapy, two times per week for about 1 month (eight sessions) under supervisor of an expert biofeedback therapist. All patients were treated under outpatient procedure. At the first session, the anatomy and physiology of the pelvic floor were explained to the patient, using diagrams and their own tests results.

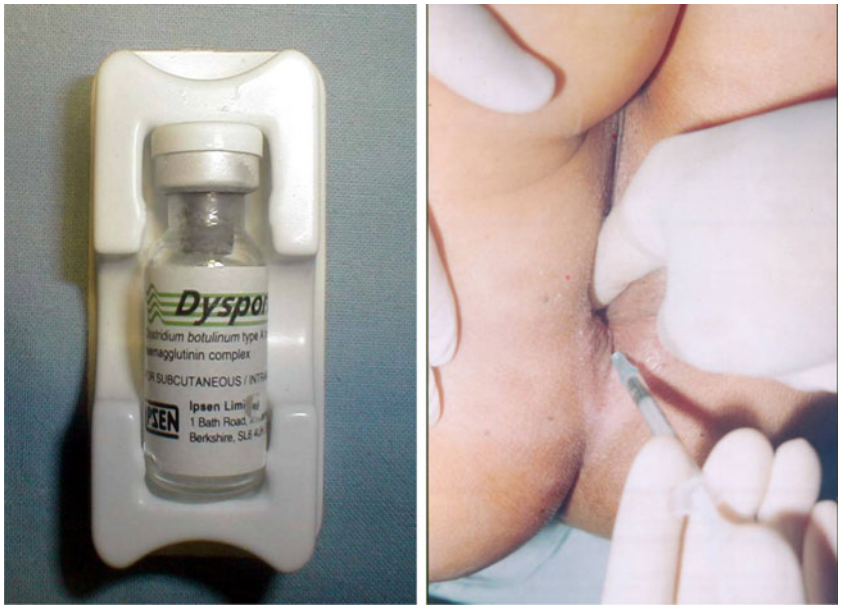

Fig. 1 Shows a vial of botulinium toxin type A "BTX-A" (dysport) (*left image*) and injection of one of the anismus patients by BTX-A (*right image*).

We used pressure-based biofeedback training, using a perfused eight-channel polyvinyl catheter with a compliant balloon at the tip (Sandhill Biofeedback programs, USA). The side holes were placed in the distal rectum and the anal canal, and the balloon attached to the tip of the catheter was used for training expulsion. Patients were told that the sphincter should relax during expulsion of the rectal balloon at the urge threshold. They should learn how to relax the pelvic floor muscles and to push down slowly using their abdominal muscles. This was accomplished by trial and error; the patient was asked to look for a monitor who gives a special figure on normal straining and another figure on false straining and ask patient repeatedly to get the normal straining figure. Straining and relaxing were repeated until a normal pattern of expulsion occurred with or without the help of a therapist.[19] The length of each BFB session was 30 min. Number of sessions was eight and at the end of each cession trial to expel a 50-ml balloon catheter was done. When BFB retraining successful, the patients were instructed and trained to continue relaxing exercise with periodic supervision every 6 months.

Group II patients (Botulinum toxin "BTX-A" injection; 20 patients) All patients were injected with BTX-A in the left lateral position; anesthesia was not required. The anal canal was cleaned with povidone iodine. A vial of Dysport, 500 µ, (Dysport, Ipsen, UK) is dissolved in 2.5 ml isotonic saline Fig. 1. A volume of 0.5 ml of dissolved toxin, i.e., 100 µ Dysport, is injected in each patient. The injection is given with an insulin syringe fitted with a needle size of 21 gauze and 3.75 lengths. The needle tip was guided by the contralateral index finger into the anal canal. BTX-A was injected into the left and right sides of the paradoxically contracting muscle, i.e., on either side of the puborectalis and the external anal sphincter at 5 and 7 o'clock in the lithotomy position. This procedure was performed as an outpatient procedure.[19] The need for further injection was assessed on each follow up. Each patient who failed the

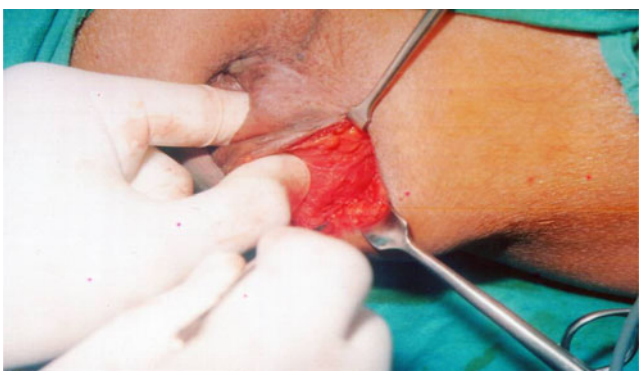

Fig. 2 Perianal incision for PDBR.

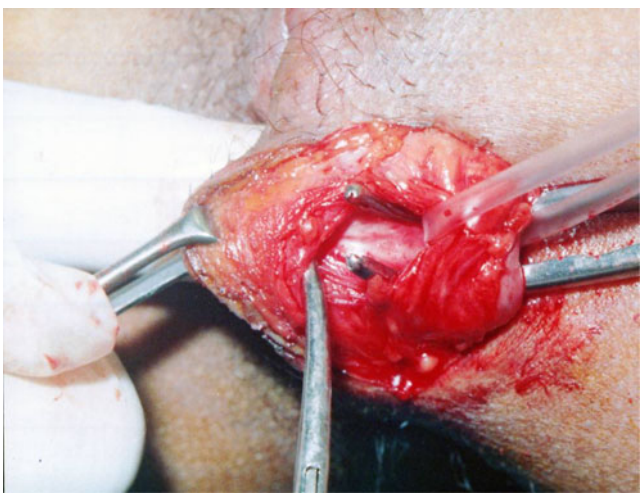

Fig. 3 Puborectalis muscle.

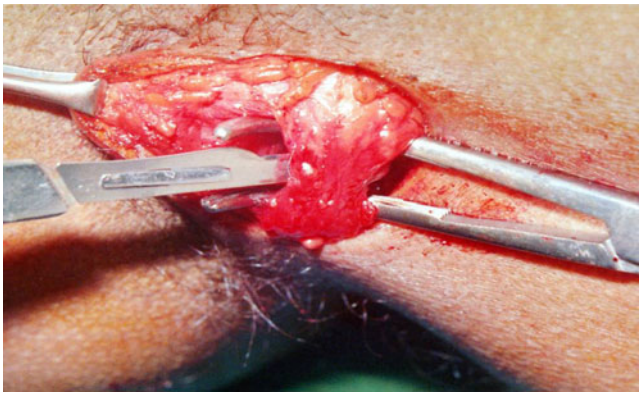

Fig. 4 Partial division of puborectalis muscle from its inner side.

first injection was given a second trial. Failing two injections was a marker for failure of the treatment. All injection was performed by the same person. Patients who wished to continue the study were assesses clinically and by manometry before the next injection.

Group III patients (Bilateral open partial division of puborectalis; 20 patients) All patients were subjected to bilateral open partial division of the puborectalis. Each patient was admitted to the hospital and subjected to routine preoperative assessment of medical fitness. In the operating room, general anesthesia was given. The patient placed in the lithotomy position. A 2-3-cm curved incision is made on either side of the anal canal along its postero-lateral aspect, each about 2.5 cm distances from the anal verge Fig. 2. After that, dissection in ischeorectal fossa was done till reaching the puborectalis sling from outside, i.e., extrasphincteric approach. Using a right angle forceps, the puborectalis sling is lifted up, guided by the contralateral index finger in the anal canal Fig. 3. Nearly the inner half of puborectalis sling was divided on each side by using a scalpel NO. 11 (Fig. 4). Complete haemostasis was followed by skin closure without drain. Postoperative wound care in the form of daily dressing and oral antibiotics (metronidazol and ciprofloxacin) was given till complete wound healing.[22]

Follow up was conducted weekly in the first month, and then monthly for about 1 year.

Assessments

All assessments were conducted by investigators who were blinded to the experimental condition. The primary endpoint was improvement in bowel habits. The patient is asked to fill up a symptom questionnaire 1 month following the therapeutic procedure and again at the end of our follow up. By this questionnaire, the degree of improvement is assessed regarding the straining severity, anorectal pain, number of weekly bowel movements, sensation of incomplete evacuation, and need for anal digitations or enema and using Agachan et al.[29] constipation score. PR examination was done in each visit to assess the relaxation of the puborectalis muscle during straining.

The term clinical improvement or success was chosen to reflect the patients who returned to normal with regard to their bowel habits (no straining, no digitations, no hard stool, no sense of anorectal obstruction, defection became more than 3 times per week)

Secondary endpoints included number of patients showing complications, recurrence, patient satisfaction using visual analogue scale (satisfaction meant a change of at least two score), postoperative incontinence, secondary outcomes were assessed 1 month after the procedure by anorectal manometry, balloon expulsion test, defecography

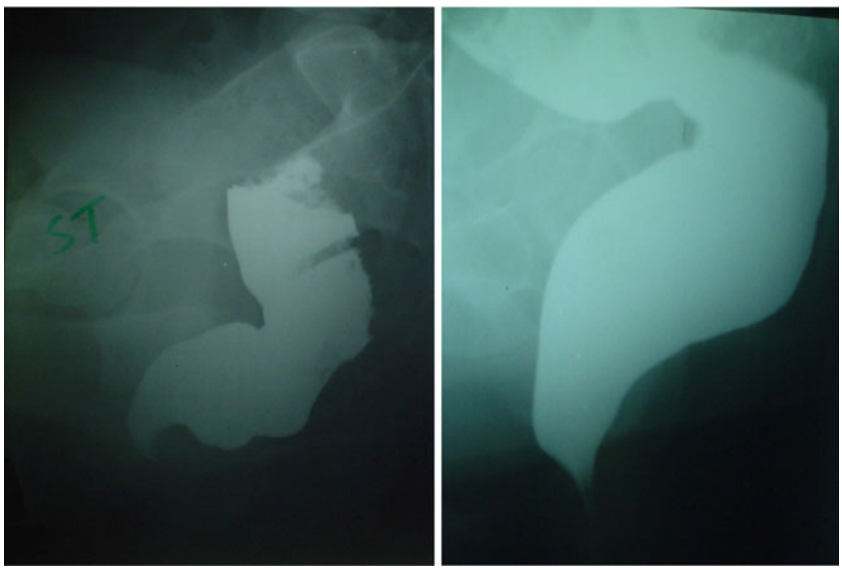

Fig. 5 Defecographic images of anismus patient during straining before (*left one*) and after (*right one*) partial division of puborectalis muscle.

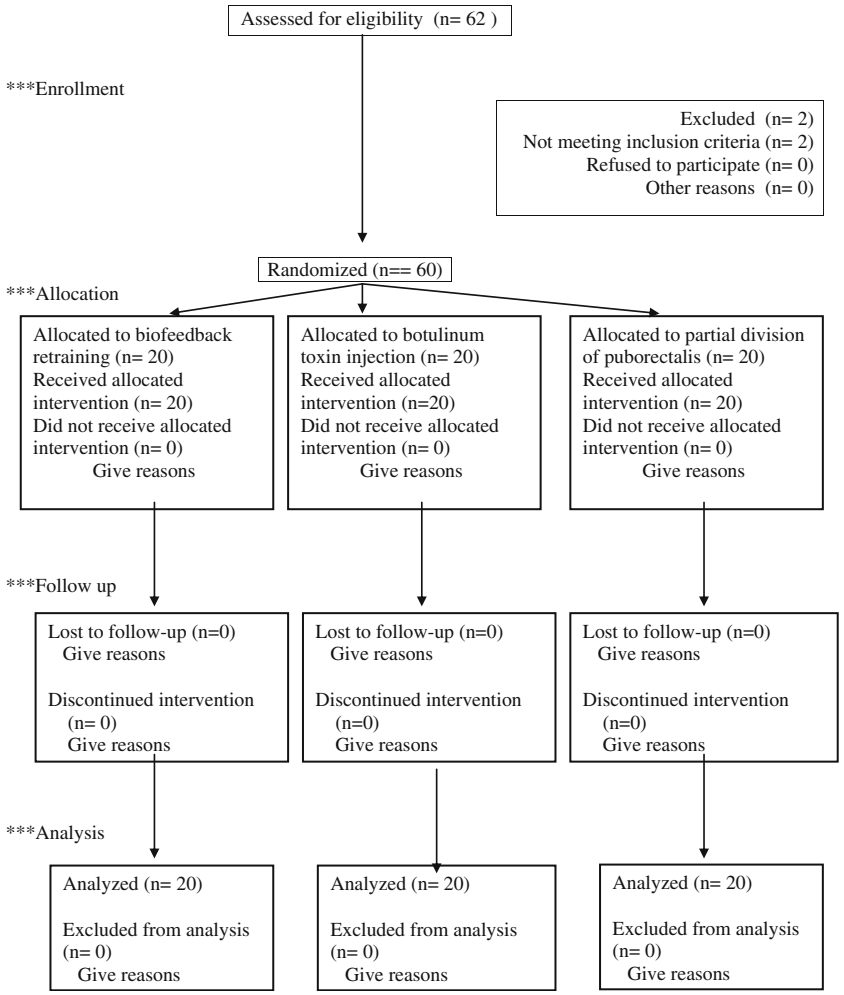

Fig. 6 Flow Diagram of the progress through the phases of a randomized trial (i.e., enrollment, intervention allocation, follow-up, and data analysis).

(Fig. 5) and EMG examination of the anal sphincter to monitor any changes in paradoxical contraction and to show whether the clinical improvement was associated with normalization of objective findings or not.

The statistical analysis of the data in this study was preferred using the SPSS version 10. Analysis of data was by intension-to-treat. For continuous variables, descriptive statistics were calculated and were reported as mean±SD. Categorical variables were described using frequency distributions. The Student's *t* test for paired sample was used to detect differences in the means of continuous variables and chi-square test was used in cases with low expected frequencies. One way ANOVA test was used to detect differences between three groups (P value <0.05 was considered to be significant).

Results

Patient Characteristics

The study flow chart is shown in Fig. 6. Of 62 consecutive patients seen during the recruiting period, 60 patients (17 men and 43 women) were eligible and entered the study. The mean age was 37.53±12.13 (range, 20–69) years. These patients were randomly divided into three

Table 1 Demographic Data of Our Patients

	BFB group	BTX-A group	Surgical group	*p*
Age (years)	39.6±15.9 (20-69)	34.7±12.3 (20-63)	38.3+8.2 (24-53)	0.33
Disease duration (years)	4.8±3.34 (1-10)	5.93±3.28 (2-12)	5.21±3.11 (1-11)	0.25
Sex				
Female	14 (70%)	15 (75%)	14 (70%)	0.25
Male	6 (30%)	5 (25%)	6 (30%)	

Table 2 Clinical Outcome after BFB Retraining, BTX-A Injection, and Surgical Groups

	BFB training	BTX-A injection	Surgical group	*p*
Initial improvement	10 (50%)	15 (75%)	19 (95%)	0.006
Long term improvement	6 (30%)	7 (35%)	14 (70%)	.02
Patient satisfaction	6 (30%)	7(35%)	14 (70%)	.05

groups. The characteristics of the three randomized groups are presented in Table 1.

Clinical Improvement

Initial improvement was recorded after the BFB retraining, BTX-A injection, and PDPR in ten (50%), 15 patients (75%), and 19 patients (95%), respectively, while long-term success was recorded in six patients (30%) after the BFB retraining, seven patients (35%) post-BTX-A injection and 14 patients (70%) post-PDPR. There was a significant difference between three groups as regards the initial success and at the end of follow up, i.e., after 1 year, surgery achieve a significant improvement initially and at end of follow up (Table 2).

Subjective measures were tested using a visual analog scale of 0-10. It was noticed that straining efforts during defecation had decreased significantly After BFB training, BTX-A injection and OPDPR in eight (40%), 12 (60%), and 19 patients (95%), respectively. This changes significantly higher post-PDPR. Anorectal pain and defecation frequency did not show significant changes after BTX-A, BFB retraining and post-PDPR. However the constipation score was significantly improved after BTX-A and PDPR but not reach the significant changes after BFB retraining.

At the end of our follow up, the overall satisfaction was assessed on VAS. Satisfaction meant a change of at least two score. Six patients (30%) were satisfied by the results of BFB retraining in contrast to seven patients (35%) after BTX-A injection and 14 patients (70%) post-PDPR were satisfied. This difference achieved a significant statistical value (Table 3).

Objective Improvement

Anorectal manometry Manometric relaxation was achieved in 15 patients (75%) treated with BTX-A injection (p= 0.001) while it resulted only in 11 patients (55%) treated by BFB retraining (p=0.04) and occurred in 19 patients (95%) post-PDPR (p=0.001). The comparison of BFB group change, BTX-A injection, and post-PDPR group change were statistically significant (Table 4).

Balloon expulsion The three groups achieved significant changes in the results of balloon expulsion test as balloon expulsion occurred in 35% of patients after BTX injection, 30% of patients after BFB retraining, and 95% of patients after PDPR, with significant difference between three groups.

The three groups produced changes in the results of EMG, defecography and per rectal examination but did not reach the statistical significance (Table 4).

The postoperative complications following PDPR were in the form of wound infection, or disruption in three patients (15%), incontinence in two patients (10%); these patients were incontinent only to flatus and rectal intussusception in two patients (10%; Table 5).

Discussion

Constipation is a common medical problem which has various etiologies among which are outlet obstruction and slow transit. Anismus is a functional disorder of the anal

Table 3 Subjective Results between BFB Group ,BTX-A Group, and Surgical Group

Variables	BFB GROUP		*P*	BTX-A GROUP		*p*	Surgical group		*p*	*P*[a]
	Pre-training	Post-training		Pre-injection	Post-injection		Pre-operative	Post-operative		
Straining pattern (VAS)	8.4±2.3	6.8±3.2	0.04	8.5±1.9	5.9±4.3	0.007	8.4±2.6	5.1±2.3	0.006	0.05
Pain pattern (VAS)	3.2±3.9	3.0±1.2	0.152	3.7±3.5	3.3±2.9	0.605	3.8±2.9	2.9±1.2	0.07	0.35
Number of bowel motion/w	4.8±2.7	5.2±2.3	0.069	4.2±2.2	5.3±2.1	0.075	4.4±2.2	5.8±2.4	0.06	0.27
Constipation score [29]	18.5±3.2	16.1±1.5	0.059	18.9±4.2	14.3±1.5	0.04	18.4±3.2	10.5 ±1.5	0.001	0.03
Overall satisfaction (VAS)	0	2.8±3.4	0.26	0	3.3±4.1	0.34	0	4.1+3.3	0.07	0.09

[a] *P* for comparison of three groups

Constipation score [29]

Table 4 Objective Results between BFB Group, BTX-A Group, and Surgical Group

Varibles	BFB group		*p*	BTX-A group		*p*	Surgical group		*p*	*P*[a]
	Pre-training	Post-training		Pre-injection	Post-injection		Pre-operative	Post-operative		
Manometeric relaxation	0	11 (55%)	0.04	0	15 (75%)	0.01	0	19 (95%)	0.001	0.05
EMG (paradoxical)	20 (100.0%)	12 (60%)	0.08	20 (100%)	9 (45%)	0.07	20 (100%)	4 (20%)	0.03	0.34
Defocogram (+ve)	18 (90%)	14 (70%)	0.085	17 (85%)	12 (60%)	0.08	19 (95%)	4 (20%)	0.01	0.17
Balloon ET (expulsion)	0	6 (30%)	0.05	0	7 (35%)	0.04	0	19 (95%)	0.01	0.03
PR (+ve)	20 (100%)	14 (70%)	0.08	20 (100%)	14 (70%)	0.02	20 (100%)	2 (10%)	0.004	0.04

[a] *P* for comparison of three groups

PR per/rectal examination, *+ve* feeling the paradoxical contraction of the puobrectalis muscle

sphincter and pelvic floor muscle in which the muscles contract, rather than relax, during attempted defecation.[1–3] Anismus is one of causes of constipation and outlet obstruction. The affected subjects strain excessively during defection with the higher centers unaware of the in coordination of pelvic floor.[19,22] Anismus affects more middle-aged women.[30] A wide variety of surgical and pharmacologic approaches have been proposed, with the aim of eliciting puborectalis muscle relaxation.[13,19,22]

Meagher et al.[31] reported that 12 patients underwent placebo treatment followed by biofeedback treatment and concluded that the clinical improvement may be in part due to placebo effect and observer bias. Gilliand et al.[18] reported that biofeedback was completely successful in only 35% of his patients, although complete success was achieved in 63% of patients who finished the prescribed training course.. Rhee et al.[32] reported that BFB was successful in 68.9% of their anismus patients. Glia et al.[30] reported that biofeedback therapy is not suitable for all patients with anismus. The low success rate may be attributed to self-discharging phenomena.

Different results between various studies are probably attributed to different case selection, different regimens, and different methods of biofeedback. Also, the absence of consensus on how to define treatment outcome.[19,33]

Table 5 Outcome of Surgical Procedure

Variables	No. of patients
Incontinence (grade 1)	2 (10%)
Rectal intussusception	2 (10%)
Wound infection	3 (15%)
Hospital stay/hours	69.6±17.1
Return to work/day	14±3.53
Initial improvement	19 (95%)
Recurrence	5 (25%)

Maria et al.[14] reported that botiulinum toxin could be a promising treatment in patients with anismus and less expensive and easier to perform than BFB training. They also reported that repeated injections could be necessary to maintain clinical improvement. Shafik and El-Sibai[34] injected 15 anismus patients with BTX-A, and noticed that improvement was recorded in 13 patients. However, improvement was maintained for a mean of only 5 months and so re-injection was necessary. Ron et al.[24] observed only 37.5% success after the first injection and 28.6% after the second. Hallan et al.[20] used the British form of BTX-A and reported that incontinence had occurred in two cases in their series.

After bilateral partial division of puborectalies, 95% of patients recorded complete clinical improvement following the operation and this improvement persist only in 14 patients (70%). The causes of recurrent symptoms were rectal intussusception in two patients and recurrence of anismus in five patients. Our results are similar to those of Wasserman [9] who describe surgical division of a part of puborectalis muscle and reported good results, also Wallace and madden [10] who reported a large series of anismus patients whom partial division of puborectalis was carried out with apparently good results. Barnes et al. [13] reported that nine women with anismus who underwent posterior division of puobrectalis muscle with success rate 22.2% and incontinence rate 55.5%. They explained that high failure rate to be related to disruption of anorectal anatomy by pervious anorectal surgery.

Our results differ from Kamm et al.[12] studied 15 patients with severe idiopathic constipation and three patients with a megarectum proved to have anismus by using balloon expulsion test and EMG. All patients underwent lateral division of puborectalis muscle and upper half of the external sphincter muscle. Twelve patients had a unilateral division and six patients had both sides divided. Four patients experienced symptomatic improvement (22.2%) and four patients experienced mild

mucus or urge incontinence (22.2%) but no patient was incontinent for solid stool. Also it is important to note that three of four improved patients were among the six who had bilateral divisions.

In this study, the high success results after PDPR as we applied a different technique and different approach which allowed us to divide the inner half of the puborectalis muscle and its fibers attached to rectal wall so increasing the anorectal angle. This resulted in a lower incontinence rate in our study; meanwhile, others used more division in order to improve the outcome.

Kawimbe et al.[23] showed that there was significant improvement in defection frequency after BEB training as it had increased from 5.2±0.8 to 8.8±1 times per week, also straining effort decreased significantly in his patients. Ron et al.[24] reported that 37.5% of his patients were satisfied with the overall results of BTX-A injection. Straining at defection decreased in 29.2% and defection frequency did not change during follow up as could be expected.

Manometric relaxation was achieved significantly post-BFB, post-BTX-A injection, and after PDPR. Ron et al.[24] reported that manometric relaxation after BTX-A injection was attained by 75% of his patients and this effect lasted throughout the entire study and follow up. Glia et al.[30] found that there was no difference in the results of anorectal manometry before and after biofeedback therapy also Kawimbe et al.[23] reported that no significant difference in the results of manometry before and after BFB training. Kamm et al.[12] reported that surgery cause a significant reduction in maximum voluntary squeeze pressure.

Ron et al.[24] found that balloon expulsion ,after BTX-A injection, achieved in 37.5% of his patients after the first injection and in 45.8% after two injection while Kawimbe et al.[23] reported that before BFB retraining, only two subjects could expel the rectal balloon, whereas after BFB retraining 13 out of 15 could do so. In our study, BFB retraining group, BTX-A injection group and in PDPR group achieved significant changes in the results of balloon expulsion test.

Joo et al.[35] reported that EMG finding accurately correlate with patients' subjective reports. Maria et al.[14] reported that paradoxical pattern at EMG were decreased in all patients and also the anorectal angle measured during straining increased significantly following injection. Our series showed also difference in EMG, defecographic finding before and after injection of BTX-A and BFB retraining but did not reach statistical significance. However, after PDPR showed significant changes.

The discrepancy between objective and subjective results may be explained by anismus is a functional disorder and there is always a possibility for placebo effect, also the non-blind fashion of follow up, which could have biased patients responses to therapy

Conclusion

Biofeedback retraining to the puborectalis muscle has a limited therapeutic effect on patients suffering from anismus. BTX-A injection could be successful for temporary treatment of anismus. However, because the mechanisms of action is short, longer term results are unsatisfactory and further controlled trials are necessary to asses the role of BTX-A in treatment of anismus. Surprisingly, bilateral partial division of puborectalis is found to be an effective method in treating patients suffering from anismus. It has a relatively lower morbidity in contrast to its higher success rate.

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ORIGINAL ARTICLE

Can Adequate Lymphadenectomy be Obtained by Laparoscopic Resection in Rectal Cancer? Results of a Case–Control Study in 200 Patients

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Abstract
Aim The aim of this study is to compare pathological findings in rectal cancer specimens obtained by laparoscopy or laparotomy.
Materials and Methods Bowel length, distal and circumferential margins, and number of total and positive nodes harvested were prospectively recorded in specimens obtained from 100 consecutive patients who had a laparoscopic total mesorectal excision for cancer. These data were compared with those extracted from a well-matched group of 100 patients who had an open procedure.
Results The mean length of the specimens was 31.04 cm in the case group and 29.45 cm in the control group (not significant (NS)). All distal margins in both groups were negative. The circumferential margin was positive in four cases in the case group and nine cases in the control group (NS). The mean number of lymph nodes harvested was 13.76 nodes/patient in the case group and 12.74 nodes/patient in the control group (NS). The mean number of involved lymph nodes was 1.18 node/case in the case group and 1.96 node/case in group 2 (NS).
Conclusion There is no difference between laparoscopic or open approaches concerning specimen's length, distal margin, circumferential margin, and total and positive lymph nodes. Laparoscopic rectal resection is not only technically feasible but it seems also oncologically safe.

Keywords Rectal cancer · Lymph nodes · Total mesorectal excision · Laparoscopy · Pathology

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Introduction

The feasibility of laparoscopic resection for rectal cancer has been proved,[1] but the place of the laparoscopic approach as a routine use in a curative intent to rectal cancer is still a matter of controversial discussion.[2] One of the most important questions is whether laparoscopic rectal resection achieves the oncological quality criteria set by open surgery.[3]

Precise clinicopathologic staging of rectal cancer is essential to advise patients about the prognosis, determine the use of adjuvant therapy, and compare outcomes between series.

In the present prospective single-center study, we investigated the specimen length, the distal and lateral margins, and the number of lymph nodes harvested in rectal cancer curative resection to compare laparoscopic and open approach performed by the same surgeons and assessed by the same pathologists.

Materials and Methods

The pathology reports from 100 consecutive primary total mesorectal excision specimens for cancer between February 2003 and June 2009 at a tertiary care teaching institution were collected. Specimens from transanal resections, complicated cancers (perforated or obstructing cancer), T4, or recurrent cancers were excluded, as were specimens containing multiple cancers. Three experienced colorectal surgeons and two experienced pathologists were involved in the study. Pathological study included standard gross examination with length of bowel specimen and identification of potential lymph nodes following a standardized harvesting technique and a quality assessment system based on the MERCURY criteria published in 2002.[4] These data were compared with those extracted from a well-matched group of 100 patients who had an open total mesorectal excision for cancer in the same institution among 224 patients operated on for rectal cancer during the period between January 1999 and July 2009. These 100 match pairs were identical regarding gender, age, American Society of Anesthesiology (ASA) score, body mass index (BMI), preoperative adjuvant radiotherapy, and tumor stage (Table 1).

Four patients from the 100 who were proposed for a laparoscopy had a conversion into laparotomy at a variable time of the procedure, for the following reasons: pathologically proved associated abdominal lymphoma, obesity (BMI at 33.5), suspected vaginal invasion, and large wedged tumor. These patients were left in the first group.

Pathologic factors including bowel length, distal and circumferential margins, tumor stage, and number of total and positive nodes identified were recorded and entered into a computer database. Group comparisons for categorical variables were carried out with Chi-square or Fisher's exact test.[5] Statistical analyses were carried out using Statview 5.0 version. Statistical significance was set at $p<0.05$.

Results

The mean length of the specimens for the whole series was 30.26 cm (range, 10–77). It was 31.04 cm (SD±9.6; range, 14 to 68) for the case group specimens and 29.45 cm (SD± 11.2; range, 10 to 77) for the control group specimens ($p=0.28$, not significant (NS)).

All distal margins in the 200 specimens were free of tumor.

Among the 200 specimens, circumferential margin was positive in 13 cases (6.5%). The involvement was considered as macroscopic in one and microscopic in 12. This circumferential margin was positive in four cases of the patients from case group and in nine cases of the patients from control group (NS).

Among the 200 specimens, 2,665 lymph nodes were identified (mean, 13.26 nodes/patient; range, 0 to 41). Based on the current American Joint Committee on Cancer recommendation[6] of 12 or more nodes, 102 patients (51%) underwent an adequate harvest. One hundred and fifty-eight patients (79%) had a harvest of at least eight lymph nodes. A total of 316 positive lymph nodes were identified (mean, 1.6 node/patient; range, 0 to 32).

The total number of lymph nodes harvested in the case group was 1,390 (mean, 13.76 nodes/patient; SD±7.6; range, 0 to 36), and the total number of lymph nodes harvested in the control group was 1,275 (mean, 12.74 nodes/patient; SD±8.0; range, 0 to 41; $p=0.36$, NS).

The number of involved lymph nodes in the case group was 119 (mean, 1.18 node/case; SD±2.5; range, 0 to 20), and the number of involved lymph nodes in the control group was 197 (mean, 1.96 node/case; SD±4.3; range, 0 to 32; $p=0.11$, NS).

Table 1 Characteristics of Patients in Both Laparoscopic ($n=100$) and Open ($n=100$) Groups

Criteria	Laparoscopic group	Open group	
Male ratio	75%	76%	NS
Mean age	63.4 years	64.0 years	NS
ASA score 3+4	26%	31%	NS
Mean BMI	24.5%	24.6%	NS
BMI over 25	35%	44%	NS
Irradiated patients	48%	49%	NS
Tumor stage pT3-ypT3	56%	62%	NS

NS not significant, *ASA* American Society of Anesthesiology, *BMI* body mass index

Discussion

The comparison between the two groups is difficult because of the absence of randomization. This is the reason why we constructed a matched-pair analysis between two groups. However, we can conclude from the present study that laparoscopic total mesorectal excision for rectal cancer appears as oncologically safe as the same operation performed by an open route, based on immediate pathologic analysis of the resected specimen.

There was no significant difference concerning specimen length, distal margin, circumferential margin, number of total lymph nodes, and number of positive lymph nodes harvested between the case and control groups. This possibly means that bowel resection and mesenteric lymphatic dissection are the same whatever the surgical approach is.

The presence of nodal metastasis is a major component of staging rectal cancer. Accurate assessment of nodal status requires sufficient nodes sampling, although the number of such nodes is a matter of debate. Recommendations from the literature vary from 6 to 17 nodes.[6–11] Current guidelines from the American Joint Committee on Cancer recommend assessment of 12 nodes for accurate staging.[6]

Data from large series have demonstrated the impact of nodal harvest on survival. A significant improvement in survival at a mean follow-up of 3.8 years was observed for patients with node-negative colon cancer who had more than seven nodes compared to those who had less nodes harvested.[12] Other studies concerning rectal cancer had the same conclusion. A nodal harvest of 14 or more nodes was associated with a significant improvement in 7.5 years survival in patients with T3 and T4 node-negative rectal cancer compared to patients with fewer nodes found (80% vs. 60%).[11] Another study of patients with T3N0 tumor again showed improvement in survival at a mean follow-up of 5 years for patients with more than ten nodes identified.[9]

To improve rectal cancer staging and reduce the consequences of tumor understaging, a better understanding of the factors that influence nodal harvest is of major impact. These factors contributing to nodal harvest are not well studied. The number of lymph nodes identified in a resected colorectal cancer might be influenced by five factors relating to the patient, operation, tumor, surgeon, and pathologist.[13]

Patient factors are sex, BMI, age, ASA score, and other anatomic variability.[14]

In this series, there was no statistical difference between the two groups concerning sex and BMI. In general, visceral fat greatly influence the technical difficulty in performing abdominal surgery and particularly colorectal surgery.[15] It has been well established that fatty tissue makes the lymph node recovery difficult,[16] especially in patients with rectal cancer.[11] Görög et al. demonstrated that a most significant reduction in the mean number of lymph nodes was observed in obese patients and that this subset of patients presented the lowest rate of nodal metastases.[17] In this series, the same proportion of obese patients was observed in both groups.

A national study on lymph node retrieval in resectional surgery for colorectal cancer conducted in Great Britain and Ireland, including 5,164 patients, showed that increasing age and ASA score were significantly associated with a reduction of lymph node harvest.[18] For the authors, the most likely reason for this finding was that a wider lymphadenectomy was more commonly performed in the young and fit patient. To avoid this bias, the 100 match pairs in our study were identical regarding age and ASA score.

Factors relating to the tumor were rather equally distributed in both laparoscopic and open groups. Neoadjuvant chemoradiotherapy has been shown to downstage tumors, with more cases of N0 staging.[19–23] Lymph nodes are both effaced and more difficult to find after chemoradiation therapy.[24,25] In our series, the same number of patients had had preoperative radiation therapy in the two groups. We excluded T4 and complicated tumors because the majority of these were treated through an open approach, as it was felt that a better control would be obtained by a wider exposition of the tumor.

Surgeon and pathologist relating factors were reduced at the minimum and had no incidence on the variables studied, as surgeons and pathologists were all experienced physicians. All surgeons performed total mesorectal excision or partial mesorectal excision depending on the site of the tumor, according to Heald[26] and Quirke[27] principles. The mesenteric lymphatic drainage was removed in each patient using a high ligation of the inferior mesenteric artery, as it has been proved that this was associated with increased survival rate and reduced recurrence rate.[28] Such a ligation was sometimes responsible for ischemia and subsequent further colonic resection, thus explaining the length of certain specimens being as high as 77 cm. Concerning the pathological features, there are of course special techniques like fat clearing methods[29,30] or ex vivo lymphatic mapping,[31] but these are laborious and time-consuming processes, so that they cannot be used in routine. All the specimens in this study were examined by two experienced pathologists who only used detection of lymph nodes in the mesenteric fat by palpation and dissection. Most of the time, they were blind to the surgical approach used.

Conclusion

In this one-single institution study, there seems to have no difference between laparoscopic or open approaches of

rectal cancer resection concerning specimen's length, distal margin, circumferential margin, total lymph nodes, and positive lymph nodes harvested, meaning that both approaches are valuable. Laparoscopic rectal resection is not only technically feasible but it seems also oncologically safe in selected patients.

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ORIGINAL ARTICLE

Expression of Cortactin Correlates with a Poor Prognosis in Patients with Stages II–III Colorectal Adenocarcinoma

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Abstract
Background The present study was designed to specifically investigate the clinicopathological role of expression of cortactin, as well as the correlation with clinical outcomes in stages II–III colorectal cancer (CRC).
Methods Two hundred and five stages II–III CRC patients were included in this study. Formalin-fixed paraffin-embedded specimens were stained for cortactin and the correlation between the staining, its clinicopathological parameters, and its prognostic power were analyzed statistically.
Results Of the 205 patients studied, 113 cases (55.1%) were strongly positive for cortactin. Cortactin expression correlated with tumor invasion (P=0.018), histological grade (P=0.004), and preoperative CEA level (P<0.001). In univariate analysis, tumor invasion, American Joint Committee on Cancer (AJCC) stage, lymphovascular invasion, preoperative CEA level, and cortactin expression were significant prognostic factors for disease-free survival (P=0.034, 0.009, 0.043, 0.004, and 0.004, respectively), while for overall survival, tumor invasion, AJCC stage, pathologic grade, preoperative CEA level, and cortactin expression were significant prognostic factors (P=0.003, 0.008, 0.038, 0.017, and <0.001, respectively). In multivariate analysis, tumor invasion, preoperative CEA level, and cortactin expression maintained their independent prognostic influence on disease-free survival (P=<0.001, 0.003, and 0.008, respectively). However, tumor invasion, AJCC stage, and cortactin expression influenced overall survival (P=0.036, <0.001, and 0.004, respectively).
Conclusions Cortactin may be a good biomarker to be applied in the clinical setting to predict the prognosis of patients with completely resected pathologic stages II–III CRC.

Keywords Colorectal adenocarcinoma · Cortactin · Prognosis

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Introduction

Colorectal cancer (CRC) is one of the three leading causes of cancer-related death among men and women worldwide.[1] It is estimated that in 2008, approximately 149,000 new cases of CRC will be diagnosed in the USA.[2] Even among patients who undergo potentially curative resection alone, 40% to 50% of them ultimately relapse and die of metastatic disease.[3] Although tumor-nodes-metastasis (TNM) classification is useful for staging colorectal cancer patients and selecting them for specific treatment,[4] it is not sufficient, as many patients at the same stage may have various outcomes, indicating that the conventional staging procedures may be

unable to precisely predict cancer prognosis.[5] Therefore, there is a great need to identify molecular markers of more aggressive colorectal tumor phenotypes to appropriately select patients for adjuvant systemic or targeted therapies.

Cortactin is an actin-binding protein that activates the Arp2/3 complex to regulate the actin cytoskeleton[6] and inhibits debranching of dendritic actin networks.[7] Cortactin is overexpressed in many types of human cancers, including head and neck and esophageal squamous carcinomas, colorectal, gastric, hepatocellular, breast and ovarian cancers.[8] Most frequently, cortactin overexpression occurs through chromosomal amplification of the 11q13 region, such as breast,[9] head/neck carcinomas,[10] and gastric adenocarcinoma,[11] however, overexpression has also been reported in tumors without that amplification.[12,13] Remodeling of the actin cytoskeleton has effects on cell migration, motility, and adhesion, as well as on tumor invasion and metastasis.[6] In some studies, overexpression of cortactin correlated with histological differentiation, T and N stage in gastric cancer,[11] depth of invasion in colorectal cancer,[14] as well as poor prognosis for patients with lymph node metastasis.[9,10,15] However, the prognostic significance of cortactin status in patients with CRC remains controversial. Thus, the present study was designed to specifically investigate the clinicopathological role of expression of cortactin, as well as the correlation with clinical outcomes in stages II–III CRC.

Materials and Methods

Patient Management and Follow-up

Two hundred and five consecutive, unselected, stages II–III CRC patients were seen at our department from March 1996 to March 2000. The histologic sections were reviewed by two expert pathologists (X.J. and Y.Z.) to verify the histologic diagnosis. All patients underwent curative-intent surgery for stages II–III CRC (according to the American Joint Committee on Cancer (AJCC) criteria). No patients with rectal cancer received neoadjuvant chemoradiotherapy in this study. All excised specimens were formalin-fixed and sliced at 10-mm intervals. Therapeutic strategies were applied according to stage of disease and presumed risk of relapse. Patients with stage II disease underwent follow-up based on history, physical examination, complete blood count, liver function tests, ultrasound scan of the abdomen, and carcinoembryonic antigen (CEA) monitoring every 3 months. Total body computed tomography scan and colonoscopy were done once a year. Patients with stage II high-risk disease (pT4 and/or gross volume tumors, perforation, obstruction, poorly differentiated histology, long-lasting symptoms, elevated CEA preoperatively, blood or lymphatic vessel invasion) were encouraged to undergo six cycles of fluorouracil-based adjuvant chemotherapy. If no contraindications were present, patients with stage III disease underwent six cycles of fluorouracil-based adjuvant chemotherapy, then were followed up. One hundred forty-four patients received fluorouracil-based adjuvant chemotherapy. Sixty-one (49.2%) patients with stage II did not receive adjuvant interventions. This study was approved by the local ethical committees.

Immunohistochemistry

Tissue microarray sections were de-waxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 5 min to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating (100°C) each section for 30 min in 0.01 mol/L sodium citrate buffer (pH6.0). After three rinses (each for 5 min in phosphate buffered saline (PBS)), sections were incubated for 1 h at room temperature with a polyclonal rabbit anti-human cortactin (clone H-191) antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) diluted in PBS. After three washes (each for 5 min in PBS), sections were incubated with biotin-labeled secondary immunoglobulin (1:100, DAKO, Glostrup, Denmark) for 1 h

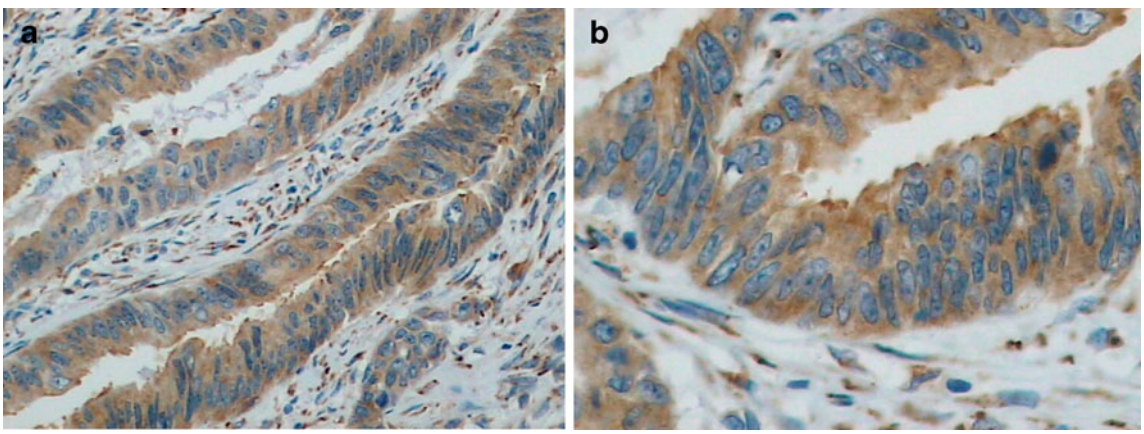

Fig. 1 Strong cortactin (*A*, ×200; *B*, ×400) immunostaining in colon adenocarcinoma.

at room temperature. After three additional washes, peroxidase activity was developed with diaminobenzidine (DAB; DAKO, Glostrup, Denmark) at room temperature.

The immunostaining intensity was separately evaluated by two pathologists (X.J. and Y.Z.) who had no knowledge of the patients' clinical status. For cortactin expression, samples were classified into two groups, positive group or negative/weak group. At least 200 tumor cells were scored per ×40 field. All sections were scored in a semiquantitative manner according to the method described previously, which reflects both the intensity and percentage of cells staining at each intensity.[16] Intensity was classified as 0 (no staining), +1 (weak staining), +2 (distinct staining), or +3 (very strong staining). A value designated the "HSCORE"

Table 1 Immunohistochemical Status of Cortactin in Patients with Stages II–III CRC (n=205)

Characteristics	No. (%)	Cortactin expression		
		Positive (n=133)	Negative/weak (n=72)	P
Median age (range), years	57 (29–78)	–	–	–
Age, years				0.955
<65	139 (67.8)	90	49	
≥65	66 (32.2)	43	23	
Gender				0.669
Male	118 (57.6)	78	40	
Female	87 (42.4)	55	32	
Location				0.374
Colon	140 (68.3)	88	52	
Rectum	65 (31.7)	45	20	
Tumor invasion				0.018
pT1	20 (9.8)	8	12	
pT2	59 (28.8)	39	20	
pT3	108 (52.7)	70	38	
pT4	18 (8.8)	16	2	
Lymph nodal status				0.114
pN0	124 (60.5)	74	50	
pN1	65 (31.7)	46	19	
pN2	16 (7.8)	13	3	
AJCC stage				0.114
II	124 (60.5)	74	50	
III	81 (39.5)	59	22	
Tumor size, cm				0.940
<2	18 (8.8)	11	7	
2–5	141 (68.8)	92	49	
>5	46 (22.4)	30	16	
Lymphovascular invasion				0.096
None	154 (75.1)	95	59	
Present	51 (24.9)	38	13	
Histologic variant				0.096
Colonic	151 (73.7)	94	59	
Mucinous	54 (26.3)	39	13	
Histologic grade				0.004
Well	11 (5.4)	3	8	
Moderate	169 (82.4)	109	60	
Poor	25 (12.2)	21	4	
Preoperative CEA level				<0.001
< 4 ng/ml	63 (30.7)	20	43	
≥4 ng/ml	142 (69.3)	113	29	
Preoperative CA 19-9 level				0.633
< 60 U/ml	138 (67.3)	88	50	
≥60 U/ml	67 (32.7)	45	22	

CRC colorectal cancer, *AJCC* American Joint Committee on Cancer, *CEA* carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9

was obtained for each slide by using the following algorithm: HSCORE=Σ(I×PC), where I and PC represent staining intensity and the percentage of cells that stain at each intensity, respectively, and the corresponding HSCOREs were calculated separately. Expression of cortactin was classified as follows: when ≥30% of the carcinoma cells in a given specimen were positively stained for cortactin, the sample was classified as cortactin positive.

Statistical Analysis

Correlations between clinicopathological factors and cortactin expression were analyzed using Fisher's exact probability test or χ^2 test. Survival was calculated by the Kaplan–Meier method, and differences in survival were determined by the log-rank analysis. A multivariable analysis of several independent prognostic factors was carried out using Cox's proportional hazards regression model.[17] Significance was defined as $P<0.05$. The statistical data were obtained using an SPSS software package (SPSS 11.5 Inc., Chicago, IL, USA).

Results

Relation of Clinicopathologic Factors According to Cortactin Expression

A total of 205 patients with completely resected pathologic stages II–III CRC were included in this study. The median age was 57 (range, 29–78) years, with 118 males and 87 females. Median follow-up time was 125.5 months (range, 108.5 to 156.5 months). Of the 205 patients studied, 113 cases (55.1%) were strongly positive for cortactin (Fig. 1a and b). Twenty-five cases (12.2%) were negative and 47 cases (22.9%) revealed unclear weak reactions. The relationship between cortactin expression and clinicopathological features is shown in Table 1. Cortactin expression correlated with tumor invasion ($P=0.018$), histological grade ($P=0.004$), and preoperative CEA level ($P<0.001$). No other clinicopathological parameter was related to cortactin expression (Table 1). Of 140, 88 (62.9%) patients with colon cancer were strongly positive for cortactin, and 45 of 65 (69.2%) patients with rectal

Table 2 Univariate Analysis of Disease-Free Survival and Overall Survival in CRC

Parameter	*P* value[a]	
	Disease-free survival	Overall survival
Age, years		
<65 vs. ≥65	0.433	0.406
Gender		
Male vs. female	0.723	0.095
Location		
Colon vs. rectum	0.667	0.493
Tumor invasion		
pT1/pT2 vs. pT3/pT4	0.034	0.003
Lymph nodal status		
pN0/pN1 vs. pN2	0.098	0.072
AJCC stage		
II vs. III	0.009	0.008
Tumor size, cm		
<2 vs. ≥2	0.243	0.117
Lymphovascular invasion		
None vs. present	0.043	0.153
Histologic variant		
Colonic vs. mucinous	0.381	0.415
Pathologic grade		
Well/moderate vs. poor	0.781	0.038
Preoperative CEA level		
< 4 ng/ml vs. ≥4 ng/ml	0.004	0.017
Preoperative CA 19-9 level		
< 60 U/ml≥60 U/ml	0.069	0.315
Cortactin expression		
Negative/weak vs. positive	0.004	<0.001

CRC colorectal cancer, *AJCC* American Joint Committee on Cancer, *CEA* carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9

[a] Log-rank test

cancer were strongly positive for cortactin, there was no statistical difference between the colon cancer group and rectal cancer group (P=0.374).

Determination of Independent Factors Affecting Prognosis

In univariate analysis by log-rank test, tumor invasion, AJCC stage, lymphovascular invasion, preoperative CEA level, and cortactin expression were significant prognostic factors for disease-free survival (P=0.034, 0.009, 0.043, 0.004, and 0.004, respectively.), while for overall survival, tumor invasion, AJCC stage, pathologic grade, preoperative CEA level, and cortactin expression were significant prognostic factors (P=0.003, 0.008, 0.038, 0.017, and <0.001, respectively.) (Table 2, Fig. 2). Cortactin expression was not a significant prognostic factor for disease-free survival in patients with rectal cancer (Fig. 3a, P=0.195) and colon cancer (Fig. 3b, P=0.464), while for overall

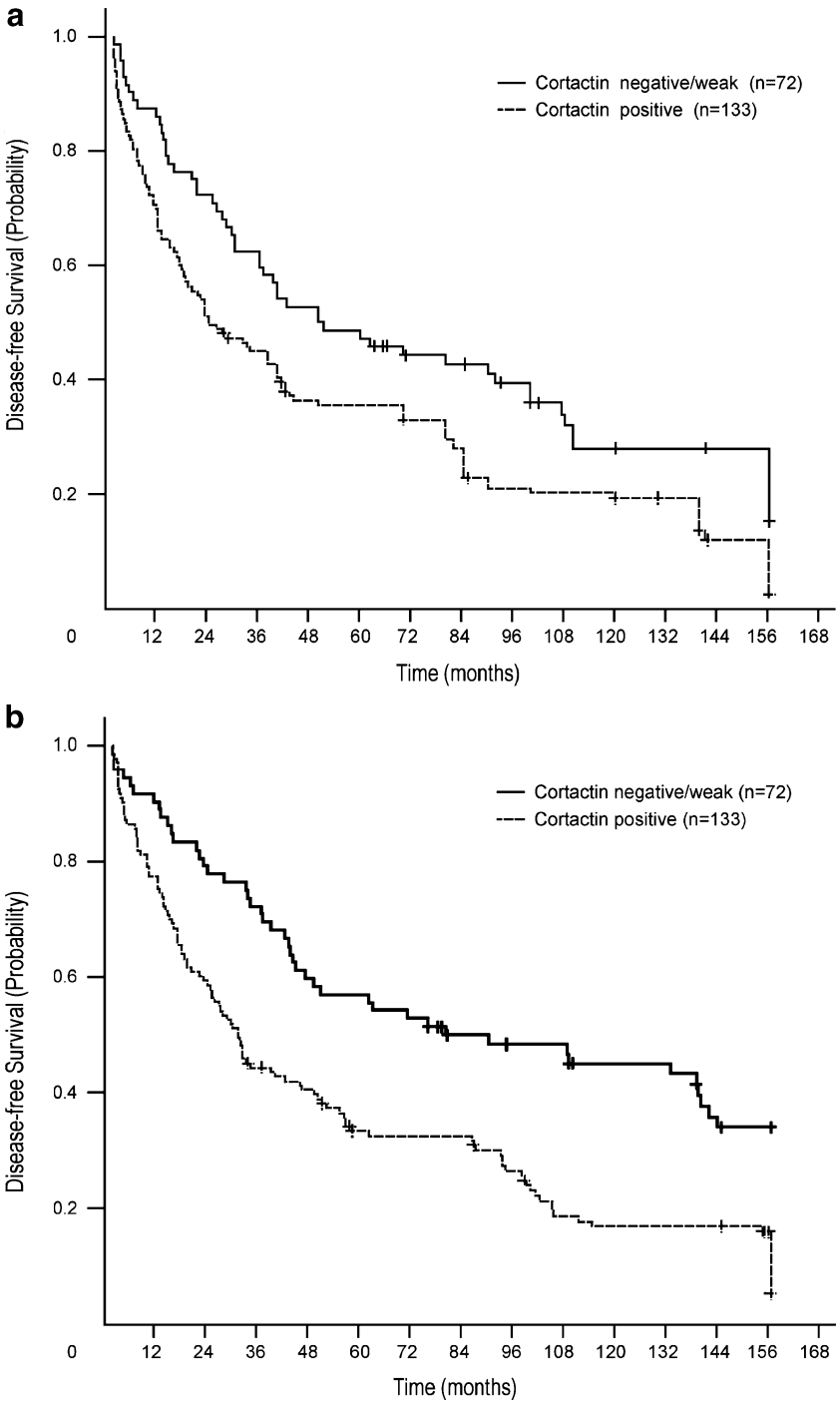

Fig. 2 Cumulative Kaplan–Meier curves for disease-free survival (**a**) and overall survival (**b**) stratified according to cortactin expression (*A*, P=0.004; *B*, P<0.001).

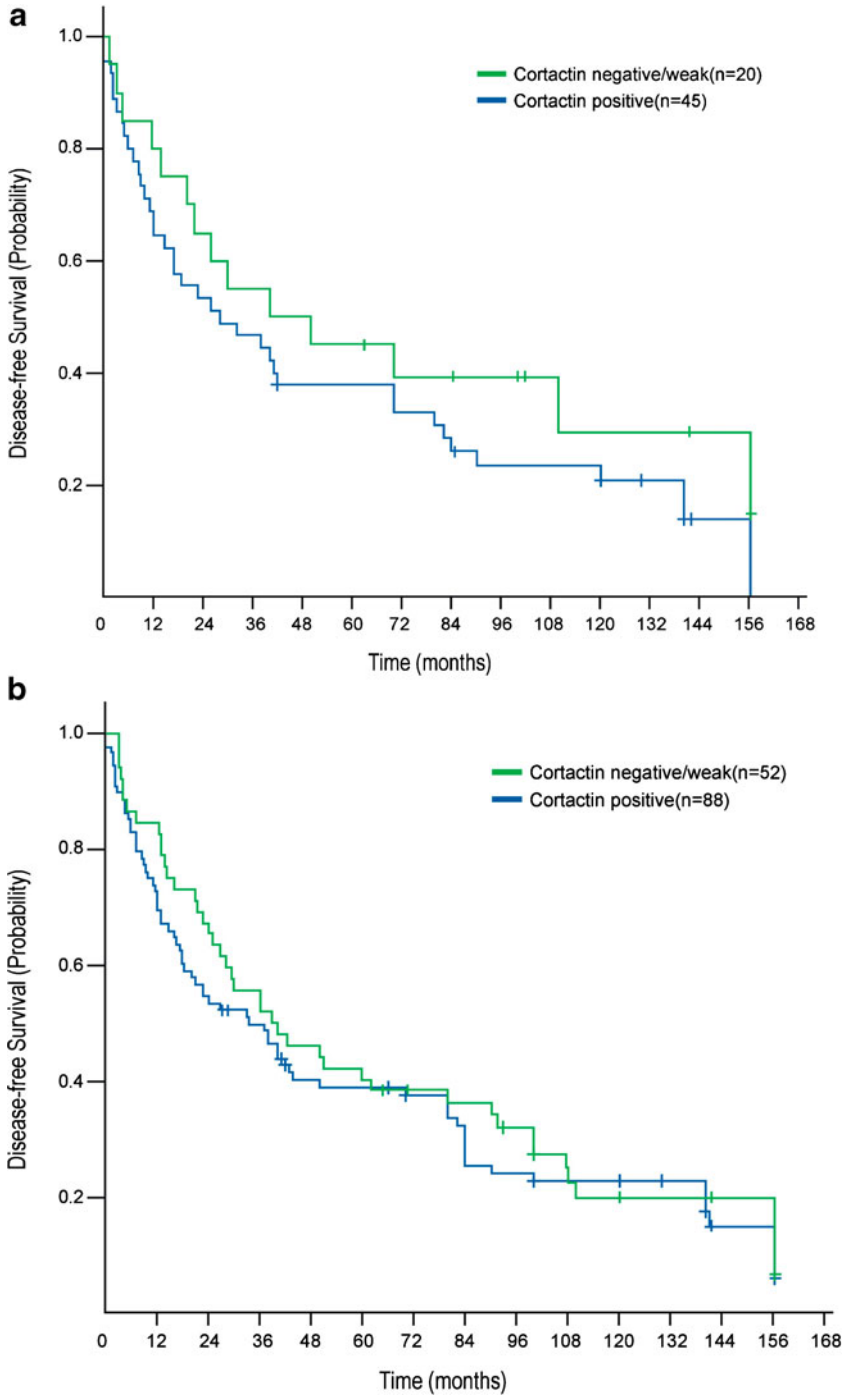

Fig. 3 Cumulative Kaplan–Meier curves for disease-free survival stratified according to cortactin expression in patients with rectal cancer (*A*, *P*=0.195) and with colon cancer (*B*, *P*=0.464).

survival, cortactin expression was a significant prognostic factor in patients with rectal cancer (Fig. 4a, *P*=0.022). However, cortactin expression was not a significant prognostic factor for overall survival in patients with colon cancer (Fig. 4b, *P*=0.102).

A multivariate analysis was performed to evaluate the independent prognostic roles of cortactin after adjusting for other significant covariates. All variables that significantly affected survival in univariate analysis were introduced into a Cox proportional-hazard model (Table 3). At the end of the stepwise process, tumor invasion, preoperative CEA level, and cortactin expression maintained their independent prognostic influence on disease-free survival (*P*=<0.001, 0.003, and 0.008, respectively). However, tumor invasion, AJCC stage, and cortactin expression influenced overall survival (*P*=0.036, <0.001, and 0.004, respectively). The 5-year survival rate for patients with cortactin negative/weak expression (*n*=72) was 55.9% (95% CI, 44.5–67.3%), whereas patients with cortactin positive expression (*n*=133) was 33.3% (95% CI, 25.3–41.3%) (*P*<0.001) (Fig. 3c).

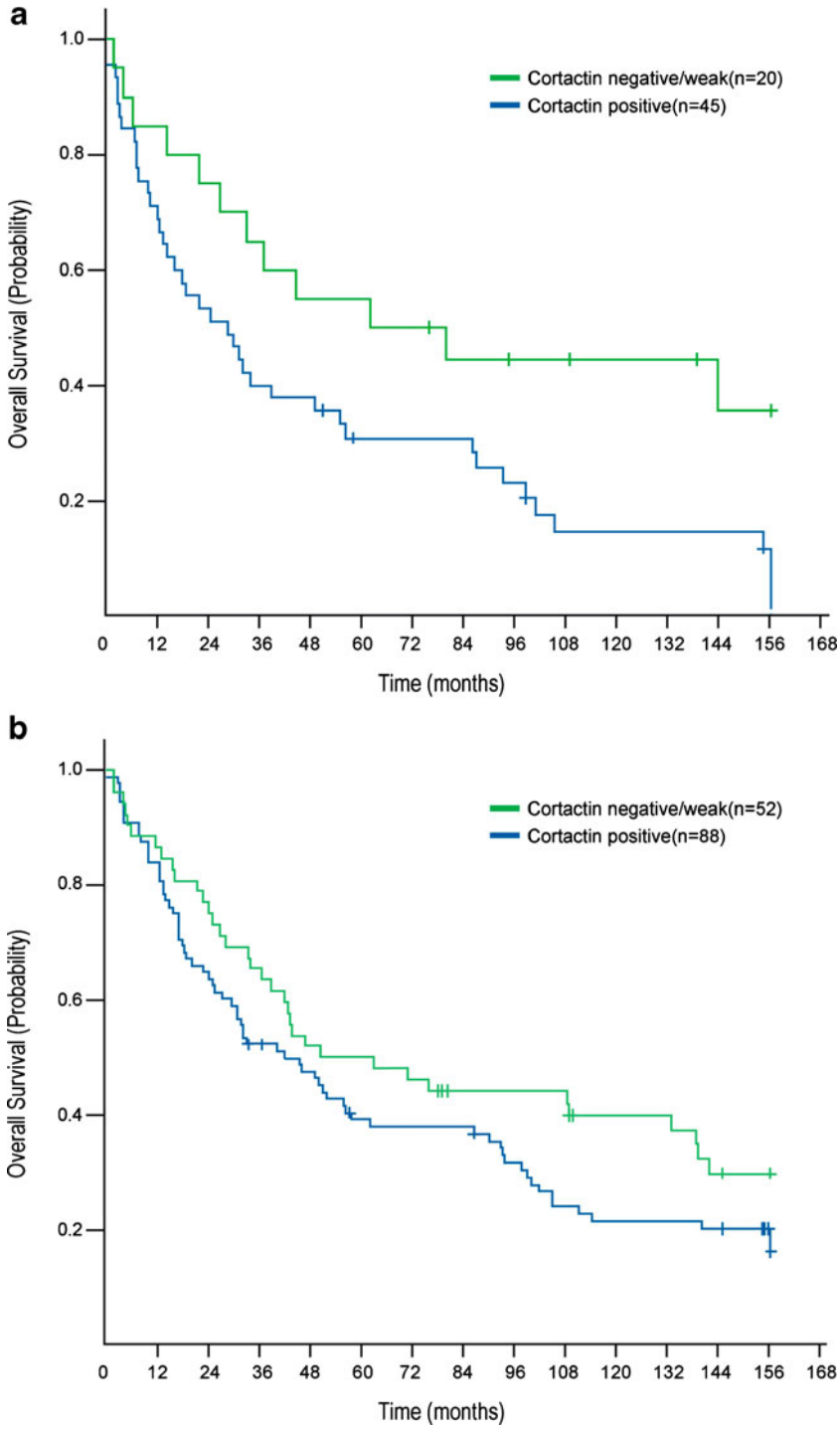

Fig. 4 Cumulative Kaplan–Meier curves for overall survival stratified according to cortactin expression in patients with rectal cancer (*A*, $P=0.022$) and with colon cancer (*B*, $P=0.102$).

Discussion

The precise preoperative staging of CRC is fundamental for surgical strategy, incomplete staging means incomplete treatment and poor outcome.[18] Large-scale clinical evaluations of predictive markers are currently in progress, including determination of their ability to predict response of patients to therapy for advanced disease and for adjuvant treatment. In the present study of 205 tumors, we demonstrated that higher cortactin expression are significantly associated with correlated with tumor invasion, histological grade, and preoperative CEA level, and found that cortactin expression status in the tumors of patients with CRC is a strong independent prognostic factor for disease-free and overall survival. Interestingly, the survival analyses performed separately for colon cancer or rectal

Table 3 Multivariate Analysis of Disease-Free Survival and Overall Survival in Colorectal Cancer

Factors	Characteristics		Hazard ratio	95% CI	*P* value
	Unfavorable	Favorable			
Disease-free survival					
Tumor invasion	pT3/pT4	pT1/ pT2	2.285	1.549–3.371	<0.001
AJCC stage	III	II	1.560	0.880–2.761	0.228
Lymphovascular invasion	Present	None	1.228	0.798–1.891	0.350
Preoperative CEA level	≥4 ng/ml	< 4 ng/ml	1.895	1.242–2.893	0.003
Cortactin expression	Positive	Negative/weak	1.510	1.115–2.045	0.008
Overall survival					
Tumor invasion	pT3/pT4	pT1/ pT2	1.513	1.032–2.224	0.036
AJCC stage	III	II	1.832	1.253–2.695	<0.001
Pathologic grade	Poor	Well/moderate	1.342	0.892–2.020	0.158
Preoperative CEA level	≥4 ng/ml	<4 ng/ml	1.130	0.582–2.194	0.717
Cortactin expression	Positive	Negative/weak	2.094	1.258–3.486	0.004

CI confidence interval, *AJCC* American Joint Committee on Cancer, *CEA* carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9

Table 4 Cortactin Expression in Human Cancers

Study	No. of patients	Cancer	Cortactin positive tumors (%)	Correlation of cortactin with clinicopathological parameters	Prognostic value of cortactin in OS	
					Univariate, *P*	Multivariate, *P*
Chuma et al, 2004[37]	152	HCC	42.1	Intrahepatic metastasis	–	–
Zhang et al, 2006[19]	58	CRC	68.3	–	–	–
Tsai et al, 2007[11]	100	GC	31	Histological differentiation, T stage, N stage, AJCC stage	<0.05	–
Gibcus et al, 2008[38]	167	LC	–	–	0.0006	0.0003
Hofman et al, 2008[21]	176	HNSCC	43.8	TNM stage, histological differentiation	0.024	0.038
Li et al, 2008[39]	509	GC	Cortactin-466: 55.2	Tumor size, depth of invasion, lymphatic and venous invasion, lymph node metastasis, UICC staging	<0.05	0.075
			Cortactin-421: 49.9	Gender, tumor size, depth of invasion, lymphatic and venous invasion, lymph node metastasis, UICC staging, Laruren's classification	<0.05	0.230
Lin et al, 2008[40]	69	SC	–	T stage, histological differentiation	>0.05	–
Hsu et al, 2008[41]	46	ESCC	43.0	–	0.018	–
Rodrigo et al, 2009[42]	86	HNSCC	43.0	Age, pN stage	0.018	–
Hsu et al, 2009[43]	46	ESCC	–	Histological differentiation	0.028	–
Lee et al, 2009[14]	94	CRC	–	T stage, M stage	0.52	–
Lin et al, 2009[44]	79	SC	–	T stage	0.48	–
	14	CCC	–	T stage, clinical AJCC stage	0.21	–
Hirakawa et al, 2009[45]	40	CRC	62.5	–	–	–
This study	205	CRC	55.1	Tumor invasion, histological grade, preoperative CEA level	<0.001	0.004

OS overall survival, *HCC* hepatocellular carcinoma, *CRC* colorectal cancer, *GC* gastric cancer, *LC* laryngeal carcinoma, *HNSCC* head and neck squamous cell carcinoma, *SC* serous cystadenocarcinomas, *ESCC* esophageal squamous cell carcinoma, *CCC* clear cell carcinomas, *AJCC* American Joint Committee on Cancer

cancer, and stratified according to cortactin expression, showed cortactin expression was only a significant prognostic factor for overall survival in patients with rectal cancer. Importantly, cortactin expression status remained an independent prognostic factor in multivariate analysis.

Recently, Lee et al. reported the expression of cortactin was higher in the colorectal adenocarcinoma and tubular adenoma than in the normal colorectal epithelia, and that higher cortactin immunostaining score was associated with more advanced stages (T and M stage).[14] However, in another study, cortactin expression was negatively correlated with TNM staging and lymphatic invasion status in CRC.[19] In previous studies, cortactin has been associated with histologic grade, T and N stage, and prognosis of gastric adenocarcinoma[11] and head/neck squamous cell carcinoma.[20,21] (Table 4) Several gain- and loss-of-function studies over the past few years have convincingly demonstrated the role of cortactin in regulation of cell motility and invasion by virtue of its role in actin remodelling, invadopodia formation, adhesion, endocytosis, and regulation of cell–cell junctions.[22] Although the acquisition of a motile phenotype has conventionally been regarded as a late event in tumor progression, it was recently proposed that aberrant cell motility may, in addition to being essential to tumor invasion and metastatic dissemination, also contributes significantly to rapid tumor growth.[23] Indeed, cortactin overexpression in our study was associated with tumor invasion, histological grade. From the pathologist's point of view, cortactin overexpression could be a new prognosis marker for CRC.

Serum levels of CEA are widely used as tumor markers in patients with gastrointestinal cancer. Several reports have suggested that the postoperative serum CEA level is a useful marker of recurrence after colorectal surgery.[24–27] Moreover, it can be measured cheaply and easily. Monitoring of the postoperative CEA level is thus commonly used in the follow-up of CRC patients.[27–31] However, there has been some controversy about the significance of the preoperative CEA level as a predictive factor of recurrence.[32,33] Recently, Takagawa et al. reported preoperative serum CEA was a reliable predictive factor of recurrence after curative surgery in CRC patients.[34] Our current results endorse these findings. We have confirmed that preoperative CEA level is an important marker for disease-free survival in CRC, which is an additional finding in the current study. As previous studies have reported, the main factor influencing prognosis in CRC is the presence or absence of metastasis in the regional lymph nodes.[35,36] However, we found there wasn't any association between lymph node status and disease-free survival and overall survival in this study, which will need to be studied in further studies.

In conclusion, cortactin may be a good biomarker to be applied in the clinical setting to predict the prognosis of patients with completely resected pathologic stages II–III CRC.

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Conflict of interest statement None declared.

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ORIGINAL ARTICLE

Impact of Resection for Primary Colorectal Cancer on Outcomes in Patients with Synchronous Colorectal Liver Metastases

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Abstract
Purpose This study was designed to evaluate the impact of resection for primary colorectal cancer on oncologic outcomes in patients with synchronous colorectal liver metastases.
Methods A retrospective analysis was performed on 91 consecutive patients with synchronous colorectal liver metastases who underwent resection of the primary colorectal cancer between December 1999 and December 2007. Of the 91 patients, 54 (59.3%) also underwent complete (R0) resection for liver metastases, and 84 (92.3%) received postoperative chemotherapy. The oncologic outcomes and prognostic factors were analyzed.
Results Operative mortality was 1.1%, and morbidity was 37.4%. The 3- and 5-year overall survival rates were 44.5% and 26.8%, respectively. A multivariate analysis revealed that residual disease after surgery (non-R0 resection; $p=0.003$), lymph node metastasis of the primary tumor ($p=0.015$), and no postoperative chemotherapy ($p=0.001$) were independent prognostic factors for poor survival. Independent predictors of an inability to achieve a complete resection were the presence of three or more liver metastases and the presence of extrahepatic disease at exploration. Significant differences in survival existed among the three risk stratification groups (no-, low-, and high-risk groups; $p<0.001$).
Conclusions The inability to safely render the liver and colon microscopically free of disease should cause a surgeon to reconsider synchronous colectomy and hepatectomy. A multidisciplinary approach that combines both complete resection of synchronous colorectal liver metastases and postoperative chemotherapy may achieve improved survival in patients with synchronous colorectal liver metastases.

Keywords Colorectal cancer · Liver metastases · Synchronous · Resection

Introduction

Recent advances in chemotherapeutic regimens using oxaliplatin- and irinotecan-based therapies have led to improved median survival in patients with metastatic colorectal cancer.[1,2] Nevertheless, complete surgical resection of hepatic metastases continues to offer the best chance of long-term survival in these patients.[3,4] Recent studies have reported 5-year overall survival rates of 35% to 58% following the resection of colorectal liver metastases.[5–7] The treatment of patients with synchronous colorectal liver metastases (SCLM) has been challenging, and several authors have suggested that patients who present with SCLM have a poorer prognosis than those who present with metachronous liver metastases.[8–10]

The present study was designed to evaluate the impact of resection for primary colorectal cancer with or without complete hepatic surgical intervention on oncologic outcomes in patients with SCLM in the era of recent advances in chemotherapy. Survival outcomes and factors associated with survival are identified, and a prognostic scoring model based on clinicopathological parameters is delineated.

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Patients and Methods

The medical records of all consecutive patients with SCLM who underwent resection of primary colorectal adenocarcinoma between December 1999 and December 2007 were retrospectively analyzed. A total of 91 patients were identified from a prospectively collected database. Cases excluded from the analysis were those involving only a bypass and/or diversion stoma for primary cancer as well as those involving palliative chemotherapy with no resection owing to the extent of the disease or the discovery of unrecognized extrahepatic disease. Cases with neoadjuvant chemotherapy or chemoradiotherapy administered prior to surgical resection were also excluded. In all of our patients, SCLM were identified before operation, and combined liver and colorectal resections were performed simultaneously. This study was approved by the institutional review board.

All patients had preoperative chest and abdominopelvic computed tomography (CT), with or without dynamic liver magnetic resonance imaging. CT portography was used as our liver-specific imaging technique to determine resectability.[11] CT combined with positron emission tomography (PET-CT) was also performed, depending on the extent of the disease. The distribution of extrahepatic disease included lung metastases and miscellaneous intra-abdominal localizations, including a porta hepatis lymph node and peritoneal carcinomatosis. Surgical resection of gastrointestinal tumors should include the complete removal of both macroscopic and microscopic disease. A minor liver resection was considered any surgical intervention, with or without radiofrequency ablation, involving two or fewer liver segments. A major resection involved three or more liver segments. The desirability of a complete (R0) resection versus an R1/R2 resection has been well described. An R0 resection is defined as one in which all margins are histologically free of tumor. In an R1 resection, microscopic residual disease is left behind, and in an R2 resection, gross residual disease remains. A multidisciplinary committee determined that 61 (67.0%) of the 91 patients in this study had resectable disease and were free of surgical contraindications preoperatively: 54 (88.5%) of the 61 underwent R0 resection of the liver metastases; in seven patients (11.5%), surgery was initiated with curative intent but resulted in only an R1 to R2 resection. The other 30 (33.0%) of the 91 patients underwent palliative surgery for the primary colorectal cancer and were offered postoperative chemotherapy without liver resection, owing to the presence of unresectable intrahepatic and/or extrahepatic dissemination of the disease; these patients were assessed every 3 months post-treatment. Overall, postoperative chemotherapy was administered in 84 (92.3%) cases. The regimen of first-line postoperative chemotherapy was quite heterogeneous: (1) FOLFOX (n=35); (2) XELOX (n=6); (3) FOLFIRI (n=18); (4) fluorouracil+leucovorin (n=16); (5) capecitabine (n=9). Oxaliplatin-based chemotherapy (49%) was the most common chemotherapeutic treatment regimen.

Postoperative mortality was defined as death within 30 days after surgery. Patients were followed every 3 months with serum carcinoembryonic antigen (CEA) testing and CT imaging of the chest and abdominopelvic region to assess disease status. For patients who did not return for observation after 1 year, information was obtained by letter or telephone. The primary end-point of this analysis was overall survival (OS). The statistical evaluation was carried out using SPSS for Windows (Version 14.0; SPSS, Chicago, IL, USA). The clinicopathological variables were compared using the chi-square test. To identify significant independent prognostic factors associated with OS, the variables with p values <0.1 by univariate analysis were entered into a multivariate analysis performed by stepwise logistic regression. Survival rates were calculated using the Kaplan–Meier method, and survival curves were compared using the log-rank test. A p value of ≤0.05 was considered statistically significant.

Results

The clinicopathological features and surgical details of the 91 patients are shown in Table 1. Fifty patients (54.9%) had a primary diagnosis of rectal cancer. Most primary colorectal tumors were staged as T3/T4 (95.6%) and were associated with metastatic nodal disease (81.4%). The mean number of liver metastases treated was two (range 1–11). Extrahepatic metastases were noted in 12 (13.2%) cases, and only one of the 12 patients underwent an R0 resection. Of the 61 patients, hepatic resection alone was performed in 45 (73.8%) patients, while the remaining 16 (26.2%) had hepatic resection combined with radiofrequency ablation with curative intent. The overall 30-day mortality rate was 1.1% (1 patient). Thirty-four patients (37.4%) experienced postoperative complications, the most common of which was small bowel obstruction (eight patients) followed by anastomotic leakage (four patients).

Table 2 summarizes the clinical and treatment variables significantly associated with OS. Variables significantly associated with higher OS included: size of primary tumor <5 cm, negative lymph node metastasis of primary tumor, size of largest hepatic metastasis <3 cm, two or fewer liver metastases, unilobar hepatic metastases, no extrahepatic metastases, preoperative CEA level <100 ng/mL, R0 resection, and receipt of postoperative chemotherapy. A multivariate analysis revealed that residual disease after surgery (non-R0 resection, p=0.003), lymph node metastasis of primary tumor (p=0.015), and no postoperative chemotherapy (p=0.001) were independent prognostic factors for poor survival (Table 3).

Table 1 Patient Characteristics and Surgical Details (n=91)

Parameter	Value (% or number)
Demographics/preoperative factor	
Mean age, years	60.6 (34–85)
Male gender	60 (65.9)
Mean CEA, ng/mL	13.6 (1.3–2628.0)
Primary tumor	
Mean size of maximum diameter, cm	4.9 (1.5–15.0)
Tumor location	
Colon	41 (45.1)
Rectum	50 (54.9)
T stage	
II	4 (4.4)
III	80 (87.9)
IV	7 (7.7)
N stage	
0	17 (18.7)
I	33 (36.3)
II	41 (45.1)
Liver metastases	
Mean size of largest hepatic lesion, cm	3.1 (0.5–10.0)
Mean number of tumors	2 (1–11)
Bilobar disease	44 (48.4)
Extrahepatic metastasis	12 (13.2)
Details of liver surgery/postoperative factors	
Minor resection	30 (33.0)
Major resection	15 (16.5)
Minor resection and radiofrequency ablation	14 (15.4)
Major resection and radiofrequency ablation	2 (2.2)
Completeness of surgery (R0)	54 (59.3)
Postoperative chemotherapy	84 (92.3)
Postoperative complications	34 (37.4)
Postoperative mortality	1 (1.1)

Minor resection one or two liver segments removed, *Major resection* three or more liver segments removed, *CEA* carcinoembryonic antigen

Median follow-up time in this series was 27.5 months (range 1–110). The 3- and 5-year OS rates were 44.5% and 26.8%, respectively. The median OS was 54 months in the patients who underwent R0 resection, but was only 16 months in those who did not (p<0.001, Fig. 1a). The OS was 70.6 months for the lymph-node-negative primary group compared with 36.5 months for the lymph-node-positive primary group (p=0.019, Fig. 1b). Median survival was 30 months in patients who received postoperative chemotherapy and 9 months in those who did not (p<0.001, Fig. 1c). Using the three independent prognostic factors for poor survival from the multivariate analysis, a prognostic grouping of the 91 patients was performed according to the following criteria: no-risk group, 0 factors; low-risk group, one factor; and high-risk group, two to three factors. According to the prognostic model, the three risk groups had significantly different survival outcomes (p<0.001, Fig. 2). Of 91 patients, 10 (11.0%), 48 (52.7%), and 33 (36.3%) patients were categorized as no-risk, low-risk, and high-risk, respectively. The 5-year OS rates for the no-, low-, and high-risk groups were 85.7%, 37.1%, and 0%, respectively.

Given the importance of R0 resection for SCLM to OS, we performed a multivariate analysis to identify factors independently associated with a patient's ability to undergo R0 resection for SCLM (Table 4). Among those patients who were operatively explored, two factors were associated with an inability to achieve an R0 resection: the presence of three or more hepatic metastases and the presence of extrahepatic disease at exploration.

Discussion

The incidence of SCLM ranges from 23.0% to 46.8% of all colorectal cancer patients with hepatic metastases.[9,12–14]

Table 2 Univariate Analysis of Factors Associated with 5-Year Overall Survival (n=91)

Factor	No.	5-Year overall survival (%)	p
Age, years			
<60	38	18.9	0.207
≥60	53	33.1	
Gender			
Male	60	30.2	0.525
Female	31	20.6	
ASA			
I+BII	70	21.9	0.551
III	21	45.4	
Maximum size of primary tumor, cm			
<5	47	42.4	0.048
≥5	44	12.2	
Primary tumor location			
Colon	41	30.3	0.213
Rectum	50	23.8	
Depth of primary tumor invasion			
II+III	84	24.6	0.504
IV	7	47.6	
Primary node status			
Negative	17	64.9	0.019
Positive	74	20.1	
Differentiation			
WD+BMD	78	21.1	0.222
PD+BMU	13	42.7	
Lymphovascular invasion			
Negative	47	25.8	0.506
Positive	44	29.8	
Perineural invasion			
Negative	45	23.5	0.801
Positive	46	31.6	
Largest hepatic lesion, cm			
<3	51	40.3	0.006
≥3	40	15.3	
No. of liver metastases			
<3	51	46.2	<0.001
≥3	40	0	
Bilobar metastases			
No	47	44.8	<0.001
Yes	44	8.3	
Extrahepatic metastases			
No	79	31.1	0.004
Yes	12	0	
Mean CEA, ng/mL			
<100	75	30.1	0.001
≥100	16	0	
Completeness of surgery (R0)			
No	37	0	<0.001
Yes	54	42.8	
Postoperative complications			
No	57	20.3	0.365
Yes	34	37.8	
Postoperative chemotherapy			
No	7	0	<0.001
Yes	84	29.7	

ASA American Society of Anesthesiologists, *WD* well differentiated, *MD* moderately differentiated, *PD* poorly differentiated, *MU* mucinous, *CEA* carcinoembryonic antigen

The possible benefit of surgical resection for asymptomatic primary colorectal cancer in patients with SCLM is still debatable. The traditional concept that surgical reduction of a tumor burden may provide an immunological benefit, and increased susceptibility to chemotherapeutic agents is still applied in treating some solid cancers.[15,16] Moreover, many surgeons still favor surgical resection of the primary tumor to prevent potential local tumor complications, and some studies have suggested that this approach may provide longer survival, better assessment of metastatic disease, and greater improvement in quality of life compared with non-resection.[17–19] Stelzner et al.[17] reported that resection of the primary tumor was an independent predictor for survival in patients with unresectable SCLM only when asymptomatic patients were considered and when in-hospital mortality was excluded.

More recently, the treatment approach for SCLM has begun to change because even in patients with unresectable colorectal liver metastases, median survival approaches 24 months in the era of chemotherapy.[1,20] Several surgical and medical oncologists recently proposed that the initial treatment should be systemic chemotherapy and that resection of the primary tumor should be reserved for the management of tumor complications only.[21–23] They suggested that early treatment with chemotherapy repre-

Table 3 Multivariate Analysis of Factors Significantly Associated with 5-Year Overall Survival (n=91)

Factor	Hazards ratio (CI)	p
Residual disease after surgery	4.179 (1.619–10.783)	0.003
Lymph node metastasis of primary tumor	3.400 (1.270–9.099)	0.015
No postoperative chemotherapy	6.203 (2.506–15.355)	0.001

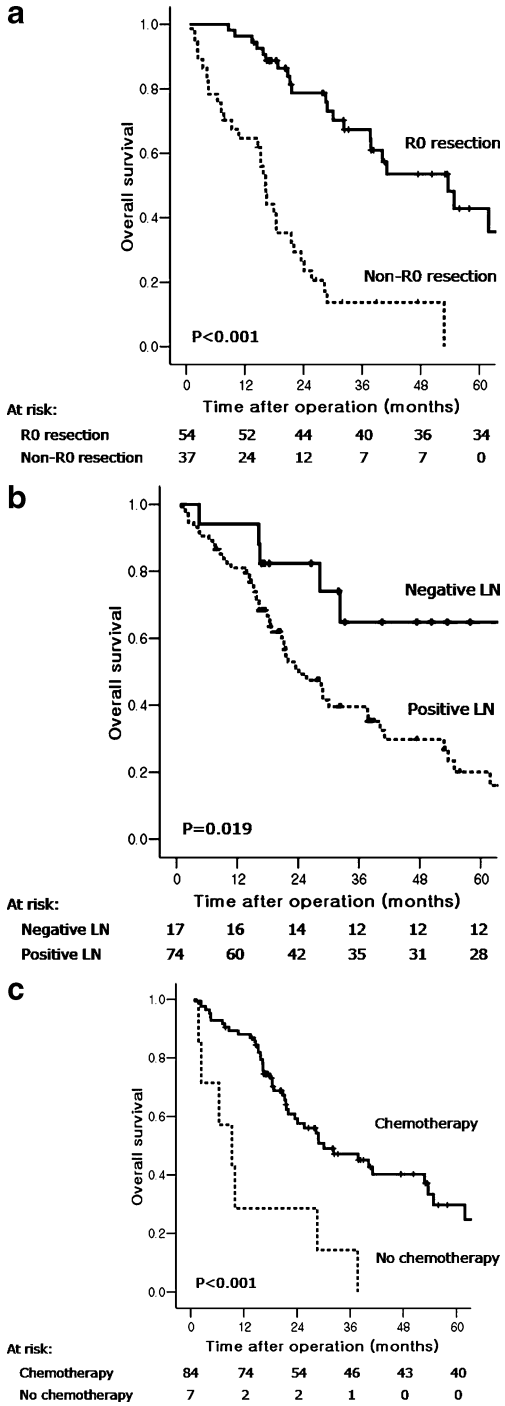

Fig. 1 **a** Survival curve according to completeness of surgery (R0). **b** Survival curve according to lymph node metastasis of primary colorectal cancer. **c** Survival curve according to postoperative chemotherapy.

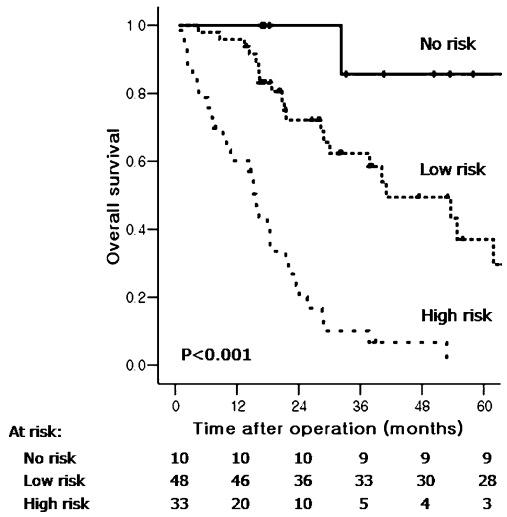

Fig. 2 Survival curve related to clinical risk score. The factors included were residual disease after surgery, lymph node metastasis of primary tumor, and no postoperative chemotherapy. The prognostic grouping was as follows: no-risk group, zero factor; low-risk group, one factor; and high-risk group, two to three factors.

sented a more logical approach to the management of stage IV disease, as survival was principally limited by the presence of metastatic disease.[24] Some authors reported that patients who underwent surgery for a primary tumor had a higher risk for morbidity, and some required reoperation.[22,25] Furthermore, it was recently demonstrated that primary resection of colorectal cancer in asymptomatic patients has no effect on long-term survival, despite the earlier use of chemotherapy.[21] The results from the present study reinforce previously published data showing that resection of primary colorectal cancer alone, without complete resection for liver metastases, provides no survival benefit in patients with SCLM.[21–23,26] Moreover, we justified complete surgical resection in patients with SCLM because the median OS of the patients who underwent R0 resection was 54 months, whereas that of patients who did not was 16 months.

The importance of various SCLM features to oncologic outcome has been inconsistent. Numerous clinicopathological features have been associated with survival-related outcomes in different cases series; these include age,[18,24,27] number of hepatic metastases,[3,27,28] size of hepatic tumors,[9,28] extent of liver disease,[4,29] lymph node status of primary tumor,[9,12,28] preoperative level of serum CEA,[27,28] residual disease after surgery,[3,9,14,30,31] and administration of postoperative chemotherapy.[32] Comparisons among these previous studies are not always feasible, given the differences in patient groupings, follow-up periods, and study designs. We identified three factors associated with a poor outcome in SCLM: residual disease after surgery, node-positive primary cancer,

Table 4 Multivariate Predictors of Complete Resection (R0) in Patients with Synchronous Stage IV Colorectal Cancer

Factor	Odds ratio (CI)	*p*
Number of liver metastases≥3	8.369 (1.548–45.246)	0.014
Extrahepatic metastases	30.095 (1.755–515.967)	0.019

and no postoperative chemotherapy. Although their validation is required, these factors accurately predicted the probability for OS in patients with SCLM. Moreover, we performed a multivariate analysis to identify factors that independently predicted a patient's ability to undergo complete resection, given the significance of this approach in improved survival. The two factors independently associated with non-R0 resection were the presence of three or more liver metastases and the presence of extrahepatic abdominal disease at the time of exploration. Fahy et al.[28] suggested that extrahepatic metastases and bilobar disease were adversely associated with completeness of hepatic resection. Together, these data indicate that extrahepatic metastases are associated with non-R0 resection and poorer survival in patients with SCLM.

In an attempt to enhance risk-based stratification for surgery, we analyzed prognostic factors for survival in patients with SCLM. Notably, patients who had no risk factors showed much superior OS in this analysis; patients categorized as high-risk had very poor survival rates. Although yet to be validated, patients suspected of having more risk factors may be treated with nonsurgical methods first, obviating unnecessary surgery. The findings of the current study are limited by the retrospective nature of the analysis and the relatively small number of patients. Although our findings are insufficient to draw concrete conclusions, they may still be valid.

In conclusion, our data showed that the inability to safely render the liver and colon microscopically free of disease should cause a surgeon to reconsider synchronous colectomy and hepatectomy. A multidisciplinary approach that combines both complete resection of SCLM and postoperative chemotherapy may achieve improved survival in patients with SCLM.

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SSAT POSTER PRESENTATION

Symptomatic Bile Duct Hamartomas: Surgical Management in an MRI Driven Practice

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Introduction

The differential diagnosis of hepatic cysts includes bile duct hamartomas (BDH), which have traditionally been thought of as simple cysts of the liver. These malformed and dilated ducts, set in the background of fibrous stroma, are thought to be constituents on the spectrum of fibropolycystic disease.[1,2] Often found as small, scattered cysts,[2–5] they have the potential to grow extremely large, becoming symptomatic and therefore prompting referral to a surgeon. Echoing this picture, the yearly incidence at our institution of non-surgical BDH and symptomatic, giant BDH is 7.3% and 0.4%, respectively.[6] Always benign, it has nevertheless been exceptionally difficult to preoperatively differentiate BDH from malignant or pre-malignant lesions. The so-called 'malignant masquerade' haunts many facets of a general surgical practice.[7,8] Indeed, it has long been presumed that BDH appear radiographically indistinguishable from cystadenomas or other lesions with potential for malignant transformation or metastases,[9,10] necessitating various degrees of hepatic resection.

In concert with our radiologists and pathologists, however, we have been able to reliably diagnose BDH radiographically and confirm that diagnosis with an intraoperative pathological consultation. BDH can, in fact, be distinguished from other hepatic lesions on the basis of particular features, seen best on MRI due to its improved soft tissue contrast. We have identified and reviewed cases of BDH for description of their optimal surgical management.

Materials and Methods

Patients were selected from an exhaustive search of electronic pathology and imaging records with assistance from both our pathologists and radiologists. After approval by the Emory IRB, a search of the electronic medical record database was performed for the text strings "biliary hamartoma" and "bile duct hamartoma" embedded within pathology and imaging reports during the time of our study. Radiology and pathology databases were cross-referenced with surgical records. These cases below represent all consecutive surgical cases for

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symptomatic cysts preoperatively termed BDH by radiologists from September 2006 to May 2009. All cysts with a radiographic diagnosis of BDH in the time under study are included. Our emphasis is on preoperative MRI; however, those BDH cases without preoperative MRI were included to make the case-series exhaustive from a surgeon's perspective. The patient characteristics listed were basic and included age, gender, symptoms at presentation and follow-up. The imaging characteristics listed include modality as well as size of identified lesions pre- and post-operatively. All available gross specimens and histology of the 25 patients that underwent hepatic resection were reviewed by two pathologists. The pathological characteristics have been pared down to the diagnosis alone, namely a benign BDH. To optimize consistency, all surgical-pathology reports, histology specimens and radiology reports and images were reviewed by two body MRI experts (DRM and BK), a hepatobiliary surgical expert (JMS), and an expert in hepatic cystic pathology (NVA). The surgical decisions were based upon an array of clinical presentations. The clinical observations specific to each patient that were used to formulate a surgical treatment strategy were extracted from the electronic charting, categorized and recorded. To minimize bias, all analysis and chart-review was conducted by a researcher independent of the clinical care (EBT). All patients had complete follow-up by outpatient clinic visits.

Results

Basic Demographics and Overview

A total of 25 patients met our inclusion criteria; six males and 19 females ranging from 40 to 85 years of age (average 62.6±11.6 years old). The men were, on average, 68.5±15.2 and the women were 60.8±10.0 years old. Demographics, clinical presentation, imaging, surgical intervention, course, and follow-up are all detailed in Table 1. Of note, we have never found a single case of malignant or pre-malignant cyst incorrectly diagnosed as benign on MRI.

Clinical Information

The commonest presentation was abdominal pain. Other presentations included one patient each with jaundice, leg swelling (compression of veins), hiccups, and pain with rupture. One patient's BDH was discovered incidentally, however his was resected at the same time as a nephrectomy for renal cell carcinoma. With few exceptions, most patients received an MRI that revealed a giant cyst accompanied by several small cysts that our radiologists could differentiate as BDH. These exceptions are two, both of which are related to the urgency of their respective cases: one patient's cyst had ruptured (only CT was available in a timely fashion) and another was transferred from an outside hospital (OSH) with sepsis and acute renal failure secondary to an infected instrumentation. In total, four patients presented to our care after failed or complicated drain placement at an OSH.

Imaging

Those lesions imaged with MRI revealed consistent features. Common to all cysts were well-defined, lobulated margins, internal fluid content, thin septations, and a hypointense T2 rim corresponding to a thin rim of peripheral enhancement (Fig. 1). Roughly two-thirds of patients had cysts with complex signal internally, thought by the radiologist to represent intracystic hemorrhage and/or proteinaceous fluid. None of the cysts (either complicated or simple) demonstrated internal enhancement on any of the postcontrast imaging to indicate any intracystic-vascularized soft tissue elements. Consistently, every patient had other cysts identified showing a range of features including smooth outer margins, round or lobulated morphology, or thin septations.

Surgical Outcomes

Surgical therapy, complications and symptomatic pain relief are summarized for all patients in Table 1. Overall, in order of the amount of liver tissue excised, 14 patients received a fenestration with or without a subsegmentectomy, three received a segmentectomies with or without a fenestration, three received sectorectomies, and five received partial hepatectomies. Seventeen of 25 patients had planned laparoscopic operations, only one of which was hand-assisted. Four laparoscopic procedures were converted to open (three for hemostasis, one to rule out bile duct injury); one of which was performed in management of hemangioma independent of the BDH. One patient received an open procedure to allow the urological service to follow with a nephrectomy for renal cell carcinoma. All told, we experienced five complications of management, none of which resulted in any long-term morbidity. Two patients experienced an abdominal abscess and sepsis; both of them had had radiological interventions prior to transfer to our institution. One patient, who would subsequently receive a histopathological diagnosis of polyarteritis nodosa, experienced intraoperative hypotension secondary to persistent bleeding from aneurysms. Another patient had a biloma and the last one had a wound infection, both of which were resolved expeditiously.

The length of stay (LOS) varied greatly dependant on the surgical approach, the analysis of which can be found in Table 2. Overall, the average post-operative LOS was

Table 1 Demographics, Presentation, Operation, and Results

Number	Age/ sex/year	Presentation	Imaging size (cm)[a]	Approach	Procedure	Complication	Length of stay (days)	Follow-up imaging	Follow-up length (weeks)	Status
1	41/♂/2006	Pain	MRI/10	Open	Partial hepatectomy	Biloma	15	CT-small cysts	70	Pain resolution
2	50♀/2007	Pain Rupture	CT/30	Open	Partial hepatectomy	none	7	CT-small cysts	40	Pain resolution
3	75/♂/2007	Jaudice	CT[b]/3	Open	Left hepatectomy[c]	Sepsis, Acute Renal Failure	17	CT-small cysts	30	Jaundice Resolution
4	60/♀/2007	Pain	MRI/22	Lap to Open	Fenestration, subsegmentectomy	None	4	cysts	29	Pain resolution
5	85/♂/2007	Pain	MRI/10	Open	Fenestration, partial left hepatectomy	Hypotension	16	MRI-small cysts	43	Pain resolution
6	72/♂/2007	Pain	MRI/11	Open	Fenestration	None	6	No Follow-up image	8	Pain resolution
7	60/♀/2007	Pain	MRI/17	Lap to Open	Fenestration, Subsegmentectomy	None	3	CT-no disease	8	Pain resolution
8	69/♀/2007	Pain	MRI/8	Lap	Fenestration	None	1	MRI-no disease	8	Pain resolution
9	53/♀/2007	Pain	MRI/5	open	Right posterior sectorectomy[c]	Intraabdominal abscess	13	CT-no disease	21	Pain resolution
10	69/♀/2007	Pain	MRI/16	Lap	Fenestration	None	3	CT-small cysts	39	Pain resolution
11	59/♀/2007	Pain	MRI/6	Lap to open	Fenestration bisegmentectomy for hemangioma	None	5	No follow-up image	18	Pain resolution
12	54/♀/2008	Pain	MRI[b]/9	Lap	Left lateral sectorectomy	None	3	CT-no disease	12	Pain resolution
13	62/♀/2008	Pain	MRI/6	Lap	Fenestration	None	1	CT-no disease	31	Pain resolution
14	76/♀/2008	Pain	MRI/10	Lap	Left lateral sectorectomy	None	1	CT-no disease	8	Pain resolution
15	75/♀/2008	Pain	MRI/15	Lap	Fenestration, subsegmentectomy	None	3	CT-small cysts	12	Pain resolution
16	45/♀/2008	Pain	MRI/13	Open	Fenestration, segmentectomy	Wound infection	6	CT-no disease	29	Pain resolution
17	57/♀/2008	Pain	MRI/14	Lap	Fenestration[c]	None	1	CT-no disease	12	Pain resolution
18	40♀/2008	Pain	MRI/9	Lap[d]	Right Trisegmentectomy	None	6	MRI-no disease	21	Pain resolution
19	63/♂/2008	Incidental	MRI/14	Open	Fenestration, subsegmentectomy, left nephrectomy[e]	None	5	No follow-up image	38	No compliants
20	67/♀/2009	Pain	CT[b]/17	Lap	Fenestration	None	0.5	CT-small cysts	12	Pain resolution
21	60/♀/2009	Pain	MRI/13	Lap to Open	Left hepatectomy	None	2	CT-no sign of disease	10	Pain resolution
22	56/♀/2009	Leg Swelling	CT[b]/13	Lap	Fenestration	None	0.5	CT-small cysts	8	No compliants
23	73/♀/2009	Pain	MRI/14	Lap	Fenestration, Subsegmentectomy	None	1	MRI-no disease	10	Pain resolution
24	75/♂/2009	Pain, Hiccups	MRI/14	Lap	Fenestration, subsegmentectomy[c]	Minor bleeding	2	CT-small cysts	8	Pain resolution
25	70/♀/2009	Pain	MRI/12	Lap	Fenestration, Subsegmentectomy	None	0.5	MRI-no disease	6	Pain resolution

Abbreviation: *Lap* laparoscopic, *CT* computed tomography, *MRI* magnetic resonance imaging, *OSH* outside hospital

[a] Size is of greatest dimension for largest cysts, if multiple

[b] Imaging at OSH, service for resection of renal cell carcinoma

[c] Drain placement at OSH

[d] Hand-assisted laparoscopy

[e] Combined operations with urology

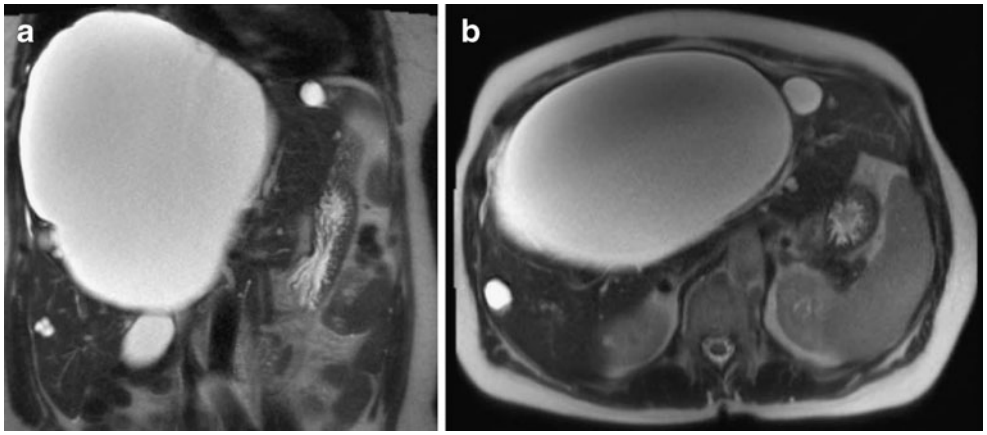

Fig. 1 Large, uncomplicated cystic bile duct hamartoma; coronal (**a**) and axial (**b**) single-shot T2-weighted images demonstrate a large hepatic cyst expanding the right hepatic lobe. The cyst shows small lobulations, simple internal fluid and a thin peripheral rim of enhancement on coronal and axial postcontrast 3D gradient echo (GRE) images. Note additional smaller, classic bile duct hamartomas in the adjacent hepatic parenchyma with similar imaging features. This patient was treated with a laparoscopic fenestration with excellent results. Without MRI, the differential diagnosis would have included a biliary cystadenoma that requires a more complex operative approach.

an average of 4.9±5.0 days with range of less than 1 to 17 days. Three features were related to a shorter LOS: a laparoscopic approach (even when it was converted to an open procedure), a minimal amount of liver resection and the year in which an operation took place. We divide our experience into the three main years of service: in 2006–2008, the average LOS was 8.2±5.9 days, in 2008–2009 the LOS was 3.3±2.2 days and in the present year the LOS was 1.1±0.7 days. In 2009, we have (as of August 2009) performed six operations for BDH: three were performed on an outpatient basis, one patient was admitted overnight for observation, one patient—75 year old male with an extensive cardiac history and ASA score of three—spent 2 days in the hospital, and one proceeded to laparoscopic left hepatectomy secondary to complexity of location. All patients faired well in their respective post-operative courses with resolution for all presenting complaints. All patients under study had complete outpatient follow-up. The average length of follow-up was 21±15.8 weeks with a range of 6 to 70 weeks. All but one patient had post-operative imaging, CT or MRI (both with contrast), which universally showed resolution of the cyst.

Histopathology

Grossly, all cysts showed a smooth lining devoid of any excrescences. Substantial hemorrhage was present in many cysts, as were fibrous septations creating a multilocular pattern along the cyst wall. Microscopic conglomerates of dilated ducts on the cyst walls were observed in half of our cases, a feature characteristic of BDH. These bile duct hamartomas along the cyst margin all gave the impression of transforming into the main cyst. No ovarian type stroma was identified. All cysts removed were confirmed as BDH on final histopathology.

Discussion

Definitively, this has been a collaborative work between three departments; radiology, pathology, and surgery. Advances in radiographical diagnosis of hepatic lesions along with rapid and accurate intraoperative pathology consultations have allowed the surgical management of giant symptomatic BDH to evolve. Accordingly, we had

Table 2 Length of Stay (LOS) in Days

	Number	LOS
Approach		
Open	8	9.3 (4.6)
Laparoscopic to open	4	3.5 (1.3)
Laparoscopic	13	2.0 (1.7)
Overall	25	5.1 (5.1)
Procedure		
Fenestration ± subsegmentectomy	14	2.3 (1.8)
Segmentectomy ± fenestration	3	5.7 (0.6)
Sectorectormy	3	5.7 (6.4)
Partial hepatectomy	5	11.4 (6.6)
Timing		
01/2006 to 12/2007	10	8.2 (5.9)
01/2008 to 12/2008	9	3.3 (2.2)
01/2009 to 05/2009	6	1.1 (0.7)

Length of stay is depicted in average days (standard deviation)

two motivations with this study. Firstly, we wanted to support the work of our radiologists and pathologists with a study devoted to the surgical management of giant BDH. Secondly, we wanted to support Gamblin's argument that a laparoscopic fenestration ought to be the standard treatment for these lesions. With this manuscript, we advance their argument by providing the preoperative imaging and histopathological features that distinguish BDH from cystadenomas.[11]

The management of BDH first requires confidence in the preoperative diagnosis. To that end, we describe consistent clinical features: our patients are, principally, elderly women who present with abdominal pain who are otherwise asymptomatic. The strongest support for this diagnosis, however, comes from our radiologists who describe consistent imaging features. Namely, BDH have well-defined, lobulated margins, thin septations, a thin rim of peripheral enhancement on T2-weighted images, internal fluid content that may contain hemorrhage and/or proteinaceaous material, and are without any intracystic-vascularized soft tissue elements. By contrast, MRI evaluation of biliary cystadenomas and cystadenocarcinomas show smooth margins, mural nodules, and papillary projections without an enhancing peripheral rim.[12] Therefore, given the appropriate clinical scenario, we recommend an MRI with contrast for evaluation of symptomatic BDH.

The value of this diagnosis is unmistakable. Nowhere is this more obvious than in the striking difference in LOS between early and later cases. On one hand, this difference represents a learning curve and a growing sense of familiarity with the giant BDH shared by three services at our academic institution. On the other, it grossly represents the shift toward laparoscopic fenestration with associated hepatic resection; we are now treating these patients on an outpatient basis. This logic is supported further by our data that shows, first, shorter LOS with a laparoscopic approach (even when converted to open for more complex cases) and, secondly, a stepwise increase in LOS in relation to the amount of liver resected. A similar experience has been reported by Gamblin et al.,[11] who showed excellent results with laparoscopic fenestrations of liver cysts. In their series, however, they note the difficulty in distinguishing cystadenoma radiographically from the benign cyst, which poses the central pitfall for this procedure. Given the potential for malignant transformation, a laparoscopic fenestration for the cystadenoma means leaving behind some tissue that will recur with an ever present malignant potential. Ten percent of cysts resected in the Gamblin series were revealed as cystadenomas on final histopathology, which they dealt with using serial follow-up CTs and the possibility of further surgery. In our series, we have shown how this risk is obviated by experience, collaboration and an accurate radiographic diagnosis obtained by MRI.

Over time, our collaborative practice has grown confident in the radiographic diagnosis as well as intraoperative consultations which allow us to offer laparoscopic procedures with associated liver resection. In addition, we also argue that given both a strong radiographic diagnosis and surgical management plan, we ought to prevent the undue complications of superfluous instrumentation and avoid placing drains in these cysts. Follow-up imaging was done using either CT or MRI (both with contrast); tracking the post-operative progress of a cyst can be accomplished with either modality.

A final word about the role of a procedure for the asymptomatic patient diagnosed incidentally. As BDH have no malignant potential and the risk of rupture is low both in our experience as well as others, we do not recommend an operation in this setting.[11]

Conclusion

The multi-disciplinary management conferences between experienced radiologists, pathologists, and surgeons at our academic tertiary referral center have been crucial to the quality improvement of the care we offer the patients with BDH. After achieving confidence in our radiographic diagnosis, the focus shifts towards management. Since all patients had the same outcome—total resolution of symptoms—then we ought to offer them the most expedient and least invasive procedure. We also believe that the laparoscopic approach should be tried as an initial step in every case, even in cysts located on segments 7 and 8, with appropriate counseling for the patient regarding further interventions.

The symptomatic bile duct hamartoma is uncommon but not rare. To treat these lesions, one must proceed from a strong radiographic diagnosis, best made using MRI. Thereafter, we recommend a laparoscopic fenestration.

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ORIGINAL ARTICLE

Risk Assessment in Cholelithiasis: Is Cholecystectomy Always to be Preferred?

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Abstract
Background As many patients with gallstone disease do not benefit from cholecystectomy, preoperative recognition of such high-risk patients is important. The aim of the study is to identify predictors of persisting symptoms at 6 months after cholecystectomy for patients with different preoperative symptomatology.
Method Participants in this prospective study were consecutive patients (n=172), age 18–65 years, with symptomatic cholelithiasis, undergoing a laparoscopic cholecystectomy. Predictors were identified using uni- and multivariate regression analyses.
Results At 6 months postcholecystectomy, patients with only preoperative biliary symptoms were most often free of symptoms (62.5%). Patients with only dyspeptic symptoms most often reported persistence of preexisting symptoms (63.2%). Preoperative non-specific symptoms predicted the report of postoperative biliary and/or dyspeptic symptoms (OR=4.5–6.1). Persistence of preexisting pattern of symptoms was predicted by the use of psychotropic medication (OR=5.3) and dyspeptic symptoms (OR=4.5). Postoperative biliary symptoms were predicted by High Trait Anxiety (HTA) (OR=10.6).
Conclusion Surgeons should take account of individual risks of patients in the management of cholelithiasis. Instead of cholecystectomy, expectative management should be the first choice in patients with non-specific symptoms, with dyspeptic symptoms only, with HTA and in patients using psychotropic medication.

Keywords Cholecystectomy · Cholelithiasis · Outcome assessment · Risk assessment · Surgery

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Introduction

Gallstone disease (cholelithiasis) is a common condition that affects 5% to 22% of the people in Western countries.[1,2] Most patients are unaware of their condition [3] and only 10–30% of these patients develop clinical symptoms,[4–7] such as classical biliary colics or other gastrointestinal symptoms. Laparoscopic cholecystectomy is the golden standard in the management of uncomplicated symptomatic cholelithiasis. As cholecystectomy entails the risk of peri- and early postoperative complications (0.2–9.4%) [5,8] and a substantial number of patients (40.4%) report negative outcome after cholecystectomy,[9,10] critical consideration of pros and cons of cholecystectomy is required. Identification of potential predictors of negative outcomes is essential for decision making in elective cholecystectomy.

Clinical characteristics, such as preoperative dyspeptic symptoms,[11–13] medication use,[10] age,[14,15] characteristics

of preoperative pain and symptoms [12,13,15,16] have been identified as associates of long- and short-term postcholecystectomy outcomes. However, the comparability of results is hampered by different criteria for inclusion, moments of follow-up, definitions and operationalisations of variables and outcomes. Besides 'hard', clinical characteristics, 'soft' predictors such as self-rated health status,[13] personality traits,[12,17–19] and other psychological variables [20] have been identified as predictors of negative outcomes as well. Despite the fact that the symptomatology of cholelithiasis is ambiguous and only the minority of patients report classical biliary colics,[5,21] preoperative symptoms (in combination with ultrasound examination) are used as a reference point for diagnosis and indication of cholecystectomy in clinical practice. Therefore, in the current study we aimed at the identification of predictors of postoperative symptoms at 6 months postcholecystectomy for patients with different preoperative symptomatology.

Methods

Patients

Patients for the current study were recruited from the Department of Surgery of the St. Elisabeth Hospital in Tilburg, the Netherlands. Consecutive patients (18–65 years) with diagnosed symptomatic cholelithiasis, awaiting an elective laparoscopic cholecystectomy who visited the hospital between March 2006 and January 2008, were eligible for the study. Exclusion criteria were: patients with ASA III or IV, undergoing an emergency procedure or intended open cholecystectomy, insufficient knowledge of the Dutch language, choledocholithiasis, cholangitis, known pregnancy, known liver-cirrhosis, history of abdominal malignancy, previous upper abdominal surgery (precluding laparoscopic approach) and psychiatric diseases. All patients underwent a standard surgical and anaesthetic procedure. The protocol of the study was approved by the local ethics committee.

Procedure

Preoperatively, participation was asked during the patients' first surgical consultation at the outpatient clinic. The surgeon introduced the study, whereas subsequently nurses informed patients about the research procedures. Patients received the first set of questionnaires, which also contained written information about the study, and signed informed consent. Medical history and comorbidities were obtained from medical records. Patients completed and returned the first questionnaires before admission for cholecystectomy. If necessary, patients received a reminder telephone call to return the questionnaires 3 to 5 days before the operation. Patients who returned the questionnaires after admission were excluded from the study. At 6 months after cholecystectomy, patients received the same questionnaire which could be returned in a prepaid envelope. If needed, patients were contacted by telephone twice, usually 2 and 4 weeks after sending the questionnaires. Patients returning their second questionnaire >9 months after surgery were considered as non-responders.

Questionnaires

The demographic questionnaire was completed preoperatively and asked about sex, age, marital status, educational level and work. Preoperatively and at 6 months postcholecystectomy, patients completed a symptom checklist based on information from focus groups of gallstone patients.[22] Patients should tick off whether they experienced biliary symptoms (upper abdominal pain, nausea, vomiting), dyspeptic symptoms (bad taste, heartburn, under abdominal pain, diarrhoea and flatulence) and non-specific symptoms (general malaise, fatigue, weight change, decrease in sexual functioning and health complaints not mentioned in the predefined checklist) in the past week. Symptoms were categorised according to a study of Weinert et al.,[13] that was based on the Minnesota Clinical Comparative Assessment Project database.[23] Furthermore, patients indicated the nature, severity, duration and frequency of pain during preoperative biliary attacks on a 100-mm visual analogue scale and on three multiple choice items. All included patients had experienced biliary or dyspeptic symptoms in medical history. After surgery, surgical reports were checked for the presence of gallstones/sludge and conversion to open surgery.

Patients completed the trait scale of the State-Trait Anxiety Inventory (STAI) preoperatively. The STAI-trait exists of 20 items with a 4-item Likert scale reflecting the extent of anxiety patients generally feel. The STAI-Trait Anxiety measure has good 3 months test–retest reliability (r=.75) and internal consistency (Cronbach's α=.84–.92).[24] Patients scoring the 80th percentile or higher were indicated as patients with 'High Trait Anxiety' (HTA), whereas patients with a score below the 80 th percentile were indicated as patients with 'Non-High Trait Anxiety'.

Statistical Procedure

Differences between groups of patients with preoperative biliary, dyspeptic or a combination of these symptoms were calculated using Chi-square tests for dichotomous and ordinal variables and one-way ANOVA between subjects

for continuous variables. Friedman's tests were used to calculate an overall difference in symptom report between the preoperative measurement and the measurement and 6 months. Changes in symptom report over a 6-month time were obtained using Wilcoxon's signed-rank tests (for ordinal variables) and McNemar's tests (for dichotomous variables). Significance was obtained from Chi-square tests and, if necessary, Binominal tests.

Persistence and emergence rates were obtained for 'any symptom'. Persistence was defined as patients reporting the same symptom preoperatively and at 6 months. An overall score was calculated form biliary, dyspeptic, and biliary/dyspeptic symptoms. Emergence was defined as patients who did not report a specific symptom preoperatively, but who reported the symptom at 6 months. Again an overall score was obtained for biliary, dyspeptic, and biliary/dyspeptic symptoms.

Univariate logistic and multinominal regression analyses, taking 'symptom free' as reference group, were performed to identify predictors of postoperative symptoms at 6 months. These analyses were used for dichotomous and categorial outcomes, respectively. Significant predictors were selected and inserted in multivariate logistic or multinominal regression analysis (Backward procedure, Method Likelihood Ratio) to identify independent predictors of postoperative symptom report. In multinominal regression analysis, the reference category was 'symptom free'. A p value<.05 indicated statistical significance. Statistical analyses were performed using SPSS version 16.0.

Results

A flow-chart of the population under study is shown in Fig. 1. In total, 253 patients received the first set of questionnaires, of which 172 patients returned their questionnaires at 6 months after cholecystectomy (response rate 68.0%). Clinical and demographic patient characteristics are shown in Table 1. The majority of patients (61.9%) reported both biliary and dyspeptic symptoms (Table 2). Moreover, 24.5% of the patients only reported biliary symptoms, and 13.6% of the patients only reported dyspeptic symptoms. In this study, groups of patients were based on the report of preoperative biliary symptoms (group 1), dyspeptic symptoms (group 2) or both (group 3). These groups did not differ on any clinical or demographic characteristics.

Six Months Benefit of Cholecystectomy

At 6 months after the operation, the majority of patients had started their normal daily activities (95.5%). Of the patients who had paid work (n=117), 99.1% had returned

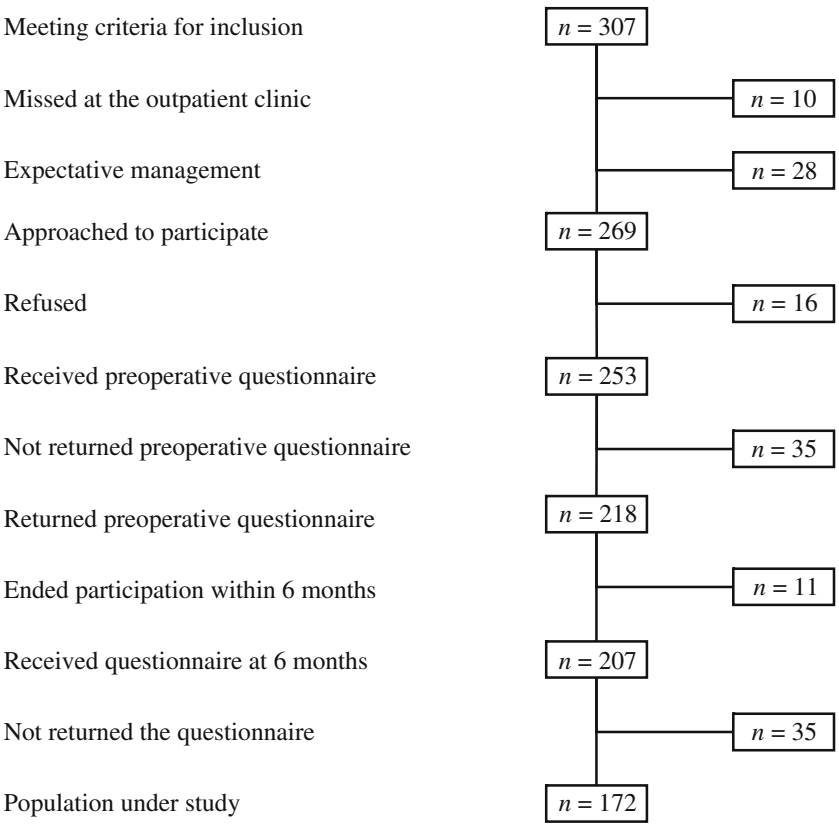

Fig. 1 Flow-chart of the population.

Table 1 Demographic and Clinical Characteristics (n=172)

Demographic characteristics	
Female patients (%)	76.2
Age (M±SD)	47.51±11.33
Highest level of education	
Lower or vocational education (%)	17.1
Secondary education (%)	49.4
Higher education (%)	6.4
Higher professional education or university (%)	27.1
Working under payment (%)	68.8
Marital status	
Single (%)	4.1
Widowed or divorced (%)	5.9
Married or cohabitant (%)	90.0
Medical characteristics	
Self-reported medication use	
Analgetics (%)	36.8
Psychotropic medication (%)	9.9
Other medication (%)	49.7
Comorbidities	
Coronary arterial disease (%)	18.0
Pneumonal disease (%)	5.8
Abdominal disease (%)	22.1
Kidney disease (%)	0.6
Urogenital disease (%)	8.1
Neurological disease (%)	9.9
Other comorbidities (%)	43.6
High trait anxiety (HTA) STAI-Trait ≥ P 80 (%)	20.1

Table 2 Preoperative Presentation of Cholelithiasis

Preoperative symptom report	
Upper abdominal pain (%)	66.5
Nausea (%)	40.4
Vomiting (%)	56.1
Bad taste (%)	21.6
Heartburn (%)	27.5
Under abdominal pain (%)	20.5
Diarrhoea (%)	18.7
Flatulence (%)	36.8
Biliary symptoms[a] only (%)	24.5
Dyspeptic symptoms[b] only (%)	13.6
Biliary[a] and dyspeptic[b] symptoms (%)	61.9
Demonstrated stones or sludge (%)	91.9
Preoperative biliary attacks (M±SD)	5.2±7.2
Pain during biliary attacks	
Pain in rest and movement (%)	94.3
Pain only in rest (%)	4.5
Pain only in movement (%)	1.2
Non-specific symptoms[c] (%)	56.1

[a] Biliary symptoms, one or more of the following symptoms: upper abdominal pain, nausea and vomiting

[b] Dyspeptic symptoms, one or more of the following symptoms: bad taste, heartburn, under abdominal pain, diarrhoea and flatulence

[c] Non-specific symptoms, one or more of the following symptoms: general malaise (9.4%), fatigue (49.7%), weight change (2.9%), decreased sexual functioning (10.5%) and other health complaints (12.3%)

to work, whereas 0.9% of the patients had not returned to work.

Six months after cholecystectomy, 47.8% of the patients were free of symptoms (see Fig. 2), whereas persistence and emergence of any symptom was reported by 17.9% and 34.0% the patients, respectively. At 6 months, a substantial group of patients still reported specific postoperative health complaints, such as wound pain (7.2%), shoulder pain (10.1%) and pain in the upper right abdomen (13.7%) at follow-up. Over 6 months time, the number of patients with biliary symptoms only and a combination of biliary and dyspeptic symptoms decreased from 24.5% to 3.1% and from 61.9% to 14.3%, respectively. However, the number of patients that reported dyspeptic symptoms increased from 13.6% preoperatively to 34.8% at 6 months postoperatively (see Fig. 2).

Patient groups were compared with regard to postoperative symptomatic outcome at six months. The overall symptomatic outcome of patients in group 2 differed significantly from the outcome of the other patient groups (χ^2=8.30, p=.040). The three patient groups differed significantly regarding the report of any postoperative symptom (biliary or dyspeptic) (χ^2=6.29, p=.043). At 6 months, postoperative symptoms were reported by 68.4% of the patients in group 2, whereas postoperative symptoms were reported by 37.5% and 60.2% of the patients in group 1 and 3, respectively. Group 3 (n=53) differed significantly from the other two groups.

Furthermore, the groups differed significantly with respect to the persistence of the same pattern of preoper-

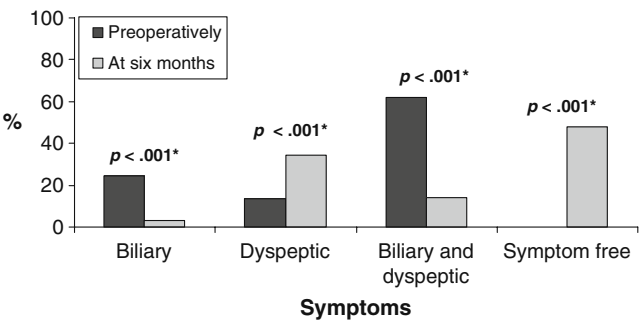

Fig. 2 Report of symptoms preoperatively and at 6 months post-cholecystectomy.

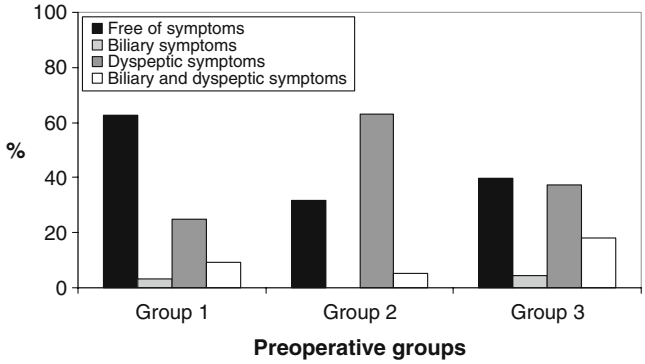

Fig. 3 Report of symptoms at 6 months after cholecystectomy.

ative symptoms (χ^2=25.02, p<.001). Preexisting symptoms persisted most often in group 2 (63.2%), followed by patients in group 3 and group 1 (18.2% and 3.1% of the patients, respectively). Furthermore, preoperative groups differed significantly regarding the development of new symptoms postoperatively (χ^2=11.64, p=.003). Patients in group 2 less often developed new symptoms after cholecystectomy than patient in the other groups (5.3% vs. 37.8% (χ^2=6.52, p=.011)). Patients in group 3 developed symptoms of another category more often than patients in the other groups (42.0% vs. 24.3% (χ^2=4.87, p=.027)). In fact, 4.5% and 37.5% of all patients in group 3, reported only postoperative biliary or only postoperative dyspeptic symptoms, respectively, which implicates that preoperative biliary symptoms subsided more often. Preoperative groups did not differ with regard to the report of postoperative non-specific symptoms.

Fig. 3 gives an overview of postoperative symptoms at 6 months for each group. Within each group, the number of patients with the prominent preoperative symptom decreased. In group 1, a significant number of patients developed a different pattern of complaints with dyspeptic symptoms only (p=.005). Furthermore, over 6 months time, the number of patients with non-specific symptoms decreased within group 1 (p=.002) (Fig. 4). In contrast, in group 2 the number of patients with non-specific symptoms did not decrease. In group 3, a decrease of patients with non-specific symptoms (p<.001) and a change to individual biliary (p=.046) or dyspeptic symptoms (p<.001) was observed at 6 months.

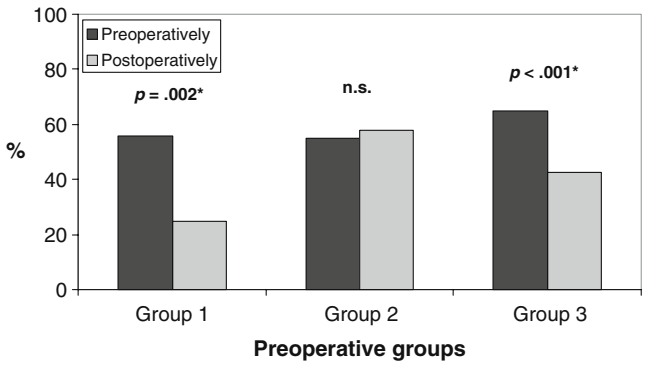

Fig. 4 Report of non-specific symptoms over 6 months time.

Predictors of 6 Months Outcome

Univariate logistic regression analyses were performed to identify predictors of the report of (any) symptoms at 6 months. These results are shown in Table 3. Multivariate regression analyses demonstrated that the postoperative report of biliary and/or dyspeptic symptoms was predicted by preoperative non-specific symptoms only (OR=3.06, p=.003; 95% CI: 1.46–6.42). When groups of patients were investigated separately, preoperative non-specific symptoms were the only predictor of any postoperative symptom in group 1 (OR=6.11, p=.043; 95% CI: 1.06–35.35) and group 2 (OR=4.53, p=.002; 95% CI: 1.77–11.60). No predictors were identified for the report of any postoperative symptom in group 3.

Predictors of persistence of existing symptoms were investigated by univariate logistic regression analyses (see Table 4). Multivariate logistic regression analysis demonstrated that the use of psychotropic medication (OR=5.27, p=.010; 95% CI: 1.50–18.55) and the preoperative report of dyspeptic symptoms only (OR=16.69, p<.001; 95% CI: 4.59–60.68) were independent predictors of persisting symptoms. Univariate and multivariate multinominal regression analyses were performed to identify predictors of postoperative biliary symptoms, dyspeptic symptoms and a combination of biliary and dyspeptic symptoms. Results of univariate regression analyses are shown in Table 5. Entering these predictors simultaneously in a multivariate multinominal regression analysis demonstrated that postoperative biliary symptoms were predicted by HTA only, whereas postoperative dyspeptic symptoms were independently predicted by preoperative dyspeptic and non-specific symptoms. Non-specific symptoms and the use of psychotropic medication predicted a postoperative course with both biliary and dyspeptic symptoms (see Table 6).

Discussion

The results of cholecystectomy are disappointing for many patients. Currently, the indication of an elective cholecystectomy is such that many patients are operated without critical consideration of individual expectations and risks. At 6 months after cholecystectomy, merely 47.8% of the patients were symptom free. In addition, 17.9% and 34.0% of the patients reported persistence and emergence of new symptoms, respectively. As it concerns a substantial number

Table 3 Univariate Predictors of Symptom Report at 6 Months after Cholecystectomy

Preoperative characteristic	OR	95% *CI*	*p* value
Psychotropic medication	3.44	1.07–11.04	.038*
Non-specific symptoms	2.85	1.49–5.45	.002*
Combination of biliary and dyspeptic symptoms	2.10	1.12–3.94	.021*
HTA	2.89	1.23–6.79	.015*
Neurologic comorbidity	4.17	1.13–15.38	.032*

Significant results are reported only

*$p<.050$ indicated significance

of patients, preoperative recognition of patients with an increased risk of negative outcome is essential to optimise the management of cholelithiasis. The present study demonstrated that patients with preoperative dyspeptic symptoms and patients using psychotropic medication are both at risk of persistence of the preexisting pattern of health complaints after cholecystectomy. Furthermore, patients with non-specific symptoms and patients using psychotropic medication are at risk of the experience of biliary and/or dyspeptic symptoms at 6 months. Patients with High Trait Anxiety (HTA) have a ten times greater chance to experience specifically postoperative biliary symptoms.

The current study investigated 6-months outcomes, as the greatest improvement after cholecystectomy is seen within this time period.[25] Overall, comparability of studies focussing at approximately 6 months is limited,[10,13,15,26] because of differences in criteria for inclusion, designs, classification, definition and operationalisation of outcomes and variability in moments of follow-up. The percentage of patients who are free of symptoms or report persisting symptoms is comparable to results of other studies focussing at 6 months.[10,13,15] The percentage of patients developing new symptoms (34.0%) is higher than percentages found in other studies (1.7–24.5%),[10,13,15,26] which may be attributed to patients in group 3 who recover partially and report only biliary or dyspeptic symptoms after cholecystectomy.

In the current study, dyspeptic symptoms persisted more often than biliary symptoms, which is in line with other studies focussing at 6 months after cholecystectomy.[13,26] Differentiating between patient groups showed that patients with only dyspeptic symptoms have the worst prognosis: dyspeptic symptoms often tended to persist (63.2%) over 6 months time. Furthermore, in patients with a combined symptom profile dyspeptic symptoms tend to persist [15] and dyspeptic symptoms developed in 25% of the patients with preoperative biliary symptoms. The latter findings support a shift towards dyspeptic symptomatology at 6 months, as mentioned in previous studies.[13,15] Reasons for this shift may be related to preexistent gastrointestinal symptoms,[26] postoperative changes in duodenogastric reflux,[26] retained stones or the formation of new gallstones,[27] or psychologic variables such as HTA.

We found that patients with preoperative non-specific symptoms are also at risk for any postoperative dyspeptic or combined dyspeptic and biliary symptoms. The cause of these non-specific symptoms is unclear; these symptoms may coincide with biliary, dyspeptic or other health symptoms, comorbid conditions or may contain a more subjective component, influenced by psychological variables, such as depressive symptomatology or personality. Weinert et al. [13] demonstrated that a subjective measure, namely self-rated preoperative health status (SF 36), predicts outcomes at 6 months after cholecystectomy. However, the SF-36 consists of items such as bodily pain which could be directly related to biliary and dyspeptic symptoms. Therefore, we believe that a parsimonious list of non-specific symptoms may be more precise and better differentiates general symptoms from pure

Table 4 Univariate Predictors of Category of Postoperative Symptoms

Postoperative outcome	Preoperative predictor	*OR*	95% CI	*p* value
Persistence of preoperative symptoms	Dyspeptic symptoms only	12.71	4.40–36.70	<.001*
	HTA	2.89	1.16–7.18	.023*
	Number of non-specific symptoms	1.50	1.02–2.20	.041*
	Psychotropic medication	5.51	1.90–15.96	.002*
	Abdominal comorbidity	2.78	1.16–6.63	.022*

Significant results are reported only

*$p<.050$ indicated significanc

Table 5 Univariate Predictors of Category of Postoperative Symptoms

Postoperative outcome	Preoperative predictor	OR	95% CI	*p* Value
Biliary symptoms only	HTA	12.19	1.76–84.30	.011*
Dyspeptic symptoms only	Dyspeptic symptoms only	3.23	1.13–9.22	.029*
	Neurologic comorbidity	4.72	1.22–18.35	.025*
	Non-specific symptoms	2.11	1.04–4.26	.038*
Biliary and dyspeptic symptoms	Psychotropic medication	9.73	2.59–36.53	.001*
	HTA	5.00	1.59–15.74	.006*
	Biliary and dyspeptic symptoms	2.74	1.01–7.42	.047*
	Non-specific symptoms	8.01	2.19–29.33	.002*

Significant results are reported only

*$p<.050$ indicated significance

biliary and dyspeptic symptoms. Although preliminary results suggest that patients with non-specific symptoms are at risk of negative postcholecystectomy outcomes, further research needs to corroborate this finding and to address the issue of subjectivity.

As persistent biliary symptoms are decisive for the subjective perception of an unsuccessful procedure,[13] special attention needs to be paid to patients with HTA, who are at risk to report persisting biliary symptoms at 6 months postcholecystectomy. So far, HTA has been identified as a possible predictor of short-term postcholecystectomy outcomes.[19,28] Patients with HTA are predisposed to react with heightened anxiety to threatening situations,[29] and may misinterpret a broad range of gastrointestinal symptoms as being of biliary nature. Misinterpretations may be mediated by the fact that HTA patients have higher pain sensitivity [30] and experience gastrointestinal symptoms more often than other patients.[31] Otherwise, because of these characteristics, HTA patients may be misdiagnosed as suffering from cholelithiasis and abusively be subjected to cholecystectomy. Consequently these patients will report persistence of preexisting symptoms. Possibly, psychosomatic mechanisms typically for HTA patients, such as heightened activity of the sympathetic nervous system,[32] production of higher levels of noradrenaline,[33] may influence digestion, wound healing or other bodily processes, which could be related to the experience of biliary-type colics after removal of the gallbladder. The role of HTA on long-term postcholecystectomy outcomes and underlying mechanisms should be further corroborated in future research.

The present study has several strengths, such as the prospective design and the participation of consecutive patients, which prevents a selection bias. In contrast to other studies,[10,13,15,26] we used strict criteria for inclusion of patients, such as symptomatic cholelithiasis, ASA I and II, limited age differences, indicated for elective cholecystectomy only, and used a big sample size ($n=172$). Follow-up was limited to the period of 6 to 9 months postoperatively. This gives a homogenous sample, which enables strong conclusions which are applicable to the field of action where critical consideration matters most: elective surgery. Another advantage of the study is the fact that the clinical presentation of preoperative symptoms was used as a basis for further investigation of predictors, which enlarges the specificity of the predictors and enables the clinical application of the results. The fact that we dichotomised many variables (yes/no) enhances the convenience to apply our findings directly in surgical practice. Alternatively, it entails the risk over oversimplification of our conclusions.

Other limitations of the study are the fact that we used a self-constructed symptom checklist, instead of a standardised gastrointestinal questionnaire. We found it legitimate to use such a checklist, because we carefully based our symptom checklist on information from focus groups,[22] other symptom checklists, and clinical experience, and currently no disease specific symptom checklist exists for cholelithiasis. The fact that we used broad symptom categories, according to a leading article of Weinert et al.,[13] increased the comparability to this study in particular. However, as categorising symptoms into

Table 6 Independent Predictors of Postoperative Symptoms (Based on Multivariate Multinominal Regression Analyses)

Postoperative symptoms	Preoperative predictor	OR	95% CI	*p* Value
Biliary symptoms	HTA	10.64	1.24–90.96	.031*
Dyspeptic symptoms	Dyspeptic symptoms only	5.69	1.50–21.63	.011*
	Non-specific symptoms	2.50	1.10–5.64	.028*
Biliary and dyspeptic symptoms	Non-specific symptoms	9.53	1.86–48.92	.007*
	Use of psychotropic medication	8.01	1.75–36.75	.007*

Significant results are reported only

*$p<.050$ indicated significance

biliary or dyspeptic symptoms is highly arbitrarily, comparability with other studies might be hampered. Given the ambiguous clinical presentation of cholelithiasis and the vague diagnostic criteria, classification of symptoms as being purely biliary or dyspeptic in nature seems to be extremely difficult. Furthermore, it should be noted that symptomatic outcome is not equivalent to broad outcome measures such as health status or quality of life, which can be easily assessed by standardised questionnaires. We recommend that future studies should investigate postcholecystectomy quality of life, especially in relation to symptomatic outcome and psychological predictors. Moreover, this study has the disadvantage that we have two separate measures, at baseline and at follow-up at 6 months. Therefore the study does not differentiate between the persistence of symptoms and the development of symptoms after initial disappearance after cholecystectomy. Comparing findings shortly after cholecystectomy (e.g. 6 weeks) with findings at 6 months may convey the course of symptoms over time. Furthermore, in the current study we did not control for confounding postoperative variables, such as comorbid diseases and major life events. Therefore, results at 6 months may be influenced by other factors than the recovery after cholecystectomy.

Implications

The results of the present study may have implications for the management of symptomatic cholelithiasis. Surgeons must be aware that less than half of their patients are free of symptoms at 6 months after cholecystectomy. Therefore, patients should be informed about the considerable risk of persistent and developing symptoms at 6 months. Generally, cholecystectomy is used to prevent further episodes of biliary colics and complications,[8] whereas expectative management is often disregarded. The use of ursodeoxycholic acids (in combination with lithotripsy) remains a matter of debate.[34] If treated expectatively, the risk of complications such as acute cholecystitis, acute pancreatitis, or biliary duct obstruction is small (1–2% a year) [35,36] and biliary pain recurs only in 31% of the patients with previous biliary attacks >1 year.[37] Most interesting is that the rate of complications, improvements of quality of life, and reduced pain over a period of 5 years are equal for expectative management and cholecystectomy. Starting expectatively, only 23–49% of the patients underwent cholecystectomy because of recurred pain and complications within 2.5–5 years.[25,38] As expectative management is safe and offers good prospectives, it should be preferred as treatment to start with in cholelithiasis especially in patients with a high risk of postoperative symptoms.

Recognition of high-risk patients is crucial to gear the treatment to the patient's characteristics. In patients with classical biliary symptoms (alone or together with dyspeptic symptoms), surgeons should be attentive to non-specific symptoms during anamnesis. In patients using psychotropic medication and patients reporting dyspeptic symptoms only, wait-and-see should be considered as the treatment of choice. Limiting our recommendations to the persistence of biliary symptoms, which are most indicative of an unsuccessful procedure, patients with HTA should be informed about their heightened risk. Furthermore, in HTA patients, the biliary or dyspeptic nature of preoperative symptoms should be addressed carefully. Expectative management should be considered especially in HTA patients with dyspeptic symptoms or using psychotropic medication. Alternatively, psychotherapeutic interventions stemming from cognitive behavioural therapy or mindfulness may teach patients how to deal with anxiety provoking situations and may reduce stress in HTA patients. Consequently, the negative influence of HTA may be reduced and postoperative symptomatic outcomes may be improved. Summarised, in cholelithiasis, surgeons should offer patients a treatment which is based on individual risks and expectations. Hopefully, the rate of unsuccessful treatments will be reduced, which will lead to greater cost-efficiency in health care.

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ORIGINAL ARTICLE

'Critical View of Safety' as an Alternative to Routine Intraoperative Cholangiography During Laparoscopic Cholecystectomy for Acute Biliary Pathology

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Abstract
Introduction The study aims to evaluate the use of "critical view of safety" (CVS) for the prevention of bile duct injuries during laparoscopic cholecystectomy for acute biliary pathology as an alternative to routine intraoperative cholangiography (IOC).
Methods A policy of routine CVS to identify biliary anatomy and selective IOC for patients suspected to have common bile duct (CBD) stone was adopted. Receiver operator curves (ROCs) were used to identify cutoff values predicting CBD stones.
Results Four hundred forty-seven consecutive, same admission laparoscopic cholecystectomies performed between August 2004 and July 2007 were reviewed. CVS was achieved in 388 (87%) patients. Where CVS was not possible, the operation was completed open. CBD stones were identified in 22/57 patients who underwent selective IOC. Preoperative liver function and CBD diameter were significantly higher in those with CBD stones ($P<.001$). ROC curve analysis identified preoperative cutoff values of bilirubin (35 μmol/L), alkaline phosphatase (250 IU/L), alanine aminotransferase (240 IU/L), and a CBD diameter of 10 mm, as predictive of CBD stones. No bile duct injuries occurred in this series.
Conclusion In acute biliary pathology, the use of CVS helps clarify the anatomy of Calot's triangle and is a suitable alternative to routine IOC. Selective cholangiography should be employed when preoperative liver function and CBD diameter are above defined thresholds.

Keywords Critical view of safety · Routine intraoperative cholangiography · Laparoscopic cholecystectomy

Introduction

Bile duct injury is the most serious complication that can occur during laparoscopic cholecystectomy. It is associated with significant morbidity, affecting quality of life, incurring high costs and sometimes litigation.[1,2] Factors considered important in minimising the incidence of bile duct injury include surgical skill and experience, as well as the use of intraoperative cholangiography (IOC) and more recently achieving a critical view of safety (CVS) before clipping and transecting the cystic duct.[3] CVS was first described by Strasberg et al.[3] in 1995. The technique is based on the principle that the Calot's triangle must be dissected free of fat, fibrous, and areolar tissue, with the lower end of the gallbladder dissected off the liver bed. At this point, there should be only two tubular structures (cystic duct and artery) entering the gallbladder directly from the hepatoduodenal ligament, with the surface of the liver bed clearly visible. This confirms absence of abnormal regional anatomy and reduces the risk of common bile duct (CBD) injury.

Large-scale epidemiological studies and several meta-analyses have shown the beneficial effect of IOC in reducing bile duct injury.[4,5] Nevertheless, the routine use of IOC during laparoscopic cholecystectomy is controversial, with opponents citing unnecessary biliary instrumentation with its own inherent morbidity and mortality[6] While CVS is increasingly employed,[7,8] there are no data available regarding its role during laparoscopic cholecys-

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tectomy for acute biliary pathology, as an alternative to routine IOC. The aim of the study was to investigate the role of CVS during laparoscopy and identify "cutoff" values for bilirubin, alkaline phosphatase, alanine transferase, and CBD dilatation, which could held predict choledocholithiasis in patients presenting with acute biliary pathology.

Materials and Methods

All same admission laparoscopic cholecystectomies performed during a 3-year period (August 2004 to July 2007) were reviewed. Patients admitted to the emergency general surgical unit at Ninewells Hospital, Dundee, Scotland, with acute gallbladder pathology (biliary colic, acute cholecystitis, and chronic cholecystitis) were considered for surgery. Cholelithiasis was confirmed by ultrasound examination in all patients. A dedicated emergency theatre was available for acute general surgery, allowing all patients to be operated on within 96 h of admission. All the surgical procedures were performed by trainees and consultants in the department of Hepato-Billiary and Upper Gastrointestinal Surgery.

A CVS was routinely attempted prior to clipping and transection of the cystic duct. The operative technique followed Strasberg's description and involved clearing Calot's triangle completely of fat and fibrous tissue, with dissection of the neck and proximal body of gallbladder from its fossa. This allowed identification of the cystic duct and artery entering the gallbladder from the hepatoduodenal ligament (Fig. 1). At this point, the cystic artery and duct were clipped and transected in order, followed by completion cholecystectomy. Where a severely inflamed Calot's triangle prevented CVS being achieved, a subtotal cholecystectomy was attempted laparoscopically. The anterior gallbladder wall was resected, allowing removal of all stones and subsequent placement of a large nonsuction drain to Hartmann's pouch. No attempt was made to ligate the cystic duct during subtotal cholecystectomy. On occasion, a retrograde open cholecystectomy with ligation of cystic duct and artery was performed, as an alternative to subtotal cholecystectomy.

IOC was performed selectively with its use restricted to patients in whom deranged liver function tests or a dilated CBD were noted on preoperative ultrasound scan. The choice of performing a preoperative magnetic resonance cholangiopancreatography (MRCP) and a subsequent endoscopic retrograde cholangiopancreatography (ERCP) in these patients was in accordance with the operating surgeon's preference.

Data regarding demographics, indication for surgery, CBD diameter, use of CVS, indication for IOC, conversion rates, reasons for conversion, and final histology were obtained from a prospectively maintained database. In addition, the cutoff levels of bilirubin (normal, 0–15 μmol/L), alkaline phosphatase (normal, 20–80 U/L), alanine aminotransferase (ALT) (normal, 12–40 U/L), and CBD diameter that predicted the presence of ductal stones were identified using receiver operator curve (ROC) analysis.

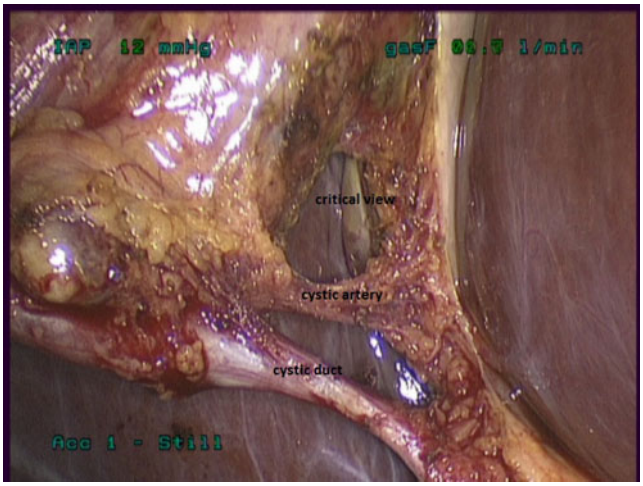

Fig. 1 The critical view of safety.

The patients were followed up for a 1-year period to assess readmission with retained CBD stones.

Statistical Methods

Statistical analyses were performed using SPSS® 14.0 statistical software (SPSS, Chicago, IL, USA). The data were analysed using the nonparametric Kruskal–Wallis test. A two-tailed Fisher exact test was employed for qualitative data. $P<.05$ was considered statistically significant. ROCs were constructed to aid prediction of CBD stones. This statistical approach is typically used to compare the diagnostic performance of two or more laboratory diagnostic tests.[9] In an ROC curve, the sensitivity rate is plotted as a function of the 100-specificity for different cutoff points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. The closer the ROC plot to the upper left corner, the higher the overall accuracy of the test.[9]

Results

Four hundred forty-seven laparoscopic cholecystectomies were performed during the study period. The mean age was 52 years. The male-to-female ratio was 1:2.5. The mean time from admission to surgery was 3 days, and the mean operating time was 122 min. Sixty-six percent (n=295) of procedures were performed by senior trainees.

Pathology

Two hundred sixty (58%) of the 447 patients had histologically proven chronic cholecystitis, 7 (2%) patients had biliary colic, and 180 (40%) patients had clinicopathological evidence of acute cholecystitis. One patient had a gallbladder carcinoma on histology.

Critical View of Safety

A CVS was achieved in 388/447 patients (87%). Three hundred seventy-eight patients subsequently underwent an uneventful laparoscopic cholecystectomy. Nine patients were found to have CBD stones after selective IOC and underwent open CBD exploration to achieve clearance. One patient had simultaneous open cholecystectomy and cystgastrostomy for a pancreatic pseudocyst.

In the remaining 59 patients (13%), a CVS technique of ductal identification was used, but CVS could not be achieved due to severe inflammation in Calot's triangle, which prevented clear anatomical delineation of the area. In 12 of these patients, the operative approach was changed to a laparoscopic retrograde subtotal cholecystectomy and the remaining 47 patients converted to open cholecystectomy. There were no reported bile duct injuries or postoperative bile leaks in the study group.

Conversions

While the overall conversion rate was 11%, it was significantly higher in those with histologically proven acute cholecystitis compared with chronic cholecystitis (18% vs. 8% $P<.001$). The reasons for conversion were (i) a thickened and inflamed Calot's triangle ($n=47$), (ii) CBD stones ($n=9$) where patients underwent open CBD exploration, and (iii) need for simultaneous cystgastrostomy ($n=1$).

IOC and CBD Stones

Fifty-seven patients had an IOC during cholecystectomy. The indications were a preoperative dilated CBD alone (72%; $n=41$; median, 9.5 mm; range, 7–20 mm), deranged liver function tests (LFTs) alone (28%, $n=16$), or a combination of dilated CBD and deranged LFTs (39%, $n=22$).

Overall, 22 (5%) patients were found to have CBD stones. Seven were identified on preoperative MRCP and underwent an ERCP with stone extraction. Fifteen were found to have stones in the CBD during IOC. Of these, three patients subsequently underwent laparoscopic CBD exploration and retrieval of stones, nine patients underwent open CBD exploration, and the remaining three patients underwent a postoperative ERCP with stone retrieval.

There was no difference in the incidence of CBD stones between patients aged over 55 years (13/227, 6%) and those less than 55 years (9/220, 4%) ($P=.5$). The mean bilirubin, alkaline phosphatase, ALT, and CBD diameter in patients with CBD stones was higher than those without CBD stones (Table 1). Statistical analysis using ROC curves (Fig. 2) identified cutoff values of 35 μmol/L for bilirubin, 250 U/L for alkaline phosphatase, 240 U/L for ALT, and 10 mm of CBD diameter, for predicting presence of CBD stones (Table 2). There were no readmissions noted during a 1-year follow-up period with retained stones in the current series.

Discussion

CVS is increasingly employed as a method of minimising the incidence of CBD injury during elective laparoscopic cholecystectomy.[7] However, there is a paucity of data regarding its role in acute biliary pathology and inflammation of Calot's triangle. The present study assessed the role of CVS in patients undergoing same admission laparoscopic cholecystectomy for acute cholecystitis and showed that the technique was feasible in the majority of patients. In addition to clarifying the anatomy of Calot's triangle, it appeared to obviate the need for routine IOC. In this study, IOC was used selectively for patients with deranged liver function and/or a dilated CBD.

While there was no evidence of CBD injury in the study group, it remains a serious complication of laparoscopic cholecystectomy, with long-term sequelae.[1] Several strategies have been employed to minimise its incidence during elective laparoscopic cholecystectomy, including the infundibular technique,[10] achieving a CVS[3] and the routine use of cholangiography.[4] The infundibular technique identifies the cystic duct by displaying the funnel-like junction of the gallbladder and cystic duct. However, this approach may be perilous in the presence of severe inflammation or when the cystic duct is hidden or effaced by a large stone resulting in a "false infundibulum," and the bile duct is at risk of injury. These conditions are visually deceptive leading to misidentification of anatomical structures and potential injury. As a technique, it is also losing favour and should probably only be used in combination with confirmatory cholangiography. The CVS on the contrary clearly delineates the structures in the Calot's triangle, but when not achieved, it should lead the surgeon to proceed to an open or laparoscopic subtotal cholecystectomy, thereby avoiding a biliary duct injury. In our institution, the infundibular technique is not used, and trainees are routinely encouraged to obtain a CVS prior to clipping the cystic duct and artery. The critical view technique is becoming popular and is now included in European protocols for elective laparoscopic cholecystec-

Table 1 Derangement of Liver Function Tests and CBD Diameter at Admission in the Presence of CBD Stones

	CBD stones	No CBD stones	*P*
Bilirubin (μmol/L)	67 (6–238)	14 (5–48)	<.001
Alkaline phosphatase (U/L)	250 (78–659)	110 (26–420)	<.001
ALT (U/L)	362 (12–1309)	68 (7–852)	.001
CBD diameter (mm)	10 (5–20)	6 (4–20)	<.001

Values are mean (range)

tomy.[11] Avgerinos et al. described its routine use in more than a thousand patients undergoing elective laparoscopic cholecystectomy.[7] No cases of CBD injury were reported in their study group. Similarly, Yegiyants and Collins[8] analysed the role of CVS in 3,000 patients undergoing elective cholecystectomy and reported one bile duct injury, which occurred during dissection of Calot's triangle, prior to achieving CVS. By comparison, the current series extends the use of CVS to patients presenting with acute biliary pathology and includes those with an increased likelihood of inflammatory involvement of Calot's triangle. CVS was achieved in 87% of patients. A policy of transecting the cystic artery before the duct also allowed better display of the triangle boundaries. This approach was supported by Wijsmuller et al.[12] who reported that the surface area of the Calot's triangle increased significantly if the artery was transected before the duct after a critical view was obtained, thereby further optimising the benefit of CVS and reducing the incidence of iatrogenic bile duct injury during cholecystectomy. For patients where the CVS technique was used but CVS was not achieved, a laparoscopic subtotal cholecystectomy or open cholecystectomy was performed. The decision to convert to open cholecystectomy was influenced by a failure to progress with the dissection and therefore an inability to obtain a CVS. Such a policy, we believe, helped minimise the incidence of CBD injury and reemphasises the need to convert on encountering increased operating difficulty during laparoscopic cholecystectomy.[13]

Recent UK data suggest that only a few perform early cholecystectomy for acute gallstone disease.[14] This limits the number of training opportunities available for surgical trainees in the acute setting. In our institution, a structured training programme was incorporated into the surgical curriculum, which allowed a stepwise progression from junior to senior trainee in the performance of same admission laparoscopic cholecystectomies.[15] As a result, 66% of the procedures in the current study were performed by trainees. While there are no UK guidelines regarding the routine use of CVS in this setting, the local policy of routine mandatory CVS for surgical trainees prior to clipping the cystic duct and artery was adopted to good effect.[15]

Several recent studies have tried to evaluate the use of IOC in preventing CBD injury. These have concluded that either routine or selective IOC was acceptable in aiding prevention of CBD injury.[16,17] Randomised trials and prospective studies on the use of cholangiography have also shown that IOC could be applied selectively.[18,19] While individual studies appeared underpowered, Ludwig et al. reported a metaanalysis of 40 case-series comprising 327,523 laparoscopic cholecystectomies. They concluded that routine use of IOC halved the rate of CBD injury.[4] However, the identification and interpretation of anatomy on IOC was subjective, and when unclear, the potential for inadvertent placement of a cholangiocatheter directly into the CBD could cause a CBD injury, rather than avoid it, although complete transection would be avoided. In our study, IOC was used selectively in patients suspected of having CBD stones preoperatively. Focusing IOC use on

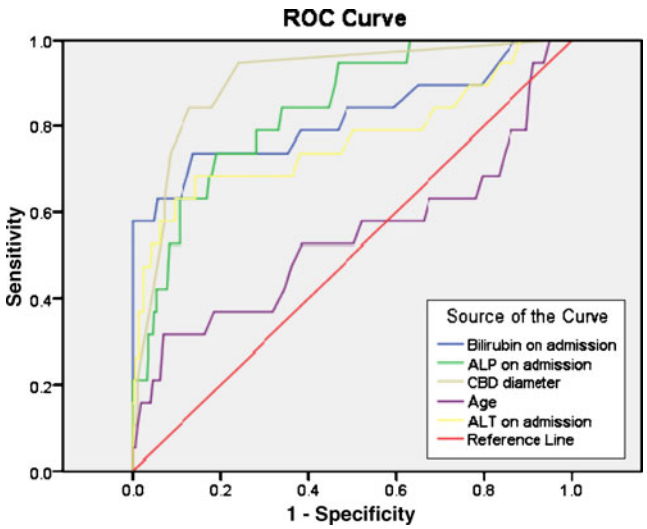

Fig. 2 ROC curves for bilirubin, alkaline phosphatase, CBD diameter, age, and ALT at admission.

Table 2 The Area Under the ROC Curve Predicting CBD Stones for Bilirubin, Alkaline Phosphatase, ALT, and CBD Diameter at Admission

Test variable	SE	*P*
Bilirubin (μmol/L)	0.66	<.001
Alkaline phosphatase (U/L)	0.43	<.001
ALT (U/L)	0.34	<.001
CBD diameter	0.84	<.001
Age	0.72	.56

detecting CBD stones was routinely used during the era of open cholecystectomy, and the present study extended this practice to laparoscopic cholecystectomy.[20] In those patients with acute biliary pathology, liver function is often deranged as a result of the inflammatory process, although it is not clear whether repeat LFT measurements remain useful in determining those patients with acute biliary pathology who are also at high risk of having CBD stones. Several previous studies have measured LFT in patients with symptomatic cholelithiasis undergoing elective cholecystectomy.[21,22] However, few have evaluated their role in acute gallstone disease. Often, results were inconclusive, although routine IOC was advocated.[23,24] The current study successfully identified "cutoff values" of LFT and CBD diameter that were predictive of CBD stones in patients with acute biliary pathology. While MRCP is normally recommended for patients suspected of having CBD stones, it is not always available in the acute setting. Consequently, an elevated bilirubin (>35 μmol/L), alkaline phosphatase (>250 U/L), ALT (>240 U/L), or dilatation of CBD (10 mm) appear predictive of ductal stones.

Conclusion

While the current series was of small number, it is also acknowledged that a large multicentre study, recruiting several thousand patients would be required to statistically demonstrate routine CVS with selective IOC, resulted in a low bile duct injury rate. However, this study has demonstrated that achieving CVS was a feasible and safe alternative to routine IOC in patients presenting with acute biliary pathology. Moreover, the measurement of preoperative liver function and CBD diameter allowed prediction of choledocholithiasis.

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ORIGINAL ARTICLE

Body Mass Index and Adverse Perioperative Outcomes Following Hepatic Resection

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Abstract
Background The effect of obesity on perioperative outcomes following hepatic resection is not clearly defined. We sought to understand the implications of obesity on post-hepatectomy outcomes in a nationally represented cohort of patients.
Methods Using a retrospective cohort design, we studied the effect of obesity on complications and 30-day mortality using multivariable logistic regression using comprehensive clinical data from the American College of Surgeons National Surgical Quality Improvement Program (2005-2008).
Results During our study period, 3,960 patients underwent hepatic resection; 32.4% had a normal body mass index (BMI; 18.5-24.9 kg/m^2), 2.5% were underweight (<18.5 kg/m^2), 33.4% were overweight (25.0-29.9 kg/m^2), and 31.7% were obese (>30.0 kg/m^2). 23.3% had at least one post-operative complication and the overall mortality rate was 2.5%. Compared to normal patients, obese patients had significantly higher unadjusted odds of having a complication (26.5% vs. 21.3%, OR 1.34, 95% CI 1.12-1.61) and dying (3.0% vs. 1.7%, OR 1.79, 95% CI 1.05-3.05). The obese were also more likely to have multiple complications compared to normal BMI patients (6.1% vs. 3.7%, OR 1.70, 95% CI 1.17-2.46). After risk adjustment, obesity was associated with attenuated but significantly higher odds of having any perioperative complication (OR 1.24, 95% CI 1.01-1.55), but was not a significant predictor of mortality.
Conclusions After adjusting for other clinical factors, the degree of obesity is independently associated with an increasing complication rate but not mortality. Risk adjustment may not capture the total clinical risk of patients at the extremes of BMI.

Keywords Body mass index · Liver resection · Hepatectomy · Complications · Outcomes · NSQIP

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Introduction

An important concern in the changing patient landscape of hepatic surgery is the increased recognition of an obesity epidemic in the United States.[1] Of the US population, 65-70% is considered to be overweight or obese,[1] and this proportion is growing. This epidemic has raised significant concerns regarding health outcomes in this population due to the strong association of obesity with cardiovascular disease, dyslipidemia, diabetes mellitus, and other co-morbidities.[2–6] The role of obesity in surgical decision-making has also received considerable attention, with efforts directed at understanding the risk of post-operative morbidity and mortality following major surgery.[2,3,7] These efforts were initiated to help clinicians stratify risk and to counsel patients considering surgery, which can be difficult

given differences between patients, procedural complexity, and the potential direct effects of obesity on the end organ.

The growing utilization of hepatobiliary surgical procedures in a more obese patient population demands that surgeons recognize the complex relationship between obesity and surgical outcomes.[8–11] Several single center studies have demonstrated that obesity has specific implications on the health of the liver, but our understanding of how obesity affects multi-system complications is limited by descriptions with modest sample size.[12–14] In order to help hepatobiliary surgeons make better decisions in the pre-operative setting with regards to patient selection and counseling as well as to properly allocate hospital resources, it is critical that the relationship between obesity and perioperative outcomes be studied using high quality multicenter data.

The American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) offers a unique opportunity to study the relationship between obesity and surgical outcomes following hepatic surgery. This database prospectively collects data on over 30,000 surgical patients in over 200 hospitals in the USA each year. It has detailed data regarding patient perioperative risk and postoperative outcomes. Using the ACS-NSQIP, we have analyzed the outcomes of nearly 4,000 patients who underwent hepatectomy over a four year period to determine how body mass index (BMI) affects postoperative risk following hepatectomy.

Methods

We obtained pooled multi-institutional data from the American College of Surgeons National Surgical Quality Improvement Program from 2005-2008. This program is the only validated and risk-adjusted outcomes based program aimed to improve the delivery of general surgical care in the US[15] These data are collected from several participating centers by trained surgical clinical reviewers at each site, and submitted to the central registry. The data collection includes 136 clinical and demographic variables on pre-operative and intra-operative care, as well as post-operative morbidity and mortality (within 30 days post-operatively). The data collection techniques are continuously monitored for internal and external validity, and inter-rater reliability.[15] This data is available through the ACS-NSQIP website to participating institutions.

The primary exposure variable was BMI, divided categorically in accordance to World Health Organization definitions of obesity, into underweight (BMI<18.5), normal (BMI 18.5-24.9), overweight (BMI 25.0-29.9), and obese (BMI>30).[6] The primary outcome measures were the rates of complications and post-operative 30-day mortality. Complications included deep and organ-space surgical site infections, pneumonia, acute renal failure, myocardial infarction, venous thromboembolism, stroke, post-operative bleeding requiring blood transfusion, septic shock, unplanned intubation, mechanical ventilation (>48 h), and fascial dehiscence.

The principal aim of this analysis was to determine if patient obesity was associated with higher complication and 30-day mortality rates. We compared patient demographics and co-morbidities by obesity class, and calculated unadjusted complication and mortality rates, using Student's t test or chi-square where appropriate. In order to determine the specific risk of obesity, we used logistic regression techniques to determine risk-adjusted complication and mortality rates, accounting for several pre-operative clinical risk factors. These factors included age, gender, race, diagnosis, type of resection, functional status, American Society of Anesthesiology (ASA) preoperative risk classification, and co-morbidity burden.

This project was approved by the University of Michigan Institutional Review Board. All statistical analyses were performed using Stata version 10.0 (StataCorp, College Station, TX, USA). Statistical significance was considered at $p<0.05$.

Results

The clinical and demographic characteristics of 3,960 patients in the study cohort are listed by BMI classification in Table 1. 2.5% were underweight ($n=98$), 32.4% had a normal body mass index ($n=1,284$), 33.4% were overweight ($n=1,323$), and 31.7% were obese ($n=1,255$). Overweight patients were the oldest of the cohort, but the average age of each group ranged from 55 to 60 years. Of the total cohort, 33.5% was male ($n=1,329$). The underweight group had the largest proportion of female individuals. The underweight classification also had a significantly greater proportion of non-white patients at 23.2%. The types of operations differed significantly by BMI classification. Obese patients accounted for 2.7-6.2% more segmental resections than other patients. More than 14% of underweight patients underwent trisegmentectomy compared to approximately 8-10% of the patients in the other groups. Only 1.5% ($n=56$) of patients were coded as having liver tumor ablation (radiofrequency, chemical, or cryo-ablation) prior to liver resection. Obese patients had a slightly higher rate of ablation compared to the other groups, but this was not significant. With regards to co-morbidities, statistically significant differences among the four BMI classifications were observed with regards to hypertension, diabetes, active smoking, dialysis dependence, recent weight loss, and ASA class. Obese patients

Table 1 Preoperative Characteristics of 3,960 Patients who underwent Hepatectomy by Obesity Classification

	Underweight BMI <18.5 (n=98)	Normal BMI 18.5-25 (n=1,284)	Overweight BMI 25-29 (n=1,323)	Obese BMI >30 (n=1,255)	p value
Age (mean ± SD)	55.1±16.0	57.4±14.8	60.0±12.8	57.5±12.8	<0.001
Gender (% male)	21.40%	29.40%	40.20%	31.63%	<0.001
Race (% non-white)	23.20%	16.20%	13.50%	13.70%	<0.017
Type of resection					0.047
Segmental resection (cpt 47120; n=2337)	60.2%	57.4%	56.7%	62.9%	
Right hepatectomy (cpt47130; n=835)	19.4%	21.7%	22.0%	19.6%	
Left hepatectomy (cpt 47125; n=401)	6.1%	10.3%	10.9%	9.4%	
Trisegmentectomy (cpt 47122; n=390)	14.3%	10.6%	10.4%	8.1%	
Pre-operative liver tumor ablation (n=56)	2.6%	3.3%	3.0%	4.3%	0.673
Functional status					<0.001
Independent	90.80%	98.52%	98.95%	97.21%	
Partially dependent	9.20%	1.32%	0.75%	2.23%	
Totally dependent	0%	0.16%	0.30%	0.56%	
Hypertension	24.49%	31.67%	46.26%	61.12%	<0.001
Diabetes	4.08%	6.54%	10.11%	18.57%	<0.001
Active smoker in last year	30.60%	18.90%	13.10%	13.80%	<0.001
Chronic obstructive pulmonary disease	37.76%	37.00%	32.38%	34.34%	0.088
Congestive heart failure	0%	0.39%	0.23%	0.16%	0.644
History of myocardial infarction	31.60%	35.10%	30.87%	31.79%	0.118
Dialysis dependence	3.06%	0.62%	0.23%	0.48%	0.002
Weight loss >10% in last 6 months	43.88%	40.31%	33.81%	33.94%	<0.001
Ascites	2.04%	1.32%	1.13%	1.75%	0.543
Esophageal varices	0.00%	0.23%	0.45%	0.64%	0.413
ASA class ≥4	5.10%	2.65%	3.00%	3.35%	<0.001
MELD score (median; IQR)	7 (6-16)	7 (6-22)	7 (6-24)	7 (6-23)	0.06
Albumin <3.5	18.37%	12.06%	10.57%	12.27%	0.095
Platelet Ct >100	94.62%	97.77%	96.87%	96.87%	0.21
Total bilirubin >2.0	19.39%	12.76%	13.43%	13.15%	0.318

were more likely to be hypertensive and diabetic, but significantly less likely to be active smokers in the last year compared to normal and underweight patients. The underweight patients had a greater propensity toward dialysis dependence, and moribund ASA class. Notably, there were no significant differences between the groups with regards to the various metrics that could be attributed to pre-existing liver disease, specifically ascites and varices.

The overall rate of complications was 23.3%, and the overall mortality rate was 2.5%. Cumulatively, obese patients had the highest unadjusted complication rate (p= 0.0051; Fig. 1). Obese patients had 34% higher odds of having a complication in an unadjusted model (OR 1.34, 95% CI 1.12-1.61), while underweight and overweight patients, respectively, did not have significantly different odds of complications compared to normal BMI patients. The obese group had the highest proportion of patients who had three or more complications (obese 6.1% vs. overweight 4.1% vs. normal 3.7% vs. underweight 5.1%). Obese patients also had greater unadjusted odds of having multiple complications (≥3 complications: OR 1.69, 95% CI 1.17-2.46). While the mortality rate across the BMI distribution was highest in the underweight group, underweight patients did not have significantly higher odds of mortality in the unadjusted regression model (OR 1.34, 95% CI 0.84-2.14), likely related to the small sample size of this group. Only obese patients had significantly higher odds of death following hepatectomy in this model (OR 1.79, 95% CI 1.05-3.05). These models suggest that the baseline morbidity and mortality risk for obese patients was substantial.

With regards to specific complications, on unadjusted analysis, obese patients had significantly higher rates of several complications (Table 2). Superficial surgical site infection and progressive renal insufficiency were greater than two-fold more common among the obese compared to

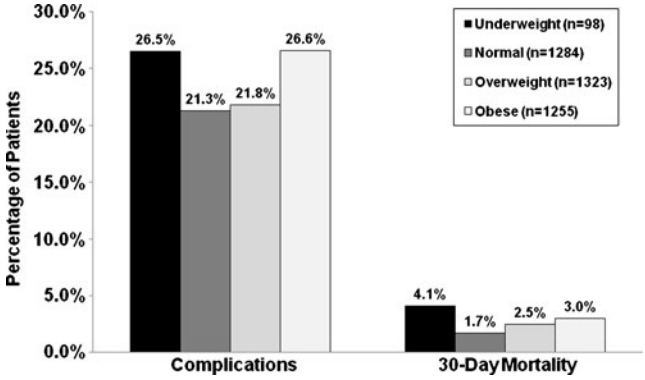

Fig. 1 Unadjusted complication and 30-day mortality rates following hepatectomy by obesity classification. By obesity classification, the frequency of having a complication and mortality follow a U-shaped distribution, respectively. Underweight and obese patients had significantly higher complication rates than normal BMI patients (p=0.0051). The 30-day postoperative mortality rate was higher among underweight, overweight, and obese patients compared to normal BMI patients, respectively.

the normal BMI patients, respectively. Underweight patients had a ten-fold higher risk of stroke compared to normal BMI patients (p=0.014). A greater proportion of obese patients required mechanical ventilation for greater than 48 hours after operation, which approached statistical significance (p=0.051). Underweight patients had a greater proportion of pneumonia, unplanned intubations, acute renal failure, organ space infections, and septic shock than other patients, but these findings did not reach statistical significance.

After adjusting for the clinical co-morbidities in Table 1, the effect of obesity on complication rates was attenuated (Fig. 2). Obese patients had 24% higher risk-adjusted odds of having a complication following hepatectomy compared to normal BMI patients (OR 1.24, 95% CI 1.01-1.55). Underweight patients also trended toward higher complication rates compared to normal BMI patients, but this was not significant (OR 1.17, 95% CI 0.67-2.06). Obese and normal patients had similar odds of having multiple complications as well (≥3 complications, obese vs. normal, OR 1.49, 95% CI 0.97-2.28). With regards to risk-adjusted mortality, as BMI increased, the risk of dying after hepatectomy appeared to increase in a stepwise fashion, but this did not reach statistical significance. Notably, obese patients trended toward an 80% higher risk of postoperative death compared to normal BMI patients (OR 1.83, 95% CI 0.98-3.46, p=0.059), but this risk was abrogated by covariate-adjustment.

We subsequently analyzed the effect of obesity on complications and mortality using the subset of hepatic surgery patients who underwent major resections (n= 1,626). In both the unadjusted and risk-adjusted models, obesity was neither associated with significantly increased complication rates nor mortality rates (Table 3).

Discussion

The growing obesity epidemic in the USA and the expansion of clinical indications for liver surgery over the last decade has greatly changed the population of patients

Table 2 Unadjusted Complication Rates Following Hepatectomy By Obesity Classification

	Underweight BMI <18.5 (n=98; %)	Normal BMI 18.5-25 (n=1,284; %)	Overweight BMI 25-29 (n=1,323; %)	Obese BMI >30 (n=1,255; %)	p value
Superficial surgical site infection	5.10	3.19	4.75	7.57	0.001
Stroke	1.02	0.08	0.60	0.08	0.014
Progressive renal insufficiency	1.02	0.62	0.53	1.59	0.020
Mechanical ventilation >48 h	5.10	3.66	3.92	5.74	0.051
Pneumonia	6.12	2.41	2.19	2.47	0.115
Unplanned intubation	6.12	3.27	3.70	3.67	0.522
Deep vein thrombosis	2.04	1.17	1.21	1.27	0.898
Urinary tract infection	6.12	4.36	3.32	4.86	0.182
Acute renal failure	2.04	1.01	1.58	1.51	0.541
Myocardial infarction	0.00	0.31	0.08	0.48	0.25
Pulmonary embolism	0.00	0.93	1.89	1.51	0.124
Postoperative bleed	0.00	0.93	0.98	0.88	0.801
Deep wound infection	1.02	0.62	0.98	1.27	0.414
Organ space infection	8.16	7.00	6.11	6.85	0.728
Septic shock	3.06	2.18	2.42	2.79	0.768
Fascial dehiscence	1.02	0.78	0.83	1.12	0.815

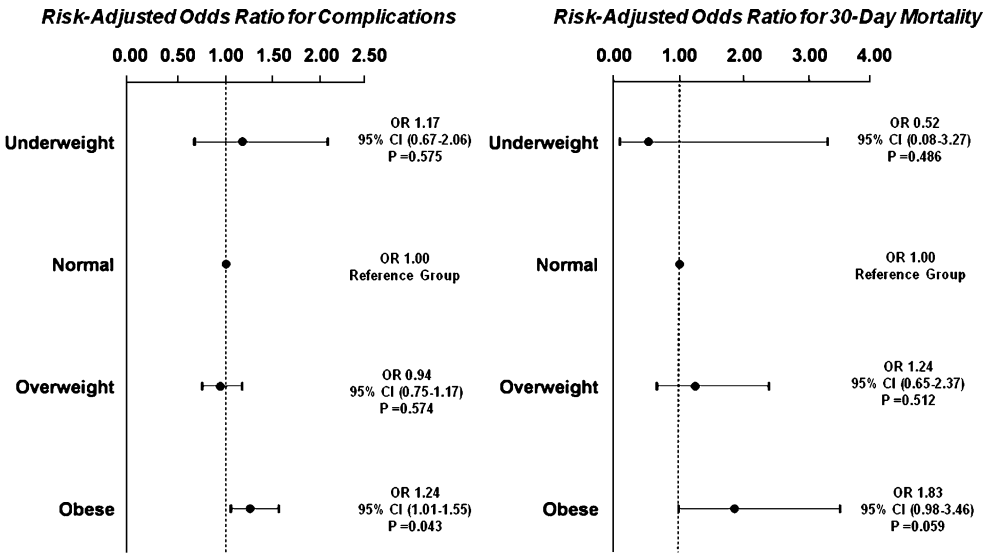

Fig. 2 Risk-adjusted odds of complications and perioperative mortality following hepatectomy by obesity classification. The risk-adjusted odds of complications and mortality are displayed in these Forest plots. Following risk-adjustment, obese patients had significantly higher, but attenuated, odds of having a complication compared to normal patients. Despite displaying higher unadjusted odds toward mortality following hepatectomy, obese patients had similar risk-adjusted mortality risk as normal BMI patients. Notably, the underweight patients did not have significantly different odds of having a complication or death than normal BMI patients after risk adjustment. This is most likely related to the small sample size in the underweight group.

undergoing hepatic resection. With growing utilization of these procedures, greater potential benefit is available to patients, and clinical risks may become redefined. We have attempted to define the risks of perioperative morbidity and mortality following hepatectomy based on BMI using a recent cohort of patients. We found that patients with BMI greater than 30 are at significantly higher risk of post-operative complications and a higher risk of mortality in the subsequent 30 days following the hepatectomy. After risk-adjusting for clinical co-morbidities, however, the potential for complications in the obese declined, and the mortality risk was similar to that of normal patients. These findings have significant clinical implications.

Several authors have identified obesity as a significant risk factor in complex general, oncologic, and vascular surgery procedures.[2–4,12,14,16–19] These studies frame a growing problem in surgery—the operative and perioperative challenges of managing an obese patient with complex surgical needs. These challenges clearly apply to the management of the obese hepatic surgery patient, and we, among others, have identified obesity as a predictor of several perioperative complications including hepatic-specific complications, biliary leaks, urosepsis, pneumonia, acute renal failure, and deep space organ infections.[5,17,20–22] The relationship between obesity and complications in these studies is largely derived from single center retrospective experiences, and has not been validated using multi-center data. The intent of our study was to stratify the independent risk of BMI in a patient population undergoing hepatic resection. Our study cohort is the largest to date with nearly 4,000 patients and represents the pooled outcomes of several centers providing hepatobiliary surgical care in the modern era. Our current findings have the most face validity as they are derived from high quality prospectively collected data specifically obtained to study clinical outcomes.

The persistence of the associations between obesity and perioperative morbidity and, arguably, mortality in our models after risk-adjusting for clinical co-morbidities is an important finding. The risk-adjustment for co-morbidities that may commonly occur in the obese may actually be adjusting away the clinical differences that we care about clinically, which signifies the importance of the magnitude of the effect observed in unadjusted analyses. The absence of any relationship between obesity and adverse outcomes in patients who had major hepatic resections suggests that careful pre-operative selection of even obese patients may not jeopardize reasonable clinical outcomes.

However, other mediators may play a role in this pathway, which may be biological or non-biological. One potential mediator is the degree of hepatic steatosis. Hepatic steatosis is associated both with high BMI and with a propensity for postoperative complications.[14,23] Unfortunately, measurement of hepatic steatosis is reliant on liver biopsy and is wrought with problems with inter-rater reliability,[24] making it difficult to use for surgical decision-making in the pre-operative setting. Further, the collection of data on the extent of hepatic steatosis by clinical registries and administrative databases is sparse at best, making it difficult to assess its

role on population-based outcomes, including this study. In order to elucidate any potential relationship between steatosis, BMI, and surgical outcomes, more robust analyses including both of these variables and outcomes must be performed to better understand this interaction, perhaps using multi-center data specifically collected for hepatobiliary surgical outcome studies.

Another important finding in this analysis relates to the risk at the extremes of body mass index. Prior to risk adjustment, we observed disproportionately high rates of overall and specific complications in the underweight group, who also displayed twofold higher mortality compared to normal patients. The risk adjustment and small sample size likely adjusted away clinically significant risk in this population in the regression models. Previous authors have also described this relationship in patients undergoing major operations for intra-abdominal cancer.[4] These findings are likely related to the frail state of underweight patients, and hepatobiliary surgeons should consider intensive pre-operative evaluation and nutritional support prior to proceeding with hepatic resections. Further, patients at the other extreme of BMI may also be at increased risk. We performed sensitivity analyses to assess the effect of morbid obesity (BMI greater than 35) on these outcomes, but did not observe a relationship. Morbidly obese patients accounted for less than 5% of the entire cohort, and the lack of an observed relationship may be either related to the selection of "healthy" morbidly obese or related to a lack of statistical power.

The findings of our study must be considered in the context of its limitations. One criticism of the ACS-NSQIP data source is the lack of data on procedure-specific complications, including liver failure and encephalopathy. This greatly limited our ability to determine the impact of obesity on liver-specific complications, which is also of great interest. Further, the risk adjustment did not include pre-operative treatments, such as neo-adjuvant chemotherapy, tumor ablation, or cancer stage, which may affect potential outcomes.[25–27] The addition of other pre-operative and post-operative procedures may induce a greater risk for hepatectomy patients, which means better data would improve risk adjustment. Another limitation is related to the non-significant relationship between obesity and mortality in the total cohort, which was attributed to the risk-adjustment schema. We were limited by the data source to examining 30-day mortality but longitudinal data or a 60-day cut point may have been more informative. Next, the association observed in this study may also be affected by variation in surgical and perioperative practice patterns. The ACS-NSQIP does not provide unique center identifiers in its public use data, and our findings could not be adjusted for the differences in perioperative management, patient selection, mortality after complications, and surgical volume that may differ across centers which may ultimately affect variation in postoperative outcomes for this patient population.[28,29]

The significance of the relationship between obesity and perioperative morbidity following hepatic resection must also be considered in the context of healthcare delivery. The association observed was a summative estimate, and the relationship may not hold true for individual patients who are unique. Obese patients can be difficult to manage perioperatively, and there may be considerable variability in the processes of care that may contribute to heterogeneity in outcomes. Further study is required in order to determine the role of center practices on outcomes of hepatobiliary surgery in the obese population, in order to assist in the development of quality improvement initiatives in centers that provide hepatobiliary surgical care.

Table 3 Complication and Mortality Risk Following Major Hepatic Resection by Obesity Classification

	Unadjusted Model			Risk-adjusted model		
	30-Day mortality					
	OR	95% CI	*p* value	OR	95% CI	*p* value
Underweight BMI <18.5 (*n*=39)	2.22	0.483-10.2	0.305	0.26	0.016-4.17	0.339
Normal BMI 18.5-25 (*n*=547)	1.00	Reference	Reference	1.00	Reference	Reference
Overweight BMI 25-29 (*n*=574)	1.56	0.77-3.15	0.214	1.28	0.53-3.11	0.584
Obese BMI>30 (*n*=466)	1.46	0.70-3.07	0.317	1.27	0.51-3.16	0.609
	Complications					
Underweight BMI <18.5 (*n*=39)	1.49	0.76-2.95	0.246	1.25	0.547-2.87	0.594
Normal BMI 18.5-25 (*n*=547)	1.00	Reference	Reference	1.00	Reference	Reference
Overweight BMI 25-29 (*n*=574)	0.92	0.71-1.21	0.581	0.82	0.60-1.12	0.209
Obese BMI>30 (*n*=466)	1.16	0.88-1.52	0.290	1.16	0.85-1.59	0.351

Major hepatectomy included right and left hepatic lobectomy and trisegmentectomy (cpt codes=47122, 47125, 47130). Segmental resections were excluded (cpt code 47120)

Regardless of the effects of centers, ultimately, surgeons bear the responsibility of counseling their patients on the risks of hepatic surgery. Any consideration of the perioperative risks identified in this study must be weighed against reasonable long-term outcomes observed elsewhere in the obese population.[17] Hepatobiliary surgeons should counsel patients in this context, and should focus on improvements in perioperative care that they may affect in order to improve the rate and management of surgical complications. With greater focus on surgical quality improvement in recent years, it is incumbent upon providers performing the most high-risk operations to evaluate practices to optimize clinical outcomes.

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SSAT POSTER PRESENTATION

An MRI-Driven Practice: a New Perspective on MRI for the Evaluation of Adenocarcinoma of the Head of the Pancreas

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Abstract
Objectives The purpose of the study was to describe the MRI-driven management of masses at the head of the pancreas.
Main outcome measures The main outcome measure was tumor resectability.
Methods A retrospective review of prospective radiographic diagnoses was undertaken.
Results Between 2004 and 2008, we have treated 124 patients for a radiographic diagnosis of adenocarcinoma of the head of the pancreas. This diagnosis was correct in 96.0% of the time. MRI was 100% sensitive in determining resectability, 73.2–78.9% specific, and had an overall accuracy of 86.3–87.5%. MRI could detect venous and arterial involvement with 95% and 95.9% accuracy, respectively, and missed only six metastases.
Conclusion MRI is a useful tool in the preoperative imaging of pancreatic head lesions that is highly sensitive and very specific for resectable disease. Prospective trials of MRI in this setting are indicated.

Keywords Pancreas · Adenocarcinoma · Pancreatic cancer · Cancer imaging · MRI · Pancreaticoduodenectomy · Head of the pancreas

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Introduction

Pancreatic cancer famously presents late in its natural history with a 15% chance of being amenable to resection.[1] Some patients, however, arrive in time for a pancreaticoduodenectomy—the Whipple—which offers the only potential cure for malignant lesions in or around the head of the pancreas. For these patients, we strive for an imaging modality that can discern the resectable from unresectable and the benign from malignant. With the emergence of borderline resectable disease as an accepted category,[2] the decision to operate demands raised standards for the resolution and detail of the tumor's advancement vis-à-vis the surrounding vasculature and potential metastasis. At the same time, the fact of post-operative histopathological findings of non-neoplastic disease in 5–14% of patients begs improvement in the diagnosis of malignancy.[3]

The debate over imaging pancreatic lesions is an old one, but it is far from over. The clinician has three major options at his or her disposal: ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). In their exhaustive meta-analysis, Bipat et al found helical CT to be the most sensitive, with no modality more specific than the other.[4] Citing this work, some believe that the role

Table 1 Demographics

	Number	Age	Men	Women
Unresectable	30	61.8±12.3	9	21
Borderline	41	66.0±10.3	20	21
Resectable	53	65.8±11.0	26	27
Overall	124	64.6±11.1	55	69

for MRI in this setting is for further characterization of small (<1 cm) hepatic lesions found on CT.[5] However, Bipat's MRI data are 8–14 years old, and their pooled sensitivity and specificity for respectability by any modality was no greater than 83% and 82%, respectively. Crucially, the fear of missed metastases on CT remains widespread, prompting some institutions to use routine staging laparoscopy.[6] Clearly, we have room to improve. Herein, we revisit the issue of MRI for the evaluation of suspected pancreatic cancer in a busy academic tertiary referral center.

Methods

A retrospective review was undertaken of all MRIs administered for suspected adenocarcinoma of the head of the pancreas at Emory University Hospital in the 4-year period between December 2004 and December 2008. These studies include all cases where MRI was utilized as the primary imaging modality including the entirety of one hepatobiliary surgeon's practice. MRI was used as a policy unless there existed a contraindication. There are no institutional guidelines in regards to the use of diagnostic laparoscopy. Its use is infrequent and varies between providers. No patients who received a pre-operative MRI received a diagnostic laparoscopy. A database was created with all patients who were evaluated and treated for this prospective radiographic diagnosis. Patient, tumor, and radiographic features were reviewed.

The radiographic features were central to the structure of the study. In concert with the surgeons, our radiologists could give one of three diagnoses: unresectable, resectable, and borderline disease. Unresectable disease is defined as the presence of metastases and significant vascular invasion or encasement that is clearly not amenable reconstructive surgery. In this case, the diagnosis of pancreatic cancer is made by either by open biopsies during a palliative procedure, CT-guided biopsy, or endoscopic brushings. Resectable denotes the absence of metastases and vascular involvement. Borderline disease denotes those tumors which significantly abut or compress otherwise patent vasculature (superior mesenteric vein, portal vein, hepatic artery, superior mesenteric artery). All radiology and pathology reports were those of our institution. The gold standard is the histopathological diagnosis. Statistical analysis was univariate, using two-tailed Student's *t* tests for continuous variables.

Results

In the 4-year period under review, 124 patients received a radiographical diagnosis of pancreatic head adenocarcinoma by pre-operative MRI which was classified as either unresectable, borderline, or resectable. One hepatobiliary surgeon (JMS) operated on 114 (92%) of these patients. Basic demographic characteristics are listed in Table 1. While the patients with unresectable disease proceeded to a biopsy in preparation for chemotherapy, the rest were offered the Whipple procedure. The outcomes of the operations performed are listed in Table 2. A basic flow diagram depicts the core outcomes in Fig. 1.

Outcomes and Histopathology

About 75% of patients were offered an operation, most of whom, in turn, accepted. Sixty-five of 94 attempted Whipples proceeded to completion. Twelve patients had positive margins, eight were retroperitoneal, three were

Table 2 Surgical Outcomes

	Resectable	Borderline	Total
Total	53	41	94
Pathology			
Benign	1	3	4
Adenocarcinoma	52	38	90
Whipple procedure			
Offered	53	41	94
Declined	3	5	8
Completed	39	26	65
Positive margin	5	7	12
Positive intraoperative	2	1	3
Negative margin	34	19	53
Node positive	21	16	37
Aborted Whipples			
Procedure			
Hepaticojejunostomy	0	1	1
Double bypass	11	8	19
Exploratory laparotomy	0	1	1
Reasons			
Venous invasion	3	4	7
Arterial invasion	3	3	6
Metastases	5	1	6
Benign pathology	0	2	2

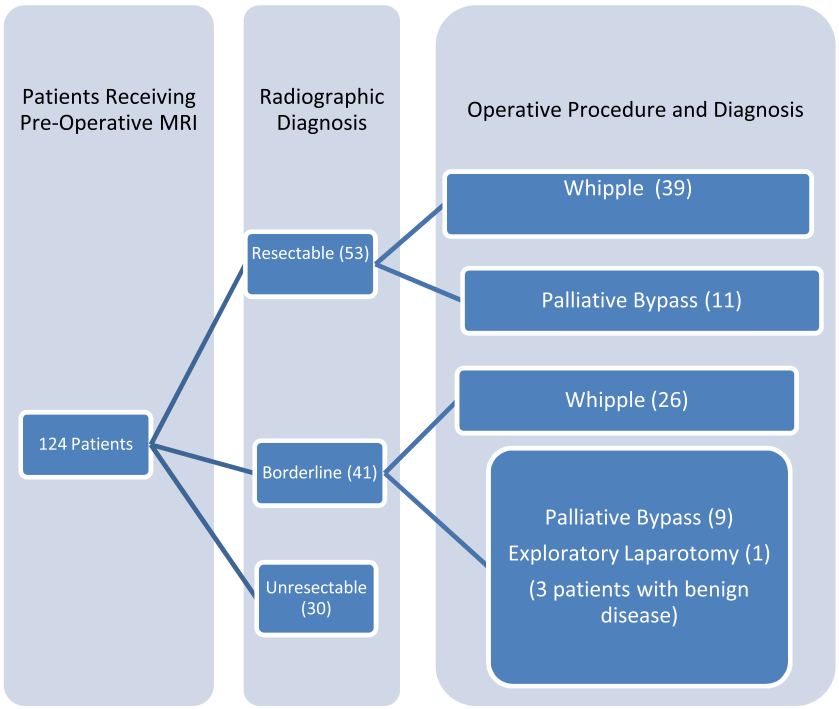

Fig. 1 Radiographic diagnoses and operations received.

known intraoperatively, and one (neck margin) was falsely reported as negative. Two positive margins were at the neck; one was at the bile duct and was positive up to the confluence. Lymph node sampling was positive for adenocarcinoma in 21 of 39 (53.8%) in the resectable group and 16 of 26 (61.5%) in the borderline group. Ultimately, pathology revealed some masses to be benign: Five lesions of the 124 evaluated (4.0%) were benign, while four of the 94 operations offered (4.3%) were for ultimately benign pathology. Of these, four were chronic pancreatitis and one was an arteriovenous malformation. MRI, therefore, correctly predicted adenocarcinoma in 96.0% of cases.

Resectability

Definitively, radiographic resectability communicates both the lack of metastases and vascular involvement that is not amenable to reconstructive surgery. We further divided these data into the categories correspondent to the two radiographical diagnoses offered operations: resectable and borderline resectable. This section will discuss the operative outcomes based on these prospective diagnoses. Beyond two cases of benign pathology (pancreatitis), the reasons for the aborted procedure were venous and arterial invasion as well as metastases (please see Table 2). The statistical analysis derived from these experiences is detailed in Table 3.

In the radiographically resectable group, 11 (22%) patients had truly unresectable disease and received palliative bypasses. Five patients had occult metastases and six patients had inoperable vascular involvement. In this group, six (10.7%) patients had significant venous involvement—three (5.7%) requiring vascular reconstruction and three for whom the Whipple was aborted—and three had hepatic arterial involvement—all of which prompted double bypass procedures—that was not picked up on MRI.

The borderline group is defined by those patients with a degree vascular involvement deemed by the radiologist and surgeon as amenable to operation. Here, venous involvement was correctly predicted 100% (38 out of 38) of the time, and arterial involvement was correctly predicted 93% of the time (26 out of 28). Six patients (14.6%) in this group required vascular reconstruction. One patient in this group had an occult metastasis.

Thirty patients had radiographically unresectable disease and were not offered an operation. The reasons were usually multiple, involving some combination of frank vascular invasion not amenable to reconstructive surgery and hepatic metastases (present in eight patients). All patients then underwent a biopsy in preparation for chemotherapy. Most received a CT-guided needle aspiration, while five patients were diagnosed with open biopsies during palliative operations and one patient was diagnosed with positive bile duct brushings. Only one patient declined a biopsy; at follow-up a year later, the disease process was discovered with follow-up imaging and biopsies to be chronic pancreatitis.

Table 3 Statistical Analysis

	Sensitivity	Specificity	PPV	NPV	Accuracy
Resectability					
Borderline	100 (89.9–100)	78.9 (72–78.9)	76.5 (68.7–76.5)	100 (91.2–100)	87.5 (79.3–87.5)
Resectable	100 (92.9–100)	73.2 (66.4–73.2)	78 (72.4–78)	100 (90.7–100)	86.3 (79.3–86.3)
Overall	100 (95.4–100)	61.2 (55.2–61.2)	77.4 (73.8–77.4)	100 (90.1–100)	83.3 (78.1–83.3)
Arterial involvement					
Borderline	91.3 (91.5–91.3)	100 (95.4–100)	100 (89.2–100)	96.1 (91.6–96.1)	97.2 (90.9–97.2)
Overall	80.8 (70.9–80.8)	100 (96.7–100)	100 (87.8–100)	95 (92.5–95)	95.9 (91.7–95.9)
Venous involvement					
Borderline	100 (95.6–100)	100 (93.7–100)	100 (93.7–100)	100 (95.6–100)	100 (90.5–95)
Overall	87.2 (91.5–87.2)	100 (96.3–100)	100 (93.4–100)	92.4 (82.4–92.4)	95 (90.5–95)

Ninety-five percent confidence intervals in parentheses

PPV positive predictive value, *NPV* negative predictive value

Metastases

The absence of metastases was correctly predicted 93.3% of the time. The six metastases found were in the liver,[3] omentum,[2] and gall bladder.[1] In 21 instances, it was decided to abort the Whipple. These patients, with one exception, received a bypass procedure to palliate their symptoms.

Occult Metastases: Retrospective Analysis of Pre-operative Imaging

Imaging was reviewed for the six patients classified as resectable by MRI that were subsequently found to have extra-pancreatic metastases by surgery. Of these six patients, two clearly demonstrated extra-pancreatic metastases upon retrospective review of the images (one patient with an omental metastasis and a second patient with a liver metastasis). These cases represent an error in image interpretation as opposed to the ability of MRI to detect metastases, and a correct interpretation would have resulted in a conversion of two of these six patients from resectable status to unresectable. The other four patients with metastases identified at surgery (one in the gallbladder, two in the liver, and the fourth patient with an omental metastasis) did not have clear evidence of extra-pancreatic disease demonstrated on MRI upon re-review. Of note, at least two of these lesions (the omental metastasis and gallbladder metastasis) were positioned in such a manner that they likely would also have been missed on laparoscopic examination.

Time to Table

We also looked that the time elapsed between the date of imaging and operation. The results of this review are listed in Table 4. There are small but insignificant differences between the groups that had negative and positive margins, those that were resected and aborted, those that had vascular invasion, but not those that had metastases.

Discussion

We undertook this study for three core reasons. Firstly, we sought to describe the results of an MRI-driven practice to add a fresh perspective to the literature. Secondly, we sought to report the value and consequences of the emerging radiographic diagnosis of borderline resectable disease at our institution. Thirdly, we wanted to find in our data whether laparoscopy has a role in our practice. Accordingly, we have three conclusions: MRI is a powerful ally in the management of pancreatic cancer, patients with borderline disease ought to be treated aggressively, and given the low incidence of missed metastases, the role for laparoscopy in an MRI-driven practice requires further study but is likely to have a marginal impact. Beyond being the newest data on MRI in almost 8 years, our series is the

Table 4 MRI to Operation in Days

	Resectable	Borderline
All	7.8 (6.4)	10 (9.9)
Resected	7.7 (6.8)	8.3 (9.3)
Negative margin	7.4 (7.1)	7.2 (7.0)
Positive margin	9.2 (3.9)	12.9 (14.6)
Aborted	8.1 (5.2)	13.8 (10.0)
Metastases	7.0 (4.1)	25 (0.0)
Venous invasion	9.7 (7.0)	12.3 (8.4)
Arterial invasion	8.3 (6.8)	13 (15.1)

Standard deviations in parentheses

largest in the English language and the first on MRI to delineate outcomes for borderline resectable disease as well as the specific sensitivities for vascular invasion.

Facing any diagnostic test is the dual burdens of sensitivity and specificity. In practice, this means providing the surgeon with detailed and accurate information about the regional involvement of the tumor, the presence or absence of metastases, and whether the tumor is malignant. For us, a radiographic diagnosis of adenocarcinoma had a positive predictive value of 96.0%. Only 4% of our patients had benign pathology, a rate that is one of the lowest reported.[3] The resectability vis-à-vis the surrounding vasculature was predicted with greater than 95% accuracy. And the absence of metastases was predicted with 93.7% accuracy. By comparison, in one of the largest series on pancreatic neoplasms, CT missed between 8.4% and 14% of metastases.[6] It is a widely held belief that the principle advantage of MRI over CT is the greater capacity for the detection of metastases.[5] We can verify that advantage, and we have found others.

The greatest advantage of MRI is its enhanced soft tissue contrast and therefore higher sensitivity for resectable disease. In general, we want to give our patients the best shot at a curative operation. At the same time, our institution strongly supports open surgical palliation as an option for our patients should their tumor turn out to be ineligible for the Whipple. Accordingly, our specificity for resectable disease was 61.2%. On the other hand, our sensitivity for resectable disease was 100%. The impact is clear. Of the cohort examined, all patients with potentially resectable disease were offered an operation. Sensitivities of up to 100% using MRI have been reported previously.[4,7–10] Yet the majority of the published literature is related to the use of CT,[3–6,11,12] from which it is common knowledge that CT is the modality of choice. The data on MRI, however, are from small series and getting old. We believe that there is a reason to continue investigating MRI in this setting. Moreover, with the emergence of borderline resectability as an accepted diagnosis[2,13] and the continued use of staging laparoscopy,[6,14,15] the stakes have been raised on our imaging modalities. The exceptional soft tissue contrast of MRI and proven ability to find even small metastases ought to re-focus our attention.

Our experience also confirms that the radiographic diagnosis of borderline resectability is a meaningful one. Compared to the resectable group, borderline resectable patients were more likely to have positive margins (26.9% vs. 12.8%), aborted Whipples (38.5% vs. 28.0%) and vascular reconstructive surgery (14.6% vs. 5.7%). It has been shown, persuasively, that there is an advantage to treating this group aggressively, with neoadjuvant chemotherapy followed by a resection.[2] Our study did not include those treated with neoadjuvant therapy. As we have shown, the majority of patients with borderline resectable disease can be treated successfully in the operating room. Still, further studies should compare life expectancy with and without neoadjuvant therapy.

Presently, there much debate about the role of laparoscopy for evaluating the resectability of pancreatic adenocarcinoma.[6,14–16] The central issue here is whether our imaging modalities are missing enough metastases to prompt a laparoscopic evaluation before ultimately committing to a laparotomy. Our experience with an MRI-driven practice does not, at first glance, support routine laparoscopy. At laparotomy, only six (6.3%) of the patients offered operations had metastases. Moreover, two had omental metastases and one had a lesion in the posterior wall of the gall bladder, lesions which are very difficult to see on laparoscopy. Furthermore, our retrospective review of the imaging yielded two lesions missed by human error. Accordingly, we arrive at a conservative estimate of four (4.3%) of patients who could have been spared a laparotomy by laparoscopy. While it is difficult to infer conclusions about diagnostic laparoscopy from a study that did not directly evaluate that procedure, we have found reason to study its role in an MRI-driven practice. A study is underway to determine the cost-effectiveness of laparoscopy.

Incidental to our primary aims, we also found that there are differences, albeit statistically insignificant, between the time to surgery for our main study groups, as well as between those with and without positive margins and vascular invasion. This finding has two implications. Clearly, a timely operation is crucial when dealing with pancreatic cancer. The difference in time for those who do and do not require reconstructive vascular surgery in our resectable group (10 vs. 3.2 days) is illustrative. Secondly, the reasons for the difference in time to surgery for those with borderline resectable disease ought to be studied. This finding could be due to chance. It could also reflect the depth of investigation and consideration that borderline findings prompt among the healthcare team. Or it could also reflect an unintended psychological impact on the patient who may hesitate in the face of the more guarded prognosis and a daunting operation. Regardless, the time to the operating table ought to be minimized. The impact of this diagnosis should be studied.

Our study has limitations. While the radiographic diagnoses are prospective, this is a fundamentally retrospective review. Secondly, we used different standards for the diagnosis of adenocarcinoma. Three points in our defense: Diagnosis in patients unsuitable for laparotomy was made in the safest and least-invasive fashion, the standards used are sufficient for medical oncologists to start chemotherapy, and the interpretations of both the CT-guided aspirates and surgical specimens were made by the

same pathologists. Thirdly, the evaluation of metastases in the unresectable group is incomplete. Save for those who had open surgery or who had their liver metastases biopsied, there is a non-zero risk of missing lesions in this group that is equal to that described above for the others.

Conclusion

MRI is a powerful ally in the management of this devastating disease, patients with borderline disease ought to be treated aggressively, and the role for laparoscopy ought to be studied but we have reason to doubt its utility in an MRI-driven practice. While further studies, prospective and otherwise, are required to further validate our findings, there is a reason to believe in a large and expanding role for MRI in the evaluation of pancreatic adenocarcinoma.

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2009 SSAT POSTER PRESENTATION

Open Cystogastrostomy, Retroperitoneal Drainage, and G-J Enteral Tube for Complex Pancreatitis-Associated Pseudocyst: 19 Patients with no Recurrence

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Abstract
Introduction Various techniques have been described to achieve definitive resolution of complex acute pancreatitis associated pseudocysts (PACs). Many of these strategies, inclusive of open, minimally invasive, and radiological procedures, are hampered by high recurrence or failed resolution, particularly for PAC near the pancreatic head. The present series describes a multimodal strategy combining a minilaparotomy for anterior gastrostomy for the creation of a stapled posterior cystogastrostomy, placement of an 8F secured silastic tube for intentional formation of a cystogastric fistula tract in combination with gastric drainage, and postduodenal enteral alimentation.
Materials and Methods Using a prospectively maintained hepatobiliary database, patients with complex PAC undergoing the above procedures were identified. PAC location, postoperative length of stay (LOS), and time to start enteral feeding were identified. PAC were assessed by computed tomography (CT) scan prior to operation, 1 month after drainage, and patients with PAC resolution were started on oral diet, with the fistula silastic tube kept in place for an additional month.
Results Over the interval 2003 to 2008, 19 patients were managed with the stated strategy. PACs were located at the pancreatic body/tail in 12 patients, and 7 patients had PAC at the level of the pancreatic head/neck area. In this cohort, prior to surgical drainage, 17/19 patients had undergone failed endoscopic retrograde cholangiopancreatography (ERCP) with decompressive stent placement and 13/19 had a failed percutaneous PAC drainage. There was no perioperative mortality after open surgical drainage. All patients started on jejunal tube feeding 24 h after surgical procedure. Median postoperative LOS was 7 days (4–13). At 1 month, 16/19 (84%) of patients showed complete resolution of the PAC on CT scan and were started on oral diet; 3/19 required additional month for complete resolution. After a mean follow-up of 31 months, there was no PAC recurrences in any of these patients demonstrated on follow-up.
Conclusion The described strategy is safe, efficient, and allows early restoration of enteral feeding with early hospital discharge. High resolution rates and absence of PAC recurrences in this series supports this approach for complex PAC.

Keywords Pancreatitis · Pseudocyst · Surgery · Cystogastrostomy · Outcome

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Introduction

Acute pancreatitis-associated pseudocyst (PAC) is a collection of serous fluid that may contain pancreatic juice as a consequence of inflammatory pancreatitis or ductal leakage that complicates about 10% of acute pancreatitis.[1] Although a watchful expectancy policy can result in resolution for 8% to 84% of uncomplicated PACs,[2,3] complex PAC including those >5 cm, compressing on surrounding structures resulting in gastric outlet or biliary obstruction and infected PAC, require drainage.[3,4]

Classical surgical drainage with either direct or Roux limb drainage though the gold standard carries substantial

morbidity and mortality of 25% and 5%, respectively.[5] Less invasive approaches including laparoscopic internal drainage[6–9] and endoscopic[10–12] and percutaneous drainage[13–15] report success in selected groups of patents. However, most series of laparoscopic drainage lack long-term follow-up[8] and report recurrence rates as high as 8%.[7] For endoscopic drainage, even though complete early resolution is achieved in 81% to 92%, the recurrence rate is as high as 23% with morbidity of 17%.[10–12] Comparable results are also obtained with percutaneous drainage with reported resolution rate of 84%, recurrence rate of 7%, and complication rate of 18%.[13]

Nowadays, the better understanding of the pathophysiology of PAC[16,17] enables the selection of optimal candidates for minimally invasive approaches, with the aim of reduced treatment failure and recurrence. For patients who have failed nonsurgical treatment (endoscopic, percutaneous) and those with PAC directly communicating to the pancreatic duct, surgical management is indicated.[18]

Herein reported is a consecutive series of patients employing a multimodal strategy applied to 19 patients with complex PAC transferred to our tertiary care facility after failure of ERCP and or percutaneous drainage. This open minilaparotomy procedure combines open anterior gastrostomy for the creation of a stapled posterior cystogastrostomy, placement of an 8F secured silastic tube for intentional formation of a cystogastric fistula tract in combination with gastric drainage, and postduodenal enteral alimentation.

Materials and Methods

Using a prospectively maintained hepatobiliary database, patients with complex PAC who had failed or were not candidates for endoscopic or percutaneous drainage, with identified pancreatic duct communication into the PAC and who underwent the combined surgical procedure as described, were identified (Table 1). Over a 5-year period (2003–2008), 19 patients (12 men/7 women) presented with complex PAC meeting the above criteria. The patients' median age was 51 years (35–75), and the etiology of pancreatitis was alcoholic in eight patients, biliary in seven, hypercholesterolemia in two, and ERCP induced in two. Twelve out of the 19 patients had PAC at the level of the pancreatic body and tail, while in the remaining 7 patients, the PAC was located at the pancreatic head–neck area. All patients were transferred to our service from other facilities after treatment failure, inclusive of 17 ERCP with decompressive stent placement and/or transgastric drainage and 13 percutaneous catheter placements. The interval elapsed between the diagnosis of the PAC, and the transfer to our service was <3 months in 12 patients and >3 months in 7 patients.

Table 1 Patient Description

Sex (M/F)	12:7
Age, median (range) (years)	51 (35–75)
Etiology of pancreatitis	Alcohol induced (n=8)
	Biliary (n=7)
	ERCP induced (n=2)
	Hypertriglyceridemia (n=2)
Location of PAC	Body–tail (n=12)
	Head–neck (n=7)
Prior attempt of treatment	ERCP (n=17)
	Percutaneous drainage (n=13)
Time from diagnosis to transfer to our service	<3 months (n=12)
	>3 months (n=7)

Upon transfer, 14/19 patients were on Nil per os (NPO) status, and nutritional repletion was by parenteral alimentation, three patients were on liquid diet, and the last two patients, though on regular diet, experienced intermittent oral intolerance. Fifteen patients were receiving somatostatin analogue, and 17 patients were receiving opioids for pain control. Fourteen patients were febrile with positive blood culture and were started upon admission on broad-spectrum intravenous β-lactam antibiotic as well as antifungal coverage as per protocol. Twelve patients had pancreatic ascites, and one patient had developed venous thromboembolic disease with pulmonary embolism complicated by gastrointestinal bleeding upon anticoagulation. Ten patients had undergone endoscopic placement of a nasojejunal feeding tube; none of which were being utilized for enteral alimentation. Similarly, ten patients were transferred with a nasogastric tube above the gastric outlet obstruction resulting from PAC compression.

On admission to our institution, all patients had an abdominal computed tomography (CT) scan with Intravenous (IV) contrast to assess the morphology of the PAC and define the relationship between the posterior gastric wall and the splenic artery (Fig. 1). The average PAC size was 12 cm (6–23 cm). The average time elapsed between admission to our service, and the open cystogastrostomy procedure was 19 days (5–27 days). Before surgery, all patients were afebrile and electrolyte abnormalities were corrected.

Technique For the surgical procedure, patients were placed in supine position under general endotracheal anesthesia; a left subcostal incision was made. Using a Bookwalter retractor, the abdominal cavity was explored. An anterior longitudinal gastrostomy was made at the widest point of the stomach body at the level of the incisura extending down to the antrum (Fig. 2). A fine needle was introduced through the posterior wall of the stomach into the retroperitoneal fluid collection confirming its location and its direct relationship to the posterior gastric wall. The

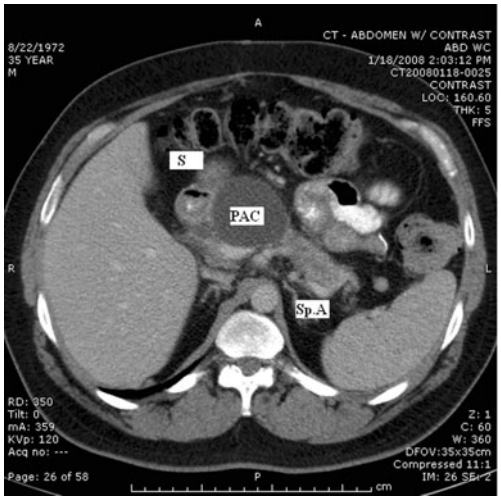

Fig. 1 Preoperative CT scan with PO and IV contrast. The pancreatic pseudocyst (PAC) is in close relationship with the posterior gastric wall (S); there is no splenic artery (SpA) interposition.

retroperitoneal space was entered through a longitudinal incision on the posterior gastric wall over the fine needle using electrocautery (Fig. 3).

Cystogastrostomy was completed to a total length of about 5 cm using one of two techniques: On the first seven patients, we used electrocautery with imbrication of the gastric–cyst edge with a running 2/0 monofilament nonabsorbable stitch. On the last 12 patients, the cystogastrostomy was completed using an endomechanical stapling device with 3.5-mm staples. Next, the posterior gastric wall was stitched on either site of the cystogastrostomy with 2/0 silk and brought to the wound to facilitate exposure. Through the cystogastrostomy, a pancreatic necrosectomy was performed. A Moss type gastric/jejunal tube was then brought through the anterior abdominal wall and then the anterior gastric wall superior to the anterior gastrostomy in Stamm fashion. Attention was placed to ensure the gastric component

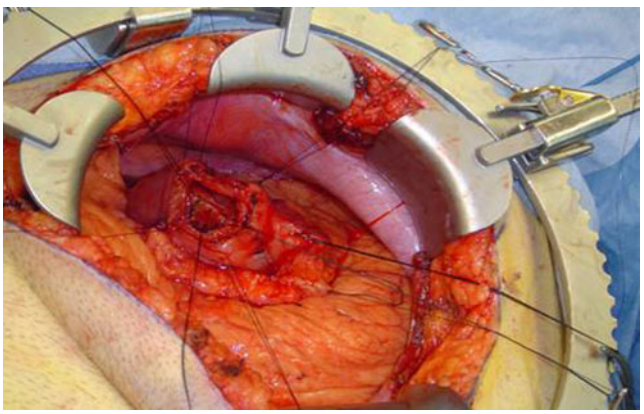

Fig. 2 Intraoperative picture: a left subcostal incision is performed and exposure facilitated with Bookwalter retractor. The anterior gastrostomy is shown at the level of the mid-to-distal stomach.

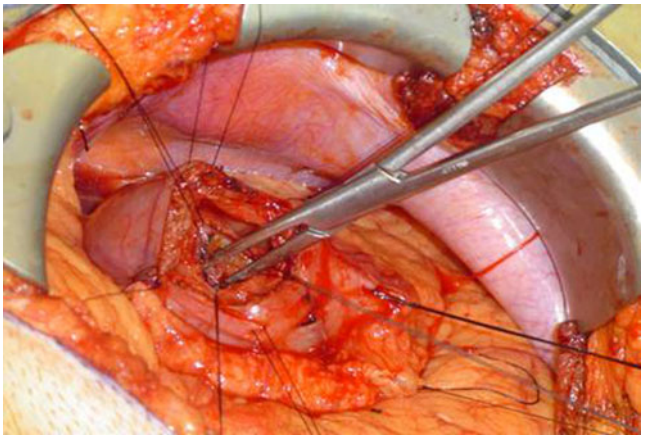

Fig. 3 The posterior gastric wall is opened and its edges were exposed and brought to the surface by silk stitches. The cystogastrostomy is started using electrocautery over a clamp.

(balloon) was within the lumen and the jejunal tube component was placed under direct vision through the pylorus and guided to the proximal jejunum. Next, through a separate stab incision on the anterior gastric wall, an 8F round silastic tube was placed through the anterior gastric wall and placed into the cystogastrostomy to externally drain the retroperitoneal space assuring continuous drainage of the communicating pancreatic ductal leak into the pseudocyst collection.

Both the Moss and the silastic tube were secured to the gastric wall (Stamm) and the skin. Next, the gastrostomy was closed in two layers using a running 3/0 monofilament absorbable stitch first, followed by interrupted 2/0 Lembert stitches. The abdominal wall was then closed in a regular fashion.

Results

All patients but two were extubated on the operating table (15/17). As per protocol, all patients were taken postoperatively to the intensive care unit (ICU) where the two patients initially requiring ventilatory support were extubated by postoperative day 2. Nasogastric tubes were removed from all patients immediately after surgery. Jejunal alimentation was begun in 17/17 patients 24 h after surgery. Fifteen patients were transferred to a regular surgical ward from the ICU after 24 h, while four patients required extra ICU care for respiratory failure (n=2), intravenous cardiac medication (n=1), and postoperative arrhythmia (n=1).

Jejunal tube feeding was tolerated by all patients and was advanced to goal (as per nutrition team) within average of 3 days from the surgery. Both the gastric tube and transgastric retroperitoneal tube were drained to gravity. Prior to hospital discharge, all patients were advanced to

water-only PO intake with the gastric/retroperitoneal tubes draining to gravity.

In this series, there was no perioperative mortality. Postoperative morbidities were graded on a scale of 1 to 5[19,20] (Table 2). Accordingly, two patients had a grade 1 complication as superficial wound infection that required bedside drainage. Two patients had grade 2 complications: postoperative pneumonia ($n=1$) and urinary tract infection ($n=1$). One patient had grade 3 complication; this patient had a complicated preoperative course marked with pulmonary embolism and deep venous thrombosis, and postoperatively required ICU care for ventilator support and developed pneumonia managed with antibiotic therapy. This same patient was readmitted approximately 3 weeks after discharge with postpneumonic empyema and required thoracotomy with decortication. There were no grade 4 or 5 complications in our series. The median LOS was 7 days (4–13 days).

One month after the surgical drainage, all patients had a CT scan to assess the PAC. In 16/19 patients (84%), the CT scan showed complete resolution of the PAC (Fig. 4). These patients were allowed a regular low-fat diet with the gastrostomy limb of the Moss tube capped for 1 month before its removal. The transgastric retroperitoneal drain was left in place for total a period of 3 months. Patients were advised to refrain entirely from alcohol consumption and follow a low-fat diet.

In the three patients whom the initial 30-day CT scan did not show complete PAC resolution, an additional month of water-only per oral intake with goal jejunal feeding was continued. Subsequent to a repeat CT, with demonstrated PAC resolution, the diet was advanced as previously described.

Clinical follow-up beyond the 6-month visit consisted of an annual visit with either CT scan or magnetic resonance imaging for the first year visit; subsequent need for imaging is symptom driven. Over a mean follow-up period of 31 months (range, 6–60 months), none of the 19 patients in this series developed PAC recurrence. Moreover, radiological evaluation at 1 year after the cystogastrostomy has demonstrated the persistence of the intended fistula tract between the stomach and the retroperitoneum, which was created by the retroperitoneal drainage catheter (Fig. 5).

Table 2 Postoperative Morbidity

Grade	No. of patients	Description
1	2	Superficial wound infection that required bedside drainage
2	2	Pneumonia
		Urinary tract infection
3	1	Postpneumonic empyema requiring thoracotomy decortication
Total	5 (26%)	

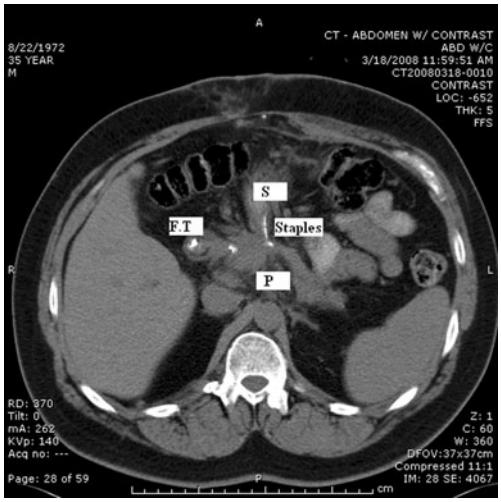

Fig. 4 Postoperative CT scan (1 month after the cystogastrostomy): the pancreatic pseudocyst is resolved. The gastric (S) wall shows the staple line of the cystogastrostomy. On the duodenum, a postpyloric feeding tube (FT) is identified.

Discussion

This modest series reports a successful treatment strategy for a complex albeit uncommon problem; patients with pancreatitis associated pseudocyst (PAC) with a pancreatic duct to cyst fistula, which have been refractory to endoscopic or percutaneous treatment. As all patients were referred to our service with the diagnosis of PAC and failed prior treatment, preoperative ERCP results were not included in this report. The vast majority of these patients

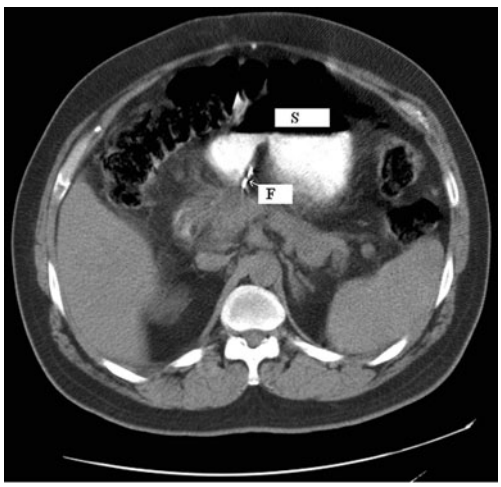

Fig. 5 CT scan 1 year after cystogastrostomy. Persistent fistula (F) is appreciated between the stomach (S) and the retroperitoneum.

had a demonstrated direct communication between the PAC and the main pancreatic duct (type VII according to Nealon classification)[17] or type II and III according to D'Egidio classification[16] likely explaining the failure nonsurgical drainage.

In this series, 19 patients with unrelieved PAC for a relative long period (7/19 patients presented after >3 months from their diagnosis) were successfully treated surgically with a median postoperative LOS of 1 week. Enteral feeding was started early, during the first 24 h after surgery through the combined gastrostomy–jejunostomy tube. Three months after the surgical procedure, 84% of the patients were tolerating regular diet with no drains. This approach was safely performed without perioperative mortality or permanent organ failure in 19 patients including 14 with document preoperative sepsis, and only five patients (26%) had grade 1 to 3 morbidities.

The authors recognize that penetration of antibiotics into pancreatic necroses is minimal and, even then, limited to a particular few. Preoperatively, patients were resuscitated and treated with a combination of broad-spectrum antibiotics (imipenem or ciprofloxacin) in combination with metronidazole, with empiric antifungal coverage. Nutritional support and repletion were initiated and continued immediately postoperatively. It is almost a certainty that there is a relationship between the contaminated or infected pancreas necrosis and the postoperative infection (n=5).

The described surgical approach was instituted by the senior author, in response to the needs of a select population transferred into a tertiary referral center; in which nonoperative treatment strategies had failed. The expectation from the perspective of an experienced hepatopancreatobiliary (HPB) team would be that for patients with demonstrated pancreatic duct to pseudocyst fistula; percutaneous drainage alone will be insufficient and often fail. While modern endoscopic techniques combining pancreatic duct stent drainage along with transgastric endoscopic pseudocyst drainage have reported improved outcomes over single modality approaches for these specific patients, the expertise for these procedures is still not widely available.

Several clinical lessons were learned through the described experience. First, it is important to have current imaging (CT), preoperatively, to define the direct relationship between the PAC and the posterior gastric wall, as well as to define the course of splenic artery within the PAC. Although not identified in any of these 19 patients, it is imperative to preoperatively exclude the presence of the splenic artery immediately adjacent to the gastric wall, at the level of the planned cystogastrostomy, as failure to recognize this would likely lead to massive intraoperative bleeding.

Next, although the PAC location is usually readily identified as a bulging through the posterior gastric wall upon opening the anterior gastric wall, it is recommended to use a fine needle to aspirate and confirm the location at which to begin the posterior gastrostomy. This technique assures the identification of a fluid containing area rather than debris and necrosis, thus, facilitating the creation of the cystogastrostomy.

In the beginning of this series, electrocautery was used to create the cystogastrostomy, and a running monofilament nonabsorbable stitch was used to imbricate its edges (n=7); in our opinion, this approach was time consuming and frankly physically uncomfortable due to the limitations of the gastrostomy and limited abdominal incision. Consequently, for the second group of patients (n=12), an endomechanical stapler was used to complete the cystogastrostomy, for reasons only of efficiency and technical ease.

Previous clinical reports have described the technique of surgical pancreatic necrosectomy and emphasized the importance of postoperative drainage to assure complete resolution. Thus, once necrosectomy was completed, the surgeon still depended on the continued support of interventional radiology through regular exchange of large-bore pancreatic drains, particularly in the presence of known or suspected pancreatic duct leak.[21] The herein described technique assures necrotic tissue drainage, a route for decompression and enteral alimentation, as well as a long-term mechanism for continued internal drainage performed as a single surgical procedure, and furthermore avoided the inconvenience of external drainage catheters.

The cornerstone concept in this treatment strategy is the silastic cystogastric tube for retroperitoneal drainage. This drain allows the direct communication between the gastric lumen and retroperitoneal compartments and prevents premature closure of the surgical cystogastrostomy. By keeping this tube in place for 3 months, there is an intentionally induced epithelialization of the cystogastrostomy to assure continued drainage, thus, in our opinion, explaining the absence of PAC recurrence in this series. The evidence to support this concept is that the imaging demonstrated persistence of this fistulous communication in the follow-up radiological studies.

As a final technical note, after discharge, when patients were allowed to start on a regular diet with a capped gastric tube, we recommend intermittent uncapping and gravity drainage of the gastric tube to assure low residual gastric content, before permanently removing the tube.

The described combined technique of wide cystogastrostomy and a transgastric retroperitoneal drainage tube was associated with complete resolution of all complex pancreatic pseudocysts without recurrence in this series. The wide cystogastrostomy promotes adequate internal drainage of the heterogeneous pancreatic pseudocyst contents, resulting in drainage and complete resolution compared to other less invasive techniques. However, this anastomosis (cystogastrostomy) will ultimately close. In these selected cases, all of

these patients had previously failed conventional nonoperative means. In the clinical scenario of suspicion or proven pancreatic duct leak into the pseudocyst, the early closure of the cystogastrostomy and/or the continued leakage of pancreatic fluid will result in the reaccumulation of trapped fluid, namely, pancreatic pseudocyst recurrence. The creation of a retroperitoneal-to-gastric drainage fistulous tract, as documented by radiological studies, has been demonstrated after 1 year to maintain adequate decompression and has precluded recurrence.

A limitation of this series is the lack of detailed pretransfer history. Consequently, it is difficult to define the exact onset of acute pancreatitis. However, the interval between the documented pancreatic pseudocyst and the transfer to our facility was <3 months in 12 patients and >3 months for 7 patients.

Another limitation is that the study was not constructed to compare this approach to other previously described strategies but rather to report the efficacy and safety of this treatment strategy when others fail. The authors acknowledge the selection bias in this report, as patients included in this study represent only patients that had already failed prior attempted treatment by various nonsurgical modalities; consequently, it would be inaccurate to advocate that the described approach should be a universal procedure for all pancreatic pseudocysts. On the other hand, we advocate that for patients with complex PAC, when other approaches fail to obtain or maintain PAC resolution, a surgical cystogastrostomy with continuous internal drainage of the retroperitoneum is safe and efficient and should be kept in the surgical armamentarium of options for these complex patients.

In the field of HPB surgery, in particular, to the care of patients with pancreatitis in all of its forms, we have fortunately come to a general agreement that for these patients, institutional or service line teams working in a coordinated fashion achieve the best outcomes. Within this manuscript, there is likely an understated importance of the nutrition support team, physical therapy, and wound care team's contributions to the comprehensive care of these complex patients; the surgical and critical care aspects of management, while important, require a multimodal group of health care practitioners to achieve the best possible outcomes.

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ORIGINAL ARTICLE

Microbiological Profile and Antimicrobial Susceptibility in Surgical Site Infections Following Hollow Viscus Injury

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Abstract
Introduction The purpose of this study was to assess the microbiological profile, antimicrobial susceptibility, and adequacy of the empiric antibiotic therapy in surgical site infections (SSI) following traumatic hollow viscus injury (HVI).
Methods This is a retrospective study of patients admitted with an HVI from March 2003 to July 2009. SSI was defined as a wound infection or intra-abdominal collection confirmed by positive cultures and requiring percutaneous or surgical drainage.
Results A total of 91 of 667 (13.6%) patients with an HVI developed an SSI confirmed by positive culture. Mean age was 33.0±14.1 years, mean Injury Severity Score (ISS) was 17.7±9.6, 91.2% were male, and 80.2% had sustained penetrating injuries. The SSI consisted of 65 intra-abdominal collections and 26 wound infections requiring intervention. The most commonly isolated species in the presence of a colonic injury was *Escherichia coli* (64.7%), *Enterococcus* spp. (41.2%), and *Bacteroides* (29.4%), and in the absence of a colonic perforation, *Enterococcus* spp. and *Enterobacter cloacae* (both 38.9%). Susceptibility rates of *E. coli* and *E. cloacae*, respectively, were 38% and 8% for ampicillin/sulbactam, 82% and 4% for cefazolin, 96% and 92% for cefoxitin, with both 92% to piperacillin/tazobactam, and 100% to ertapenem. The initial empirical antibiotic therapy adequately targeted the pathogens in 51.6% of patients who developed an SSI.
Conclusion The distribution of the microorganisms isolated from SSIs differed significantly according to whether or not a colonic injury was present. Empiric antibiotic treatment was inadequate in upwards of 50% of patients who developed an SSI. Further investigation is warranted to determine the optimal empiric antibiotic regimen for reducing the rate of postoperative SSI.

Keywords Microbiological profile · Antimicrobial susceptibility · Surgical site infection · Hollow viscus injury · Trauma

Introduction

The incidence of surgical site infections (SSI) following hollow viscus injury (HVI) remains high despite perioperative antimicrobial administration, ranging from 4% to 31%.[1–10] There is an extensive body of literature examining the risk factors associated with an SSI following trauma, which include gross contamination by colonic content, advanced age, requirement for blood product transfusion, and concomitant stomach injury.[1,2,5–7,9] Multiple investigations have also attempted to assess the efficacy of various antimicrobial regimens aimed at reducing the incidence of postoperative infections after abdominal surgery.[3,4,10–19]

A clear understanding of the microbiological profile of SSI after HVI is important for guiding empiric therapy for

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these injuries. This is made more difficult by the fact that this is a fast-moving target. In particular, the increasing incidence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, β-lactam- and vancomycin-resistant *Enterococci* (VRE), and methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA/ORSA) is of major concern for clinicians. These resistant microorganisms have been observed not only in hospital environments but also in community-acquired intra-abdominal infections.[20,21] For trauma patients, however, our understanding of the microbiological profiles and antimicrobial susceptibility patterns of SSI after HVI remains poor.

The purpose of this study was to investigate the pathogens responsible for the SSI, the antimicrobial susceptibility patterns of these pathogens, and the adequacy of the empiric antibiotic therapy utilized.

Methods

After IRB approval, the Los Angeles County+University of Southern California Medical Center (LAC+USC) trauma registry was queried for patients sustaining a stomach, small bowel, or colonic injury from March 2003 to July 2009 using the International Classification of Diseases-Ninth Edition (ICD-9) codes. Patient data including demographics and clinical characteristics on admission were collected using a computerized spreadsheet (Microsoft Excel 2003, Microsoft Corporation, Redmond, WA, USA). SSI was defined as a wound infection or intra-abdominal collection confirmed by positive cultures and requiring percutaneous or surgical drainage. In-hospital occurrence of an abdominal SSI was documented from the discharge and microbiological reports. Specimen cultures included samples of fluid or wound cultures or percutaneous aspiration of intra-abdominal collections. Microbiological and in vitro antimicrobial susceptibility profiles of isolated pathogens were abstracted. Per protocol, at the LAC+USC Medical Center, all trauma patients undergoing an emergent laparotomy are administered 24 h of a perioperative broad-spectrum empiric antibiotic at the discretion of the treating physician. In cases of severe abdominal contamination, the skin of the abdominal incision is left open to prevent wound infection and is managed per protocol by delayed primarily closure at bedside.[22]

The microbiological profiles of patients with and without colonic perforations were compared. In addition, the microbiological profiles of early (within 7 days from injury) versus late (after 7 days) SSI as well as wound infections versus intra-abdominal collections were compared. Furthermore, the adequacy of the initial empiric antibiotic therapy was assessed. Empiric coverage was considered to be inadequate when the antibiotic therapy failed to adequately cover at least one of the isolated pathogens.

Values are reported as means±standard deviation (SD), means±interquartile (IQ), or percentages. *P* values were obtained from Chi-square test for proportions and from Mann–Whitney test for means. All statistical analyses was performed using the Statistical Package for Social Sciences (SPSS Windows©), version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

During the 77-month study period, 91 of 667 patients (13.6%) with an HVI developed an SSI, confirmed by positive microbiological cultures. Their mean age was 33.0 ±14.1 years, and the mean ISS was 17.7±9.6. A total of 91.2% were male, and 80.2% sustained a penetrating injury. There were a total of 68 patients with a colonic injury, and 23 patients had a small bowel and/or stomach injury without colonic perforation. All patients underwent emergent open surgical treatment of the HVI (resection and primary anastomosis (n=60), direct suture repair (n=26), or temporary/definitive stoma (n=5)).

The mean time from laparotomy to the definitive diagnosis of the SSI was 9.8 days (IQ range, 6.0–11.3). The SSI consisted of 65 (71.4%) intra-abdominal collections and 26 (28.6%) deep wound infections mandating intervention. Fourteen of the 26 (53.8%) wound infections were associated with a fascial dehiscence.

Overall mortality was 3.3% (three of 91). The three deaths occurred on hospital days 12, 20, and 111. All three patients had hospital courses complicated with ongoing abdominal sepsis related to the SSIs, resulting in subsequent abdominal compartment syndrome and multiple organ failure.

Microbiological Profiles

A total of 222 microorganisms were isolated from the SSI samples in the 91 patients. *Enterobacteriaceae* were the most common pathogens found in 73.6% of patients. Of those, *Escherichia coli* (54.9%) was the predominate isolate, followed by *Enterobacter cloacae* (26.4%), *Klebsiella* spp. (16.5%), and *Proteus mirabilis* (4.4%; Fig. 1).

E. coli and *Bacteroides* spp. were significantly more common in patients with colonic injuries compared to those without colonic lesions (64.7% vs. 26.1%, p=0.001, and 29.4% vs. 8.7%, p=0.045, respectively; Fig. 1). In contrast, *E. cloacae* and *Klebsiella* spp. were significantly more common in patients with stomach and/or small bowel injuries without colonic perforation (39.1% vs. 17.6%, p= 0.035, and 30.4% vs. 11.8%, p=0.037, respectively; Fig. 1). Fifteen of 91 (16.5%) patients with SSI had *isolated* small bowel perforations. The most common cultured microorganisms in this subgroup were *E. cloacae* (53.3%, n=8),

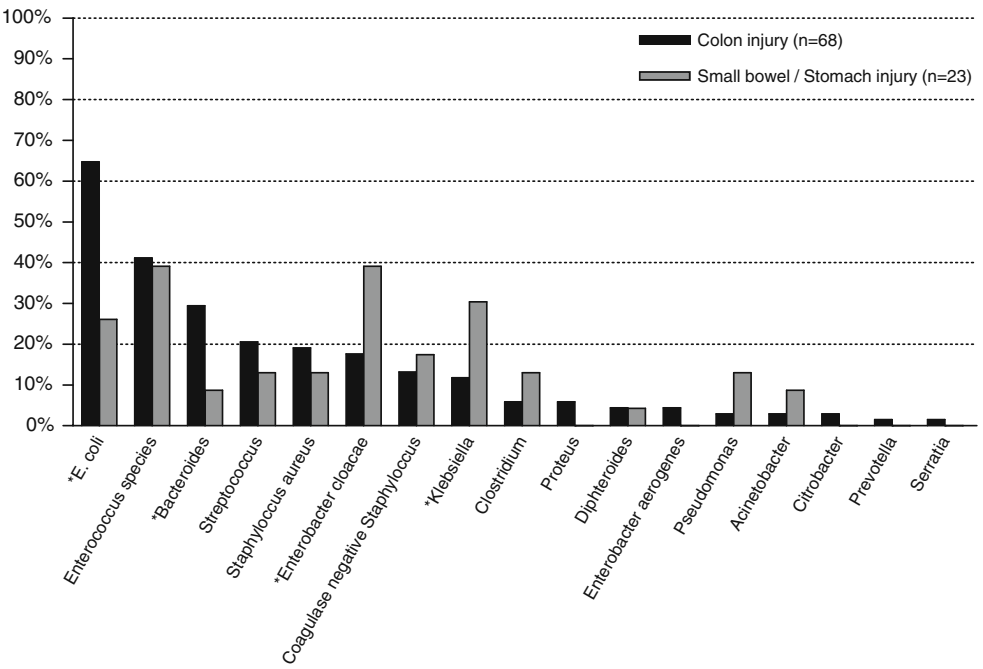

Fig. 1 Microbiological profile of surgical site infections in patients with hollow viscus injuries with or without colonic perforation. *Asterisk* statistically significant difference ($p<0.05$, Chi-square).

*statistically significant difference ($p<0.05$, Chi-square)

followed by *Enterococcus* spp., *E. coli*, and *Klebsiella* (each 33.3%, $n=5$). In three patients with *isolated* stomach injuries, two intra-abdominal collections and one deep wound infection with fascial dehiscence occurred. In these patients, only *Enterococcus* spp. was isolated.

The overall second most common isolates were *Enterococcus* spp., which were cultured in 37 of 91 patients (40.7%; Table 1). *Enterococcus* spp. was significantly more often isolated from intra-abdominal collections compared to wound infections (47.7% vs. 23.1%, $p=0.031$). The incidences of all the remaining pathogens did not differ between intra-abdominal collections and wound infections.

Klebsiella and *E. cloacae* were significantly more common in early SSI (within 7 days from admission) compared to late SSI (27.0% vs. 8.2%, $p=0.036$, and 37.8% vs. 14.3%, $p=0.021$). The incidences of the other microorganisms did not differ between early and late SSI.

Yeast was isolated in a total of 19 (20.9%) patients. No difference in the occurrence of yeast was found when comparing patients with or without colonic injuries (20.6% vs. 21.7%, $p=0.907$) or early versus late SSI (23.1% vs. 19.6%, $p=0.689$).

Antimicrobial Susceptibility Patterns

Table 1 summarizes the results from the in vitro activity of the antimicrobials tested against the most frequently isolated microorganisms. Ertapenem was consistently the most active agent against the three most commonly isolated *Enterobacteriaceae* (*E. coli*, *E. cloacae*, and *Klebsiella* spp.). Piperacillin/tazobactam and the second and third generation cephalosporins were >90% active, whereas ampicillin/sulbactam was the least active agent against these *Enterobacteriaceae* (Table 1). Ampicillin/sulbactam-resistant *E. coli* was isolated in 13 patients with early and in 18 patients with late SSI ($p=0.707$). None of the microorganisms was suspicious for being an ESBL-producing *Enterobacteriaceae*. However, *E. coli* and *E. cloacae* showed sporadic resistance to second and third cephalosporins. All isolated *Klebsiella* cultures were susceptible to the first generation cephalosporin cefazolin. By linear regression, no significant change was noted in the resistance patterns of *E. coli* to ampicillin/sulbactam over the 77-month study period ($p=0.258$, $R^2=0.251$; Fig. 2).

Four of 37 (10.8%) isolated *Enterococci* spp. were resistant to vancomycin (VRE). These four VREs were also resistant to penicillins, aminoglycosides, and fluoroquinolones. Two of the VREs were isolated from early (within 7 days) and two from late (after 7 days) SSI ($p=0.627$).

Of the isolated *S. aureus*, 38.9% (7 of 18) were ORSA. In four patients, *ORSA* was cultured from intraperitoneal aspirates and in three cases from deep wound cultures. *ORSA* was isolated in two patients with early (within 7 days) and in five patients with late (after 7 days) SSI ($p=0.637$).

Adequacy of the Empiric Antibiotic Treatment

Overall, ampicillin/sulbactam (46.0%) and piperacillin/tazobactam (20.7%) were the most commonly used empiric

Table 1 Antibiogram (Reported as Percent Susceptible)

	Gram-negative aerobic and facultative			Gram-positive aerobic and facultative	
Number of isolates	*E. coli* *N*=50	*Enterobacter* *N*=24	*Klebsiella* *N*=15	*Enterococcus* *N*=37	*S. aureus* *N*=18
Penicillin	–	–	–	86	11
Oxacillin	–	–	–	–	61
Ampicillin	–	83	–	97	–
Ampicillin/sulbactam	38	8	93	–	–
Piperacillin/tazobactam	92	92	100	–	–
Cefazolin	82	4	100	–	61
Cefotaxime	100	96	–	–	–
Cefotetan	100	96	–	–	–
Cefoxitin	96	92	–	–	–
Ceftazidime	98	92	–	–	–
Ceftriaxone	100	96	–	–	–
Ciprofloxacin	96	–	–	–	–
Clindamycin	–	–	–	–	89
Erythromycin	–	–	–	–	67
Gentamicin	88	96	100	86	100
Tobramycin	90	100	100	–	–
Ertapenem	100	100	100	–	–
Levofloxacin	88	100	100	92	50
Linezolid	–	–	–	100	100
Streptomycin	–	–	–	89	–
Tetracycline	–	–	–	97	94
Trimethoprim/sulfamethoxazole	52	96	100	–	100
Vancomycin	–	–	–	89	100

antibiotics. Less frequently administered empiric antibiotics were cefazolin (11.8%), cefoxitin (7.3%), carbapenems (3.6%), and others (10.5%). Patients who developed an SSI tended to get more ampicillin/sulbactam (54.9% vs. 44.6%, $p=0.066$) and less piperacillin/tazobactam (17.6% vs. 21.2%, $p=0.431$) compared to those without a SSI.

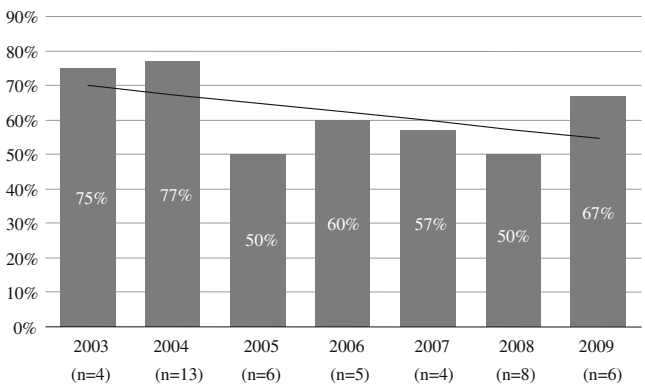

Fig. 2 Ampicillin/sulbactam resistance over the study period of *Escherichia coli* isolated from surgical site infections after traumatic hollow viscus injury (percent resistance).

Fig. 3 illustrates the perioperative empiric antibiotics administered in the 91 patients with SSI. After checking those against the susceptibility pattern of the isolated pathogens, it was found that the initial empiric antibiotic therapy inadequately targeted the pathogens in 48.4% (44 of 91) of patients. In 61.4% (27 of 44) of cases, this was due to inadequate coverage of *E. coli* and *Enterobacter* spp. by ampicillin/sulbactam. The remaining 38.6% (17 of 44) was attributed to *S. aureus* not adequately covered by ampicillin/sulbactam or piperacillin/tazobactam (15.9%, $n=7$) and *Enterobacteriaceae* not covered by cefazolin (9.1%, $n=4$).

Discussion

In the present study, 14% of trauma patients with an HVI developed clinically significant postoperative wound infections or intra-abdominal collections, confirmed by a positive microbiological culture. This rate is within the one reported in the literature (4–31%).[1–10] For patients with SSI, although statistically not significant, there was a trend towards more

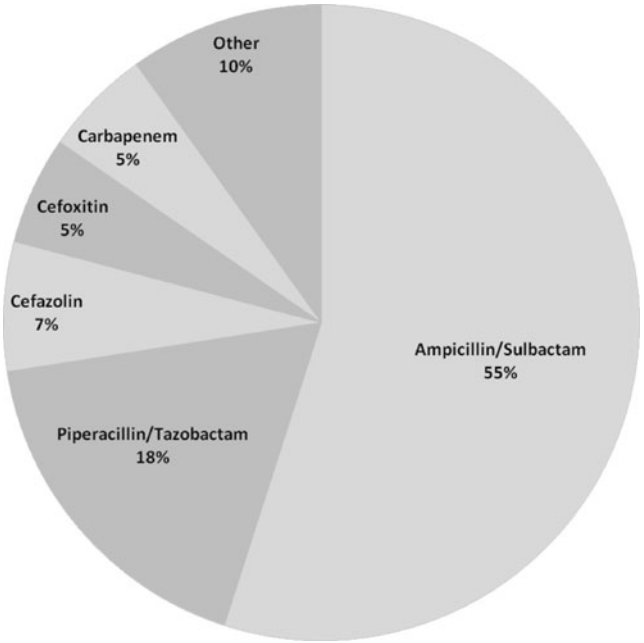

Fig. 3 Antibiotics used for perioperative empiric treatment.

ampicillin/sulbactam administration compared to those without SSI (54.9% vs. 44.6%, $p=0.066$).

As expected, *Enterobacteriaceae* were the most common isolates comprising almost three quarters of the total. Likewise, other investigators have previously found similar microbiological patterns; however, those studies examined heterogeneous cohorts with both community- and hospital-acquired intra-abdominal infections.[20,21] The present study, however, is a single-center study focused on a relatively young and previously healthy trauma cohort, in whom resistant pathogens and immunologic deficiencies are most likely absent.

E. coli was significantly more frequent in patients sustaining colonic perforation, whereas in patients with stomach and/or small bowel injuries without colonic injury, *E. cloacae* and *Klebsiella* were isolated more often (Fig. 1). Such observations are not surprising since the normal intestinal flora of the stomach, small bowel, and the colon differs significantly. Trauma patients are often not fasting on admission. Fluid aspirated from the stomach is usually sterile, except after meals, which temporarily neutralizes the pH. This allows organisms from the mouth and ingested food to remain in the stomach.[23–25] Aerobic bacteria, mainly *Streptococcus* spp., dominate in the proximal small bowel. However, with increasing distance from the pylorus, the numbers of *Enterobacteriaceae* increase. *E. coli*, *E. cloacae*, and *Klebsiella* were found in the small bowel in 7%, 15%, and 35% of duodenal, jejunal, and ileal samples, respectively.[26] Finally, the microflora in the distal ileum is quite similar to that in the colon. In the present study, a similar localization of pathogens according to the site of injury was found. However, the numbers of patients with *isolated* stomach, small bowel, or colonic perforation were limited, precluding any meaningful statistical analysis.

During the recent decade, global antimicrobial surveillance studies have shown increasing resistance patterns in *Enterobacteriaceae* and other Gram-negative *Bacilli* isolated from community- and hospital-acquired intra-abdominal infections.[27–32] Such developments limit the effectiveness of the empiric antimicrobial therapy and may lead to more clinical failures in treating these patients.[31] Similar to other reports, ertapenem was the most consistently active antimicrobial agent against *Enterobacteriaceae* in vitro.[20,21] In contrast, ampicillin/sulbactam, although the most frequently used empiric antibiotic agent at our institution, demonstrated poor coverage of *Enterobacteriaceae* (8–93%), leading to inadequate empiric therapy in almost a third (27 of 91) of the study cohort. Whether the trend towards more ampicillin/sulbactam administration in patients with SSI compared to those without SSI is responsible for the infection is uncertain.

The high proportion of *E. coli* strains resistant to moderate-spectrum β-lactam penicillins (62%) reported here is in line with previous reports.[16,20,33] Although none of the antibiograms in this cohort were suspicious for ESBL-producing *Enterobacteriaceae*, sporadic resistance to second and third generation cephalosporins of *E. coli* and *Enterobacter* spp. occurred. Nevertheless, in contrast to ampicillin/sulbactam, broad-spectrum empiric therapy for 24 h with a second generation cephalosporin or piperacillin/tazobactam appears to be adequate.[3,8,34] However, ESBL-producing microorganisms should be anticipated, since recent data has demonstrated that both hospital and community-acquired intra-abdominal infections can be caused by these resistant pathogens.[20,21]

Empiric coverage of *Enterococci* remains controversial. The Infectious Diseases Society of America (IDSA) guidelines recommend the treatment of *Enterococcus* in nosocomial peritonitis, but not in community-acquired intra-abdominal infections.[35] Several investigators have found increased complications in patients with peritonitis in the presence of *Enterococci*,[36–38] but an increased mortality was only noted in two studies.[33,39] *Enterococci* were isolated in 41% of SSIs in the current series. This is a considerably higher proportion than the approximately 10% reported in the contemporary non-trauma literature.[21,40,41]

Widespread antimicrobial therapy with vancomycin has contributed to a significant increase in VRE infections. An analysis of the US National Hospital Discharge Survey found for the year 2004 an overall abdominal VRE infection rate of almost 20%.[42] In our series, 11% isolated *Enterococci* were VRE. One of these patients had a VRE isolate from the intraperitoneal fluid 5 days after admission.

This patient eventually died after 111 days following a complicated in-hospital course of ongoing abdominal sepsis leading to multiple organ failure. VRE infections are an increasing burden and can be very difficult to treat because they are often resistant to multiple antimicrobial drugs and often require the use of linezolid, quinupristin/dalfopristin, or other more costly antibiotics.[42–45]

S. aureus, resistant to newer narrow-spectrum β-lactam penicillins (e.g., methicillin or oxacillin), became endemic in many US hospitals with incidences of up to 60% in intensive care units.[46] In the present study, *S. aureus* was cultured from almost 20% of SSIs, of which seven (39%) were ORSA. Similar to the presented series, Eagye et al. found *S. aureus* in 18% of 34 abdominal SSIs, of which 45% were MRSA.[34] Whether colonization of intra-abdominal SSIs with MRSA/ORSA affects mortality remains unclear. Although the overall incidence of these multiresistant pathogens has increased over the last decade, no detrimental impact on mortality by MRSA was documented in a recent nationwide review.[47]

Conclusion

The distribution of the microorganisms isolated from traumatic SSIs differed significantly according to whether or not the colon was injured. *Enterobacteriaceae* were the most common isolates, comprising almost three quarters of the total. Empiric antibiotic treatment was inadequate in upwards of 50% of patients who developed an SSI, and 61% of failures were linked to *E. coli* and *E. cloacae* not being adequately targeted by ampicillin/sulbactam. The selected empiric antibiotic treatment should be individualized according the local antibiogram. Based on the results presented, the empiric treatment will be changed to cefoxitin+metronidazole or piperacillin/tazobactam. Further prospective investigation is warranted to determine the optimal empiric antibiotic regimen for reducing the rate of postoperative SSI.

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ORIGINAL ARTICLE

Role of Factor VII in Correcting Dilutional Coagulopathy and Reducing Re-operations for Bleeding Following Non-traumatic Major Gastrointestinal and Abdominal Surgery

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Abstract
Objective The objective of this study is to evaluate the effectiveness of rfVIIa in reducing blood product requirements and re-operation for postoperative bleeding after major abdominal surgery.
Background Hemorrhage is a significant complication after major gastrointestinal and abdominal surgery. Clinically significant bleeding can lead to shock, transfusion of blood products, and re-operation. Recent reports suggest that activated rfVIIa may be effective in correcting coagulopathy and decreasing the need for re-operation.
Methods This study was a retrospective review over a 4-year period of 17 consecutive bleeding postoperative patients who received rfVIIa to control hemorrhage and avoid re-operation. Outcome measures were blood and clotting factor transfusions, deaths, thromboembolic complications, and number of re-operations for bleeding.
Results Seventeen patients with postoperative hemorrhage following major abdominal gastrointestinal surgery (nine pancreas, four sarcoma, two gastric, one carcinoid, and one fistula) were treated with rfVIIa. In these 17 patients, rfVIIa was administered for 18 episodes of bleeding (dose 2,400-9,600 mcg, 29.8-100.8 mcg/kg). Transfusion requirement of pRBC and FFP were each significantly less than pre-rfVIIa. Out of the 18 episodes, bleeding was controlled in 17 (94%) without surgery, and only one patient returned to the operating room for hemorrhage. There were no deaths and two thrombotic complications. Coagulopathy was corrected by rfVIIa from 1.37 to 0.96 ($p<0.0001$).
Conclusion Use of rfVIIa in resuscitation for hemorrhage after non-traumatic major abdominal and gastrointestinal surgery can correct dilutional coagulopathy, reducing blood product requirements and need for re-operation.

Keywords Reoperation · Factor VII · Postoperative complications · General surgery · Coagulopathy

Introduction

Activated factor VII (rfVIIa) plays a key role in hemostasis by activating the extrinsic pathway of coagulation. Factor VIIa initiates coagulation by interacting with exposed tissue factor, in turn activating factor X to produce thrombin.[1] Thrombin then causes a conformational change of circulating platelets, and the activated surface of platelets further activates the coagulation cascade to produce stable fibrin clots.[2] Injured sub-endothelial cells express tissue factor. Activation of factor VII by exposed tissue factor occurs at sites of local tissue injury and endothelial damage, making factor VII an attractive therapeutic agent for active hemorrhage following surgical injury.

In 1999, the US Food and Drug administration approved the use of recombinant rfVIIa for the treatment of the inherited bleeding disorders hemophilia A and B as well as inhibitors to factor VIII or IX. Because rfVIIa targets areas of active bleeding, it has been used to treat non-hemophiliac cases of coagulopathy or refractory hemorrhage in trauma, neurosurgery, cardiac surgery, and liver transplantation.[3,4] In randomized, placebo-controlled trials

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among trauma patients, rfVIIa was shown to significantly decrease units of red blood cells (RBC) transfused.[5,6] Multiple case reports and case series have since suggested the effectiveness of rfVIIa in treating hemorrhage during surgery.[7–10]

Although these studies have shown significant reductions in bleeding, there are no studies clearly evaluating the role of rfVIIa in preventing re-operations for hemorrhage following major abdominal gastrointestinal surgical procedures. Although sepsis and anastamotic leaks are the most common indications for re-operation following gastrointestinal surgery, bleeding accounts for as much as18.5% of urgent abdominal re-explorations.[11,12] Because these re-operations represent additional trauma to the patient and family and may result in increased morbidity and mortality, surgeons are faced with the dilemma of continuing potentially futile resuscitation with blood products or performing a potentially harmful repeat operation. In general, in these situations re-operation has been recommended. However, we hypothesized that rfVIIa would control bleeding by correcting dilutional coagulopathy after surgery without significant complications. To evaluate the role of rfVIIa in preventing re-operations for hemorrhage following major abdominal and gastrointestinal surgery, we performed a retrospective chart review of 17 consecutive patients treated by a single surgeon with rfVIIa to avoid re-operation.

Methods

This is a retrospective case series from a single surgeon in a large tertiary referral medical center who prospectively planned to first use rfVIIa (rather than return immediately to the operating room) if significant bleeding occurred in the postoperative setting following major gastrointestinal and abdominal surgery. Postoperative patients were identified by surgical residents who notified the attending surgeon that there was significant bleeding and may need to return to the operating room (OR). Instead, each patient received rfVIIa for attempted control of hemorrhage without surgery. Inclusion criteria included patients with bleeding less than 7 days from date of operation, 24-h blood loss greater than 4 units packed red blood cells (pRBC), and clinical evidence of hemorrhagic shock (systolic blood pressure <100 mm Hg, pulse >100, and urine output <400 cc/24 h). There was no threshold for Jackson-Pratt drain output, but increasing output and frank blood instead of serosanguinous output were considered by surgical residents as evidence of postoperative hemorrhage. Exclusion criteria included postoperative blood loss occurring greater than 7 days from date of surgery and patients receiving prophylactic or intraoperative rfVIIa. Patient records from November 2004-November 2008 were reviewed, and 17 patients who met these criteria were identified.

The primary outcome was whether a patient needed surgical re-exploration for hemorrhage within 3 days following administration of rfVIIa. Secondary outcomes included units of packed red blood cells, fresh frozen plasma, cryoprecipitate, and platelets administered. Blood components were calculated as total units in the 24-h period prior to and following rfVIIa administration. Postoperative blood loss was calculated by subtracting immediate postoperative hematocrit by immediate pre-rfVIIa hematocrit. This should be considered as an approximation of actual postoperative blood loss as peri-operative fluid shifts, IV fluid administration, and variability in timing of lab samples preclude definitive characterization of peri-operative blood loss based on this calculation. Additionally, mortality and thromboembolic complications following administration of rfVIIa were assessed. Diagnostic criteria for thromboembolic complications included diagnosis of a lower extremity deep venous thrombosis by duplex ultrasound, hemorrhagic or embolic stroke on head CT, acute myocardial infarction by EKG and serial troponin measurement, and limb or mesenteric ischemia. Given the complexity of operations contributing to the sample, we included portal vein or SMV thrombosis diagnosed by CT as thromboembolic complications.

P values reported for transfusion requirements, postoperative blood loss, and coagulopathy were calculated using Wilcoxon rank sum tests. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA).

This study did not require IRB review and approval because data were extracted from the medical record in a de-identified blinded fashion.

Results

Seventeen patients with postoperative hemorrhage following major abdominal surgery were treated with activated recombinant factor VII. Out of these 17 patients, nine cases were due to pancreatic tumors requiring a Whipple procedure or a distal pancreatectomy, four cases were due to sarcomas requiring resection of the mass and involved gastrointestinal structures, two cases were due to gastric cancer requiring gastrectomy and Roux-en-Y gastrojejunostomy, one case was due to goblet cell carcinoid requiring a right hemicolectomy, and one case was due to chylous ascites requiring laparotomy and ligation of the cisterna chyli (Table 1). Average case length was 252 min. Average estimated blood loss was 1.5 L, requiring 5.1± 0.8 L crystalloid,.8±0.2 L colloid, 3.5±1.6 units pRBC, and 1.6±1.0 units fresh frozen plasma (FFP; Table 2). The

Table 1 Clinical Characteristics

Age	Weight	Diagnosis	Primary procedure	Thromboembolic complications	Mortality	Re-operation for bleeding
32.00	66.6	Left flank Ewing's sarcoma	Resection of mass+hernia repair with mesh and latissimus, gluteal, and paraspinous muscle flaps	No	No	Yes
74.00	94	IPMT	Whipple	No	No	No
52.00	52.3	Pancreatic cyst, pancreatitis	Pancreatic neck dissection with Roux-en-Y pancreaticojejunostomy	No	No	No
56.00	92.7	Adenocarcinoma of the duodenum, sigmoid colon	Pancreaticoduodenectomy, sigmoid resection with rectal anastamosis. SMV injured intraop and reconstructed.	Yes	No	No
69.00	69.1	Cystic mucinous tumor	Distal pancreatectomy, splenectomy	No	No	No
53.00	78.2	Intra-abdominal liposarcoma	Resection of mass	No	No	No
47.00	50	Left buttock neurofibroma with AV malformation and hematoma	Resection of mass	No	No	No
49.00	72.7	Chylous ascites	Laparotomy, ligation of cisterna chyli	No	No	No
67.00	79.5	Serous cystadenoma of pancreas	Distal pancreatectomy and splenectomy	No	No	No
72.00	40.9	Gastric outlet obstruction with afferent loop syndrome	Resection of Bilroth II gastrojejunostomy with new Roux-en-Y gastro jejunostomy and jejunal tube	No	No	No
85.00	76.4	Retroperitoneal liposarcoma	Resection of mass, Left hemicolectomy	No	No	No
76.00	49.9	Goblet cell carcinoid tumor appendix, intraabdominal abscess	Right hemicolectomy	No	No	No
63.00	74	Pancreatic NET	Pancreaticoduodenectomy with SMA and SMV reconstruction	Yes	No	No
71.00	47.6	Mucinous cystic neoplasm pancreas	Pancreaticoduodenectomy	No	No	No
76.00	56	IPMT	Laparotomy, subtotal pancreatectomy, splenectomy, partial gastrectomy, transverse colectomy	No	No	No
78.00	57.2	Gastric cancer	Total gastrectomy, roux-en-y	No	No	No
88.00	50.8	Pancreatic cancer	Distal pancreatectomy, splenectomy, L nephrectomy	No	No	No

high volume of intraoperative crystalloid and pRBC/FFP may have contributed to postoperative dilutional coagulopathy as immediate postoperative hematocrit was 30.4±1.5.

In 16/17 patients, there was a single episode of bleeding occurring within 7 days of the operation. One patient had two episodes of bleeding in the defined post-operative period; therefore, rfVIIa was administered for 18 episodes of bleeding (doses 2,400-9,600 mcg, 29.8-100.8 mcg/kg). The patient who had two episodes of bleeding was a 74-year-old female undergoing Whipple pancreaticoduodenectomy for an intraductal papillary mucinous tumor who had bleeding on post-operative days 0 and 2. In all cases, there was clinical evidence of hemorrhage necessitating resuscitation with blood component products and the possibility of return to the OR. Postoperatively, average blood loss resulted in a reduction of the hematocrit from a 30.4 postoperatively to 22.6 when the decision for rfVIIa was made (Table 3). Post-rfVIIa hematocrit of 32.1 ($p<$

Table 2 Operative Characteristics

Patient	Duration of case (min)	Intraoperative EBL	Intraoperative crystalloid	Intraoperative colloid	Intraoperative pRBC	Intraoperative FFP	ASA class	Immediate postoperative Hct
1	304	4,500	7,000	250	8	2	3	31.0
2	396	1,000	4,200	1,000	0	0	2	35.2
3	315	500	4,200	0	0	0	2	29.0
4	237	1,200	11,000	1,250	4	0	4	35.2
5	150	250	4,000	0	0	0	3	32.7
6	288	700	8,000	2,000	2	0	3	28.8
7	108	250	1,200	500	0	0	2	14.6.0
8	180	500	5,700	1,000	2	2	3	32.2
9	245	1,000	8,000	1,500	2	0	2	31.2
10	203	200	3,000	500	0	0	3	36.4
11	270	3,000	6,000	3,500	10	2	4	41.0
12	140	250	800	0	2	0	3	33.5
13	535	8,000	13,000	1,000	26	17	2	28.7
14	324	200	2,500	1,000	0	0	3	24.6
15	269	1,900	4,400	1,000	4	4	2	21.2
16	162	2,000	0	0	0	0	2	28.5
17	158	250	4,000	0	0	0	3	33.1
Median	245	700	4,200	1,000	2	0	3	31.2
Mean	252	1,512	5,118	853	3.5	1.6	3	30.4
Standard error	25.9	495.4	845.0	220.8	1.6	1.0	0.2	1.5

Fluids are in milliliter. Blood components are in units transfused

ASA American Society of Anesthesiologist

0.00001) was significantly higher than pre-rfVIIa. There was also a significant reduction in the INR ($p<0.01$).

In the 24 h pre-rfVIIa administration, a mean of 5.2 units pRBCs, 2.0 units FFP, 33 units cryoprecipitate, and 0.39 units platelets were administered. Median values for pRBC, FFP, cryoprecipitate, and platelets were 4, 1.5, 0, and 0, respectively. In the 24 h post-rfVIIa administration, a mean of 0.83 units pRBC, 0.38 units FFP, 0 units Cryo, and 0.06 units platelets were given. Median values for all blood products post-rfVIIa were 0 (Table 4). Transfusion of pRBC and FFP was significantly less than pre-rfVIIa amount by Wilcoxon rank sum test ($p<0.00001$ and $p<0.01$), and transfusion of cryoprecipitate and platelets were not significantly different ($p=0.06$ and $p=0.09$).

Out of the 18 episodes, bleeding was immediately controlled in 17 (94%) without surgery. One patient returned to the OR for surgical control of bleeding (retroperitoneal hemorrhage following flank sarcoma resection and hernia repair). There were no deaths. There were two thrombotic complications involving major veins, one with thrombosis of the superior mesenteric vein and another with thrombosis of the portal vein. Both of these veins had been partially reconstructed during the surgical procedure. Neither patient developed long-term complications from the

Table 3 Postoperative Blood Loss and Coagulopathy Corrected by rfVIIa

	Postop Blood Loss	Pre-FVII	Post-FVII	*p*
Hematocrit	−7.8 (−7)	22.6 (22.7)	32.1 (32.6)	<0.00001[a]
Platelets	-	212 (188)	172 (142)	0.40
INR	-	1.37 (1.4)	0.96 (0.9)	<0.01[a]

Units reported are mean and (median)

Unit for hematocrit is% of blood volume occupied by red blood cells. Unit for platelets is 10^3 /uL

[a] Statistically significant

Table 4 Transfusion Requirements pre- and post-rfVIIa Administration

	24h pre-factor VIIa, mean (median) units	24h post-factor VII (median units)	*P* value
pRBC	5.2 (4)	0.83 (0)	<0.00001[a]
FFP	2 (1.5)	0.38 (0)	<0.01[a]
Cryo	0.33 (0)	0 (0)	=0.06
Platelet	0.39 (0)	0.06 (0)	=0.09

pRBC packed red blood cell, *FFP* fresh frozen plasma, *Cryo* cryoprecipitate

[a] Statistically significant

thrombosis. There were otherwise no cases of stroke, myocardial infarction, or pulmonary embolism. The dose of rfVIIa given ranged from 29.8 to 100.8 mcg/kg, with an average of 78.0 mcg/kg. Bleeding was controlled in 8/18 episodes (44.4%) with the initial dose of rfVIIa, while 10/18 (55.6%) bleeding episodes required two or more doses. We subsequently routinely planned to give two doses separated by 2 h, and in the latter half of the study period (2006-2008), 7/9 (77.8%) bleeding episodes were treated with two doses.

Discussion

Current dogma in surgery differentiates between surgical and non-surgical causes of bleeding. As a cause of surgical bleeding, postoperative hemorrhage has been traditionally managed by re-operation. However, reoperation costs a significant amount in money, time, energy, and potential patient and family suffering. This study demonstrates the ability of rfVIIa in conjunction with component resuscitation to control hemorrhage and avoid reoperation for bleeding following complex major abdominal and gastrointestinal surgery.

Postoperative hemorrhage following major abdominal surgical procedures such as pancreatectomy and pancreatoduodenectomy, the most common operations in our case series, represent a challenging complication, with a mortality rate as high as 43%.[13] Likewise, for gastrointestinal procedures such as gastrectomies, reoperation for postoperative bleeding has also been shown to be associated with increased hospital length of stay as well as morbidity.[14] The primary intervention for control of post-operative hemorrhage is ether transfusion of blood products or re-operation. From a review of the literature, in 47.2-69% of cases blood product transfusion alone was unable to cease hemorrhage, necessitating urgent surgical re-exploration for hemostatic control.[13,15–17] However, both surgical re-exploration and excessive blood product replacement have been associated with increased infection risk, multiorgan dysfunction, increased hospital length of stay, and mortality.[11–13,18–20] More recently, interventional radiology has been used for treatment of postoperative hemorrhage, but in terms of hemostasis, morbidity, and mortality, it is comparable to surgical re-exploration.[13] Therefore, reducing the amount of blood products transfused and avoiding a re-operation presents a real benefit to patents.

The administration of rfVIIa was effective in arresting hemorrhage and preventing re-operation in 17/18 episodes (94.4%). Further, it resulted in a reduction of blood products transfused for all patients. Additionally, our results show that rfVIIa administration was associated minimal morbidity. Given that the average patient in our series had a class 3 physical status by the American Society of Anesthesiologist, reoperation would present increased risks.

Although there are many studies evaluating the use of rfVIIa in the perioperative setting,[21,22] there are none that evaluate the use of rfVIIa specifically in the postoperative setting in major gastrointestinal and abdominal cases.[8,10] Most previous reports have focused on vascular and cardiac surgery.[23–25] Because the current study excluded any patient who received prophylactic and intra-operative rfVIIa, we were able to assess the role of rfVIIa to prevent re-operation in the postoperative setting for postoperative hemorrhage, the primary outcome measurement of this study. Administration of rfVIIa appeared to be an effective therapy as it prevented re-operation in 94.4% of cases, normalized coagulopathy, and raised the hematocrit. The single case that returned to the OR for hemostatic control involved a patient undergoing a complicated operation involving resection of a large left flank Ewing's sarcoma; hernia repair with mesh; and latissimus, gluteal, and paraspinous muscle flaps. In this patient, we wanted to avoid a hematoma that would have compromised the integrity of the hernia repair with mesh and flaps.

Administration of rfVIIa also significantly reduced blood transfusion requirement for red blood cells, fresh frozen plasma, cryoprecipitate, and platelets. This is a similar finding to other studies that have evaluated the efficacy of rfVIIa in controlling hemorrhage among trauma, cardiac, vascular, and liver cases.[3,5,6,26,27] The average dose of rfVIIa was 78.0 mcg/kg, and this is consistent with recent studies showing efficacy of doses of rfVIIa >50 mcg/kg.[28] However, the wide range of dosing among various studies occurs because of limited initial experience with the drug. Eventually, the dose for rfVIIa was standardized to >50 mcg/kg for two doses, and this was done in the last ten patients.

Thrombosis is a potential complication of this drug. Thrombosis of two major veins that had been reconstructed

was observed. It is unclear if the thrombosis was a complication of the procedure itself or to rfVIIa. However, both patients stopped bleeding without reoperation and neither patient had any long-term sequellae from the thrombosis. There is a lack of consensus in the literature regarding the association of rfVIIa with thromboembolic complications with some studies concluding an increased association[3] while others conclude no association.[7,28] In a meta-analysis of rfVIIa used peri-operatively among abdominal surgery patients, rfVIIa was not observed to increase thromboembolic risk. Nevertheless, thromboembolic complications should be considered, and the surgeon must weigh the benefits of avoiding re-operation to the potential risk of thromboembolism. Based on its efficacy and the limited morbidity, we have continued to choose rfVIIa to control postoperative bleeding and avoid re-operation.

Given the high cost of rfVIIa, other staff surgeons and pharmacists have been cautious about its use. Using the standard pharmacy *Red Book* reference and pharmacy department at our institution, the price of rfVIIa is $1.08 per mcg, supplied in 1,200; 2,400; and 4,800 mcg vials. For a 4,800-mcg dose (68.6 mcg/kg for a 70-kg patient), the purchasing cost is $5,184. Patient charges at our institution for 2 h of OR time is $17,640, aggregating anesthesia, surgery, and OR time charges. Although a full cost-effectiveness analysis was not undertaken as part of this retrospective study, a basic cost analysis would suggest that rfVIIa should be considered when surgical re-exploration is the other alternative given the high cost of re-operation. Further, we did not include the cost of continued transfusion of blood and clotting factors that the use of rfVIIa effectively reduced. Although this was not included in our analysis, transfusion of blood products carry significant risk for infections and increased length of stay, also arguing for the use of rfVIIa to avoid reoperation. One cost-benefit analysis has suggested that rfVIIa is only favorable if the patient is expected to receive 40 units of RBC or one whole blood transfusion (RBC, clotting factors, and platelets).[28] Loudon and Smith have proposed that rfVIIa is cost-effective after transfusion of 14 units of RBC.[29] Including the averted cost of re-operation, the cost of additional blood products plus the cost of other related complications would elucidate whether rfVIIa is potentially cost saving in the setting of postoperative hemorrhage. Our results suggest that a full cost-effectiveness analysis using decision analytic models would provide important information for surgeons and pharmacists when deciding to use rfVIIa.

Algorithms for the use of rfVIIa for postoperative hemorrhage following non-traumatic major abdominal and gastrointestinal surgery need to be developed. Clark and colleagues defined three criteria for the administration of rfVIIa: (1) transfusions of RBC to at least 1.5-fold the blood volume (>15 units), (2) persistence of bleeding despite conventional therapy, and (3) no foreseeable immediate surgical bleeding control.[30] However, this algorithm ignores the benefit posed by preventing the high morbidities associated with surgical re-operation, which is consistently recommended in the surgical literature. Instead, Von Heymann and colleagues suggest that in situations of persistent massive postoperative bleeding where the remaining options are only rfVIIa or surgical re-exploration, a 90 mcg/kg of rfVIIa should be given, repeated by another dose one hour later if bleeding persists.[7] This algorithm implies that successful treatment with rfVIIa must be accompanied by correction of acidosis (pH>7.2), fibrinogen (>100 mg/dL), platelets (>50,000/uL), and hematocrit (>24%), each of which may impact the efficacy of rfVIIa.[31] We also have tried to correct these factors before administering rfVIIa perhaps contributing to the effectiveness of rfVIIa for preventing re-operation in our experience.

Factor VII has been extensively studied among the trauma surgery literature.[5,6,32] Additionally, the role of increased FFP to PRBC ratio has also been examined in massive transfusion protocols for civilian trauma.[33,34] A massive transfusion protocol was implemented at our institution in July 2005 supporting a 1:1.5 FFP/PRBC ratio, improved communications, and enhanced systems flow to optimize rapid blood product availability. Activation is recommended for greater than 4 units PRBCs transfused in the first hour or expected transfusion requirements in excess of 10 units in a 12-h period. In our clinical algorithm, factor VII is recommended for consideration if there is persistent coagulopathy or hemorrhage after administration of two massive transfusion packs or 12 units pRBCS and 8 units of FFP.

Studies have shown that civilian trauma patients undergoing massive resuscitation often arrive with significant dilutional coagulopathy from excessive crystalloid administration.[35] Likewise, the patients in our sample may have also had significant dilutional coagulopathy as they received a large amount of intraoperative crystalloid, with a mean of 5.1 L resulting in mean immediate postoperative hematocrit of 30.4. They received relatively few intraoperative pRBC (3.5±1.6 units) and FFP (1.6±1.0 units) given the large estimated blood loss (1,512±495.4 cc) for a pRBC/FFP ratio of 2:2. Although the interpretation of these data is challenged by the small sample size and large standard error, these findings nevertheless highlight that the patients in this sample could have had significant dilution of clotting factors secondary to large intraoperative crystalloid and high pRBC relative to FFP transfusion. This may be a common problem among patients undergoing complex and long gastrointestinal and tumor resection surgical procedures. This dilutional coagulopathy may explain the success of rfVIIa in stopping bleeding in our patients.

Implementation of our massive transfusion protocol (MTP) among trauma patients at our institution has been shown to improve mortality.[36] Given that the trauma literature suggests improved outcomes with a 1:1 ratio of FFP to pRBC, perhaps the need for rfVIIa could have been averted had a 1:1 pRBC/FFP ratio been administered instead of the ratio of 2:2 intra-operatively and 2:6 post-operatively in this series. However, this is complicated by data from our institution showing increased survival due to decreased time to transfusion, as component ratios did not change in the periods prior to and post-implementation of the MTP.[36] Because our review extended to patients in 2004, five patients were resuscitated prior to the advent of our institution's massive transfusion protocol. Additionally, the patients in this study did not meet our criteria for MTP, since average blood loss in the 24-h period prior to rfVIIa administration was 5.2 units pRBC. Therefore, for the patients in our series, the amount and endpoints of component resuscitation and rfVIIa occurred as a result of direct decision making of the surgeon and not as a consequence of the institutional MTP. This study suggests the inadequacy of applying trauma resuscitation protocols to patients undergoing major abdominal tumor resections as patient characteristics such as age, type of operation, and comorbidities clearly differ. Clinical decision-making algorithms should be derived specifically for the setting of postoperative bleeding and coagulopathy following non-traumatic abdominal and gastrointestinal procedures.

Deficiencies of this study include small sample size ($n=17$) and study design (retrospective case series). Similar to previous studies of the use of rfVIIa among surgical patients, a more robust case-control analysis was not possible because of the heterogeneity of diagnoses and operations.[8] An advantage of this study was that there was no operator variability in surgical technique or clinical decision making, since all patients were under the care of one surgeon. In each instance if rfVIIa had not been used, the attending surgeon deemed that the patient would have needed to undergo surgical re-exploration.

Currently, there are no indications for the administration rfVIIa for hemorrhage following major abdominal and gastrointestinal surgery in the absence of inherited coagulation disorders. However, this and other studies have suggested that rfVIIa reduces blood transfusion requirements, posing both clinical and pharmaco-economic benefit. Additionally, rfVIIa should be considered as an alternative to re-operation for surgical control of bleeding in cases of postoperative hemorrhage refractory to blood products. However, thromboembolic complications may be a risk of this approach.

Randomized controlled studies and evaluations of clinical algorithms are needed to provide a prospective assessment of the role of rfVIIa in the common surgical challenge of postoperative bleeding. This study highlights that for patients undergoing long and complex gastrointestinal surgical procedures, dilutional coagulopathy from intraoperative resuscitation may be an under-recognized condition that exacerbates postoperative bleeding. For this subset of patients, rfVIIa in conjunction with traditional blood component resuscitation can control bleeding and decrease the probability of return to the OR in patients for whom re-operation would pose significant risk of complications. However, given the small sample size and limitations of this study, further research is warranted to describe the subset of patients who would benefit most and the cost-effectiveness of this approach.

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ORIGINAL ARTICLE

Impact on Dyslipidemia of the Laparoscopic Ileal Interposition Associated to Sleeve Gastrectomy in Type 2 Diabetic Patients

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Abstract
Background Dyslipidemia is known to increase significantly the odds of major cardiovascular events in the general population. Its control becomes even more important in the type 2 diabetic (T2DM) population. Bariatric surgeries, especially gastric bypass, are effective in achieving long-term control of dyslipidemia in morbidly obese patients.
Objective The objective of the study was to evaluate the control of dyslipidemia in patients with T2DM and BMI below 30 that were submitted to the laparoscopic ileal interposition associated to sleeve gastrectomy.
Methods An observational transversal study was performed in a tertiary care hospital, between June 2005 and August 2007. Mean follow-up was 24.5 months (range 12–38). The procedure was performed in 72 patients: 51 were men and 21 were women. Mean age was 53.1 years (38–66). Mean BMI was 27 kg/m^2 (22.1–29.4). Mean duration of T2DM was 10.5 years (3–22). Mean HbA1c was 8.5%. Hypercholesterolemia was diagnosed in 68% of the patients and hypertriglyceridemia in 63.9%.
Results Mean postoperative BMI was 21.2.kg/m^2 (17–26.7). Mean postoperative HbA1c was 6.1%, ranging 4.4% to 8.3%. Overall, 86.1% of the patients achieved an adequate glycemic control (HbA1c<7) without anti-diabetic medication. HbA1c below 6 was achieved by 50%, 36.1% had HbA1c between 6 and 7, and 13.9% had HbA1c above 7. Total hypercholesterolemia was normalized in 91.8% and hypertriglyceridemia in 89.1% of patients. Low-density lipoprotein below 100 mg/dl was seen in 85.7%.
Conclusions The laparoscopic ileal interposition associated to sleeve gastrectomy was an effective operation for the regression of dyslipidemia and T2DM in a non-obese population.

Keywords Dyslipidemia · Type 2 diabetes mellitus · Cardiovascular disease · Ileal interposition · Metabolic syndrome · Metabolic surgery

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Introduction

Patients with type 2 diabetes mellitus (T2DM) have an increased prevalence of lipid abnormalities, which contributes to higher rates of cardiovascular disease (CVD). Most of the complications and hospitalizations in diabetic patients are related to atherosclerosis, which is responsible for about 80% of all diabetic mortality. It is believed that as many as 50% of patients with newly diagnosed T2DM already have cardiovascular disease.[1]

Persons with low HDL cholesterol levels (less than 40 mg/dl [1.03 mmol/l] in men and less than 50 mg/dl [1.30 mmol/l] in women) are at increased risk of coronary heart disease,[2] and death from cardiovascular causes, especially if such persons have diabetes.[3] Epidemiologic studies and studies in animals suggest that raising the levels of HDL cholesterol may retard the development of atherosclerosis. In humans, each increase in baseline HDL cholesterol of 1 mg/dl (0.03 mmol/l) is associated with a 6% decrease in the risk of death from coronary disease or myocardial infarction.[4]

Intervention trials using statins to lower low-density lipoprotein (LDL) cholesterol have consistently shown substantial reductions in major cardiovascular events in the treated groups.[5] Furthermore, the magnitude of the reduction in events is a function of the extent of LDL cholesterol lowering, with each decrease of 40 mg/dl (1.0 mmol/l) in LDL cholesterol corresponding to a 24% reduction in major cardiovascular events.[6]

Hypertriglyceridemia is a common form of dyslipidemia that is frequently associated with premature coronary artery disease.[7] Whether hypertriglyceridemia causes coronary artery disease or is a marker for other lipoprotein abnormalities that cause premature coronary artery disease remains controversial.[8]

Bariatric surgery, especially gastric bypass[9] and malabsorptive surgeries,[10] are effective in achieving long-term control of obesity, T2DM, and dyslipidemia in morbidly obese patients. For non-morbidly obese patients, with BMI 23.6 to 34.4, the metabolic syndrome (MS) could no longer be characterized in 95% of the patients following the laparoscopic ileal interposition associated to a sleeve gastrectomy. The impact of this operation on the individual components factors of the MS was variable.[11]

The objective of this study is to evaluate the control of dyslipidemia in patients with T2DM and BMI below 30 that were submitted to the laparoscopic ileal interposition associated to a sleeve gastrectomy (LII-SG).

Materials and Methods

Seventy-two patients with dyslipidemia and T2DM were consecutively submitted to the laparoscopic ileal interposition associated to a sleeve gastrectomy (II-SV) between June 2005 and August 2007. There were 51 (70.8%) men and 21 (29.2%) women. Mean age was 53.1±6.4 years (range 38–66 years). Preoperative BMI ranged from 22.1 to 29.4, with a mean of 27±2.5 kg/m^2. Mean duration of T2DM was 10.5±4.7 years, ranged 3–22. Preoperatively, oral hypoglycemic agents were used by 52.8% of the patients (mean number of 2.4) and insulin by 47.2%. The demographics are better illustrated in Table 1.

Table 1 Demographics Data

	Total
Patients (*n*)	72
BMI (kg/m^2)	
Mean	27±2.5
Range	21.8–29.4
Age (years)	
Mean	53.1±6.4
Range	38–66
Gender	
Male	51
Female	21
Follow-up (months)	
Mean	24.5±3.8
Range	12–38
T2DM duration (years)	
Mean	10.5±4.7
Range	3–22
Treatment	
OHA	52.8%
Insulin and OHA	34.7%
Insulin only	12.5%

BMI body mass index

The diagnosis of T2DM was established according to the American Diabetes Association.[12] Inclusion criteria included documentation of hemoglobin A1c (HbA1c) above 7.5% for at least 3 months, stable weight, defined as no significant change, more than 3%, over the 3 months prior to enrollment, and evidence of stable treatment with oral hypoglycemic therapy and or insulin for at least 12 months.

The exclusion criteria included C-peptide levels below 0.5 nmol/l, elderly (>66 years), previous major upper abdominal surgery, pregnancy, malignant or debilitating diseases, severe pulmonary or cardiac diseases, severe renal disease (glomerular filtration rate below 30 ml/min), taking appetite suppressant medication, eating disorder such as bulimia or binge eating, and obesity due to any other endocrine disorder. There were no special criteria for the indication of the two different configurations of the procedures, although we assumed that the diverted version would be more effective in controlling T2DM.

An altered lipid profile was observed in 75% of patients. Hypercholesterolemia was diagnosed in 68% of the patients and hypertriglyceridemia in 63.9%. LDL was abnormal in 68% of the patients and HDL in 34.7%. According to the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (NECP/ATP III) guidelines, suggested target lipid levels would be LDL cholesterol below 100 mg/dl, triglycerides below 150 mg/dl, and HDL cholesterol above 40 mg/dl.[13] Specific preoperative tests included nonesterified

fatty acids, test for microalbuminuria, serum creatinine, and estimation of glomerular filtration rate. Other tests included Doppler study of the carotid arteries, retinopathy screening, and detailed cardiac evaluation. Biochemical markers of T2DM were obtained and included fasting plasma glucose, postprandial plasma glucose, glycosylated hemoglobin (HbA1c), fasting plasma insulin, the homeostasis model assessment of insulin resistance (Homa-R), and C-peptide. Plasma glucose concentrations were measured by a glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Model YSI 2300 STAT plus analyzer; YSI, Inc., Yellow Springs, OH, USA). Plasma insulin and C-peptide concentrations were measured by auto-DELPHIA automatic fluoroimmunoassay (Wallac, Inc., Turku, Finland).

Preoperative preparation for surgery included clear liquids for 48 h prior to the operation in association with regular insulin according to capillary glucose. Preoperative bowel cleansing, perioperative antibiotics, and low molecular weight heparin were administered.

The technique consisted in the interposition of a segment of ileum up to the jejunum or up to the duodenum associated to a sleeve gastrectomy. The sleeve gastrectomy was performed after devascularization of the greater curvature. The gastric resection started 2 to 3 cm above the incisura angularis up to the angle of Hiss. For the ileal interposition, the ligament of Treitz was identified, and the jejunum was divided 20 to 30 cm distally. Then, the cecum was identified, and the distal ileum transected 30 cm proximal to the ileocecal valve. A 150 to 170 cm of ileum was measured proximally and transected. This segment of ileum was interposed in an isoperistaltic way into the proximal jejunum, previously divided. Next, we performed three side-to-side enteroanastomosis. The first one was the ileo-ileostomy, then the jejuno-ileostomy, and finally, the ileo-jejunostomy. All three mesenteric defects were closed with interrupted sutures. To interpose the segment of ileum into the duodenum, the devascularization along the greater curvature of the stomach continued to the duodenum, 3–4 cm beyond the pylorus. The sleeve gastrectomy was performed as mentioned above. The duodenum was transected. An ileal segment of 170 cm was created 30 cm proximal to the ileocecal valve as described above. Next, we performed three anastomosis. The first was an ileo-ileostomy. Then, the segment of ileum was interposed in an isoperistaltic way into the proximal duodenum, previously divided, and the second anastomosis was done between the first portion of the duodenum and the proximal part of the interposed ileum. A point in the jejunum 50 cm from the ligament of Treitz was measured and anastomosed to the distal part of the interposed ileum. The three mesenteric defects were closed (Fig. 1). A detailed description was published elsewhere.[14]

Postoperatively, all patients were monitored three times daily through capillary plasma glucose and were under insulin therapy according to a sliding scale for 1 month. An appropriate and restricted diet was suggested. Following that, all anti-diabetic medications were to be discontinued until 18 months after the operation. Outcome measures were collected prospectively. The main parameters included evaluation of the total cholesterol, HDL, LDL, triglycerides, NEFFA, fasting and postprandial glucose, HbA1c, diabetes medications usage (agents, doses, and frequency), weight loss (expressed in BMI and percentage of weight loss), resolution or improvement of associated diseases and complications, reoperation rate, and morbidity–mortality of the procedure. Patient and laboratory evaluation were scheduled for every 3 months during the first year after surgery and yearly until 60 months of the operation.

The Hospital's Institutional Review Board approved the study, and all patients gave written informed consent.

Statistical Analysis

Statistical analysis was done using exact Fisher's test and Student t test according to the data. A significance level of 0.05 (α=5%) was adopted, and levels below this were considered significant.

Results

All 72 patients underwent laparoscopic ileal interposition associated to sleeve gastrectomy. Intraoperative complications (8.3%) included resection of an isquemic segment of 5 cm of the transposed ileum (one), hypertensive crisis (one), increased intraoperative bleeding due to intraoperative usage of aspirin^{R} (two), and cardiac arrhythmia (two). Associated procedures included eight (11.1%) cholecystectomies with cholangiography, three (4.2%) hiatal hernia repair, three (4.2%) extensive lysis of adhesions, and seven (9.7%) hepatic biopsies. Mean operative time was 155 min (range 120–220 min) for the II-SG and 175 min for the II-DSG (range 160–260 min). Overall, the mean operative time was 175 min. There was no conversion to open surgery. The median hospital stay was 4.8 days (range 2–51 days).

There was no mortality. Major postoperative complications occurred in 10 patients (13.9%) and included gastrointestinal bleeding that required blood transfusion (one), massive subcutaneous hematoma (two), gastric fistula (one), intra-abdominal abscess (one), prolonged ileus (three), acute renal failure (one), and severe cardiac arrhythmia (one). Two patients (2.8%) needed to be reoperated, one with gastric fistula on the 12th postoperative day and one intraabdominal abscess. Prolonged hospitalization was required for six patients (8.3%).

Following discharge from the hospital, during the first postoperative month, postprandial discomfort, early satiety,

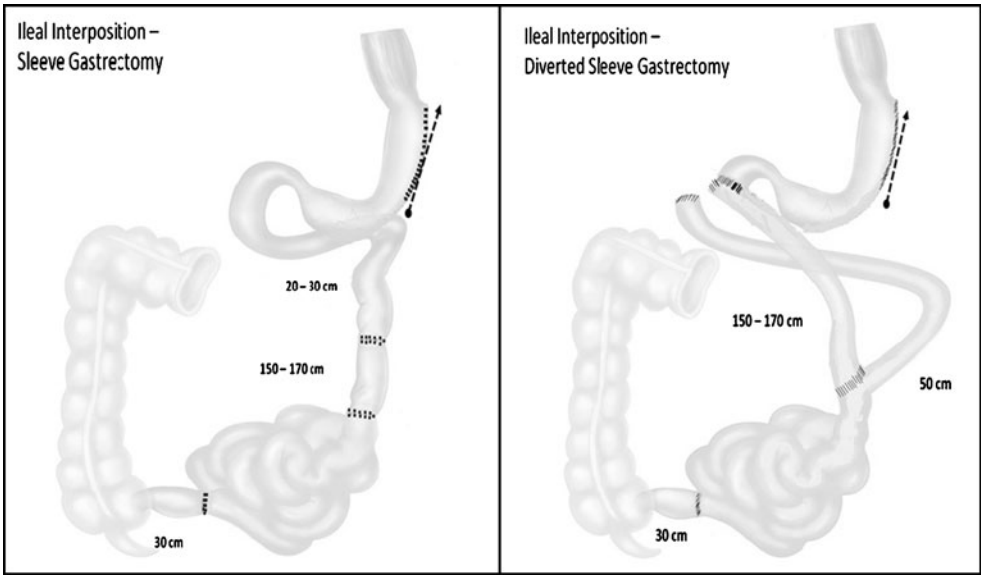

Fig. 1 Ileal interposition associated to a sleeve gastrectomy.

nausea, anorexia, heartburn, and discomfort in the lower abdomen were the most frequent complains. Two patients (2.8%) were readmitted into the hospital due to persistent vomiting. Both had uneventful resolution with appropriate therapy. There was a progressive improvement of these early symptoms late in the follow-up, with heartburn, anorexia, and food intolerance being the most important symptoms. Persistent diarrhea was observed in two patients (2.8%). There were six (8.3%) late hospitalizations: two due to acute cholecystitis that were submitted to a laparoscopic cholecystectomy, one acute appendicitis submitted to a laparoscopic appendectomy, one pneumonia, and two urinary tract infections.

All 72 patients were followed up a mean of 24.5 months (range 12–38).

The operation adequately treated dyslipidemia. Postoperatively, all patients achieved better parameters than preoperative levels, without medications. Overall, dyslipidemia could no longer be characterized in 90.7% of the patients. Evaluation at different moments, range 1 to 36 months, demonstrated a total cholesterol decrease from a mean of 204.4±57 to 157.4±22.8 mg/dl ($p<0.001$). These results were achieved as early as the first evaluation at 1 month following the operation. Up to 36 months, the results were nearly the same (Fig. 2). Hypercholesterolemia was diagnosed in 68% of the patients. Postoperatively, normal levels were observed in 91.8% of these patients. LDL decreased from a mean of 116±54.7 to 84.4±27.4 mg/dl ($p=0.014$) and had the same pattern of decrease as the total cholesterol (Fig. 3). Preoperative abnormal levels were seen in 68% of patients, while 85.7% had LDL below 100 mg/dl in the postoperative period. Triglycerides decreased from a mean of 241.5±208.4 mg/dl in the preoperative period to 109.8± 46.1 mg/dl postoperatively ($p=0.014$) (Fig. 4). Hypertriglyceridemia was diagnosed in 63.9% of the patients before surgery. It was normalized in 89.1% of the patients following the operations, while 4.3% still had triglycerides above 200 mg/dl. HDL increased from a mean of 45.5±5.6 to 47.5±5.2 mg/dl ($p=0.526$) (Fig. 5). HDL was altered in 34.7% of the patients and normalized in 68%. Five (6.9%) patients still had low levels of HDL and HbA1c above 6%. Nonesterified free fatty acids decreased from a mean of 0.68 mmol/l (0.4–1.41) to a mean of 0.54 mmol/l, $p=0.542$.

Mean HbA1c decreased from 8.5±1.8% to 6.1 ± 0.9% ($p < 0.001$), range 4.4% to 8.2%. Overall, HbA1c<7% was achieved by 86.1% of the patients without anti-diabetic medication. Normalization, HbA1c<6%, was reached by 50% of the patients. Glycemic control, HbA1c between 6% and 7%, was achieved by 36.1% of patients and improvement by 13.9% of the patients (Table 2). Nearly 86% of patients permanently discontinued preoperative oral hypoglycemic agents and/or insulin. The other 14% of patients were under oral agents. There was no patient requiring regular insulin therapy. Only 18 patients (25%) had HbA1c above 6.5%. For these patients, the use of metformin or sulfonylurea was implemented. Adjustment of these medications, as monotherapy, on an individual basis, determined a better HbA1c control with all patients achieving levels below 6.5%.

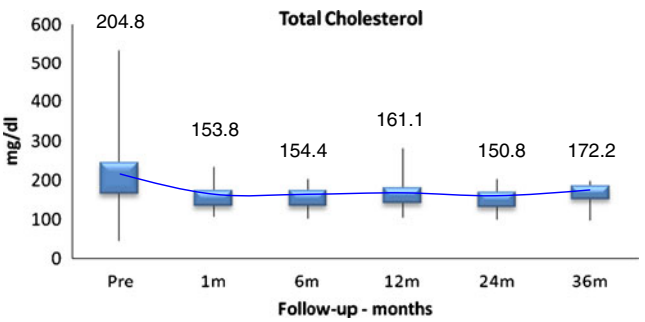

Fig. 2 Total cholesterol ($p<0.001$).

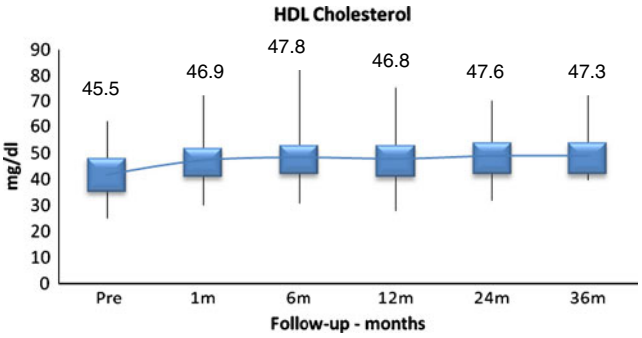

Fig. 3 HDL cholesterol (p=0.526).

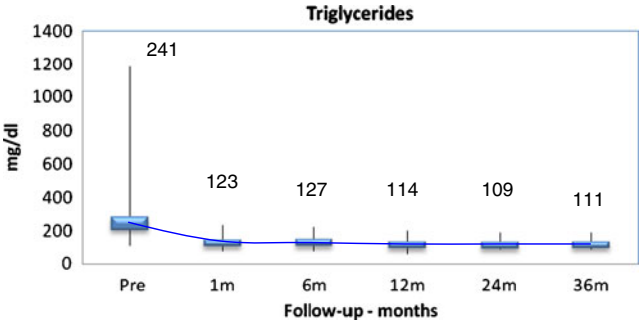

Fig. 5 Triglycerides (p=0.014).

Fasting plasma glucose decreased from 195.8±70.1 to 105.1 ± 23.1 mg/dl($p < 0.001$), and postprandial plasma glucose from 282.1±99.6 to 142.4±48.4 mg/dl (p<0.001) (Fig. 6). Fasting plasma insulin decreased from 17.6±19.5 to 3.9 ± 3.7 mU/ml($p < 0.001$). The homeostasis model assessment of insulin resistance decreased from preoperative levels of 6.4±8.2 to 1.2 ± 1.1($p < 0.001$). C-peptide decreased from 3.2±1.8 to 1.7 ± 0.8 ng/ml($p < 0.001$).

Mean BMI decreased from 27±2.5 kg/m^2 in the preoperative to 21.3 ± 2.3 kg/m^2($p < 0.001$) ranging from 17.8 to 26.2 kg/m^2 (Fig. 7). Mean percentage of weight loss was 22±3.6% of the initial weight. (p<0.001).

Preoperatively, hypertension was present in 55.5% of the patients by means of a casual blood pressure measurement. All patients were using anti-hypertensive medications, mean of 2.1. The blood pressure normalized (≤130/≤85 mmHg), without medication, in 92.5% of the patients (casual blood pressure measurement).

Preoperative nephropathy was diagnosed in 37.5% of the patients. Microalbuminuria was observed in 29.2% of the patients and macroalbuminuria in 8.3%, while 4.2% had glomerular filtration rate between 60 and 89 ml/min. Postoperatively, there was a substantial improvement in the renal function. Mean microalbuminuria decreased from 57.4± 138.3 to 27.6±82.2 µg/min (p=0.026). Microalbuminuria was still present in eight patients (11.1%) and macroalbuminuria in 4.2%. All patients with GFR between 60 and 89 normalized their filtration rate (>90 ml/min).

Coronary heart disease was identified in 20.8% of patients. Postoperatively, a major adverse cardiovascular event (fatal and nonfatal myocardial infarction, coronary insufficiency, heart failure, cardiovascular death, and stroke) was not characterized up to now. Carotid artery evaluation was abnormal in 43.1% of the patients, ranging from intimal–medial thickness to partial stenosis, up to 40% of the arterial lumen. Postoperative routine evaluation at 24 months demonstrated minor improvement.

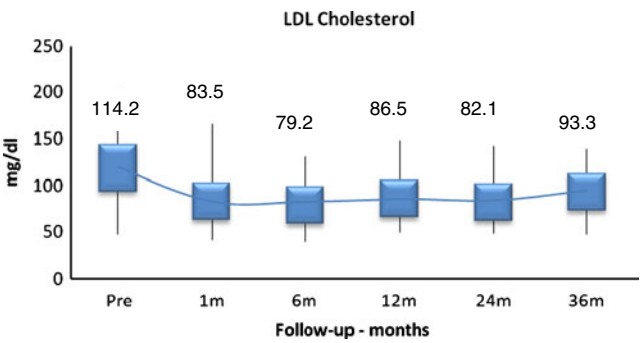

Fig. 4 LDL cholesterol (p=0.014).

Discussion

We found in this diverse cohort of type 2 diabetic patients with BMI below 30 that laparoscopic ileal interposition associated to a sleeve gastrectomy was an effective tool for the treatment of dyslipidemia. Overall, dyslipidemia could no longer be characterized in 90.7% of the patients. The impact of this operation on the individual components of dyslipidemia was variable.

Hypertriglyceridemia was normalized in 89.1% of the patients. We hypothesize that diverting the duodenum may alter the GIP resistant state of these T2DM patients. Zhou et al.[15] demonstrated that GIP plays a crucial role in switching from fat oxidation to fat accumulation under the diminished insulin action, and inhibition of GIP signaling ameliorated insulin resistance. Central obesity paired with insulin resistance is probably a major factor contributing to

Table 2 Type 2 Diabetes Mellitus Resolution According to Different Procedures

	Remission A1c≤6%	Control A1c 6.1–7%	Improvement A1c>7%
Total	50.0%	36.1%	13.9%

A1c glycated hemoglobin

hypertriglyceridemia.[16] Decrease in insulin resistance and central obesity was observed in almost all of these patients. A first step in evaluating patients with hypertriglyceridemia is to obtain an extensive family history. Hypertriglyceridemia is frequently associated with premature coronary artery disease. A family history of premature coronary artery disease would suggest familial combined hyperlipidemia or familial hypoalphalipoproteinemia.[17] This was suspected in 13% of these patients. All but one achieved an adequate control of triglycerides.

In the Framingham Heart Study, HDL cholesterol level was more potent as a risk factor for coronary heart disease than was the level of LDL cholesterol.[18] A non-significant rise of HDL was observed following both operations. The Expert Group on HDL recommends considering the addition of a fibrate or niacin for persons whose HDL cholesterol level is less than 40 mg/dl and who have diabetes.[19] In the postoperative period, five (6.9%) patients still had low levels of HDL and HbA1c above 6%, so lifestyle modifications, followed by the consideration of pharmacotherapy in these high-risk patients, were suggested.

In individuals without overt cardiovascular disease, the primary goal in treating dyslipidemia is LDL cholesterol below 100 mg/dl. Following these operations, 85.7% of patients with preoperative abnormal levels had LDL below 100 mg/dl, while 34.7% had LDL below 70 mg/dl. A reduction in LDL cholesterol to a goal of <70 mg/dl is an option in very high-risk diabetic patients with overt CVD.[20] Hypercholesterolemia resolution was achieved in nearly 92% of patients. In a study in rats, Tsuchiya et al.[21] demonstrated that ileal transposition to the upper jejunum affected lipid and bile salt absorption, attenuating cholesterol absorption and transport, possibly by promoting premature absorption of bile salts.

Cardiovascular disease is the major cause of morbidity and mortality for individuals with T2DM and the largest contributor to the direct and indirect costs of diabetes. Dyslipidemia and hypertension are coexisting conditions with T2DM, and benefits are seen when multiple risk factors are addressed globally.[22] Postoperatively, we found that 66.7% of patients had normal lipid profile, adequate blood pressure levels, and normal HbA1c, fasting and postprandial glucose. Individually, HbA1c below 7% was achieved by 86.1% of patients and control of hypertension in 92.5%. Lipid management aimed at lowering LDL cholesterol, raising HDL cholesterol, and lowering triglycerides has been shown to reduce macrovascular disease and mortality in patients with T2DM, particularly in those who have had prior cardiovascular events.[23] This was the case in 20.8% of the patients of this study. There was an early, as soon as 1 month, adequate, and stable control of postprandial glucose throughout the follow-up. A relation between mortality and plasma glucose at 2 h is observed and is independent of fasting glucose.[24] The postprandial plasma glucose and not fasting plasma glucose is associated with cardiovascular disease.[25]

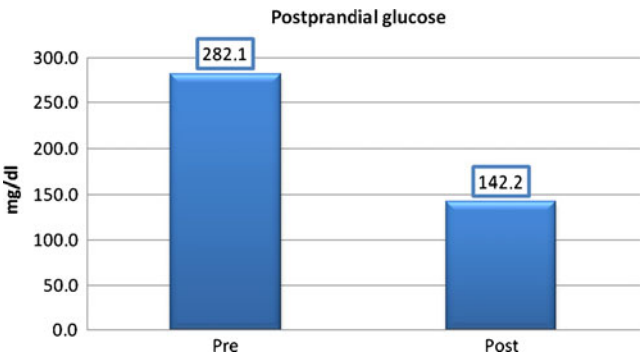

Fig. 6 Postprandial glucose ($p<0.001$).

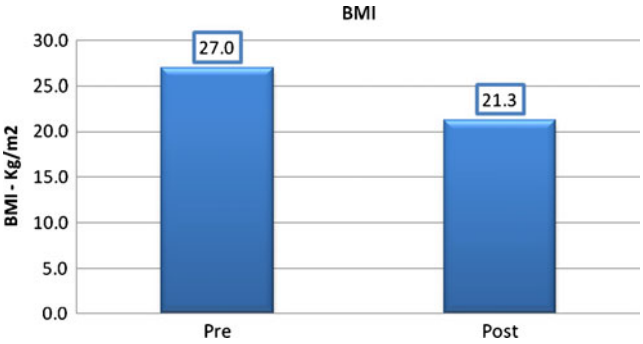

Fig. 7 Pre- and postoperative BMI ($p<0.001$).

It has been already demonstrated in previous published studies that this operation made an almost universal improvement in cholesterol and triglycerides levels,[26] and also that incretins hormones are impacted by these operations and that GLP-1 exhibited a statistically significant increase in response to meal stimulation. The observed preoperative GLP-1 blunted response was markedly changed, suggesting that levels of meal-stimulated GLP-1 may be restored toward normal with consequent possible improvement control of diabetes, especially postprandial glucose.[27] Kumar et al.[28] reported their preliminary results confirming the feasibility, safety, and efficacy of this surgical procedure in type 2 diabetic patients. Although we do not want to draw any definitive conclusion, the objective finding of no major cardiac events during this follow-up period may be the result of the global beneficial of the laparoscopic ileal interposition in different cardiovascular risks, including regression of dyslipidemia.

Disclosure statement The authors declare that they have no conflict of interest.

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HOW I DO IT

Robotic Distal Splenopancreatectomy: Bridging the Gap Between Pancreatic and Minimal Access Surgery

Dimitrios Ntourakis · Ettore Marzano · Patricia Alexandra Lopez Penza · Philippe Bachellier · Daniel Jaeck · Patrick Pessaux

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Abstract
Introduction Almost 10 years have passed since computer-aided, most commonly known as robotic surgery, has emerged gaining slowly but steadily its place within minimally invasive surgical procedures. Nevertheless, pancreatic surgeons only recently have started incorporating it into current practice.
Methods In this 'how I do it' article, we describe our method for robotic distal splenopancreatectomy, focusing on its technical advantages, as well as its drawbacks. Furthermore, we describe some pitfalls commonly encountered during the procedure and we propose ways to avoid them.
Conclusion Pancreatic robotic-assisted surgery is offering many practical advantages over the "classic" laparoscopic approach. Even though a difficult procedure to master, it may have the potential to establish the concept of minimally invasive surgery in areas where it is nonexistent as in pancreatic surgery.

Keywords Robotic surgery · Computer-assisted surgery · Pancreas · Pancreatic surgery · Minimal invasive surgery

Introduction

Pancreatic surgery remains a surgical field unreceptive to minimal invasive surgical techniques. A few possible explanations for this are the already demanding technical aspects of pancreatic surgery, its high operative morbidity and the poor survival of patients with pancreatic cancer.[1] Recent advances both in technology and surgical skills have made possible the endeavor of laparoscopic duodeno-pancreatectomy and laparoscopic distal splenopancreatectomy (DSP) in specialized centers.[2] However, its uses are still limited by a steep learning curve and a lack of data regarding its safety regarding long-term oncologic outcome. The increasing use of computer-assisted, or as more commonly known robotic surgery, in other fields of general surgery has eventually led to the first robot-assisted pancreatic resections.[3, 4] These first publications are rather optimistic, advocating that the benefits are important enough to uplift the concept of minimally invasive pancreatic surgery.

In this short report, we describe our technique for the robotic-assisted DSP, focusing on the technical aspects of the procedure.

Surgical Technique

The operation starts with patient under general anesthesia, in the supine anti-Trendelenburg position with a wedge support behind his left flank. The robotic system Da Vinci S (Intuitive Surgical, Sunnyvale, CA, USA) is placed on the upper left side of the patient. The assistant is positioned on the right side of the patient and the scrub nurse on the left. The operating theater setup is illustrated in Fig. 1.

A pneumoperitoneum of 12 mmHg is achieved by placing a 12-mm optical port in the left para-umbilical area, with the

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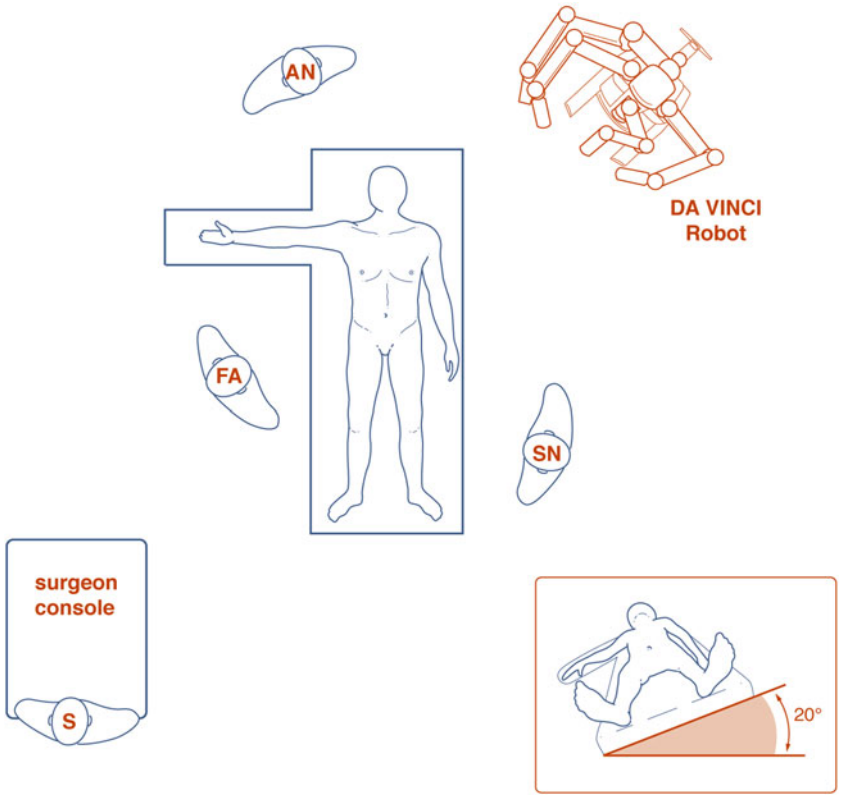

Fig. 1 Operating theater setup for robotic distal splenopancreatectomy. Patient supine with the right arm extended. *S* surgeon (at the operating console), *FA* first assistant, *SN* scrub nurse, *AN* anesthesiologist.

Hasson technique. Six ports are used in total, placed as shown in Fig. 2. The 12 mm 30° optics laparoscope is preferred.

After careful exploration of the peritoneal cavity and peritoneal viscera in order to rule out metastatic disease, the major omentum is retracted upwards and the lesser sac is entered as, in open surgery, through extensive division of the colo-epiploic ligament. Then the splenocolic ligament is divided and the left colic flexure is mobilized and retracted inferiorly. The posterior gastric wall is grasped by the robot arm no. 3 and retracted cephalad exposing the anterior border and the inferior margin of the pancreas.

Using ultrasonic shears—Harmonic scalpel (Intuitive Surgical, Sunnyvale, CA, USA)—the peritoneum at the inferior border of the pancreas is dissected, until the superior mesenteric vein (SMV) is recognized. Then the dissection is continued cephalad, following the anterior border of the SMV (Fig. 3). The pancreas is retracted upwards by the robot arm no. 3 and blunt dissection is continued between the SMV and the posterior pancreatic surface creating a retropancreatic tunnel. A surgical tape is passed around the neck of the pancreas which is retracted upwards by the assistant facilitating the exposure of the inferior pancreatic border. The splenic–inferior mesenteric vein confluence is exposed. The proximal splenic vein is dissected free from the pancreas, and is placed on a vessel loop (Fig. 4). The pancreas is retracted caudally by the assistant, and the dissection is continued at the superior pancreatic border. The splenic artery is recognized and isolated with a vessel loop (Fig. 5). Care must be exercised to prevent inadvertent injury of the common hepatic artery during this step.

The splenic artery is secured with three absorbable vascular clips and transected. A linear EndoGIA stapler (Covidien, Dublin, Ireland) loaded with a white vascular cartridge is used through the assistant port A to control and divide the splenic vein. Finally, the pancreas is transected by EndoGIA stapler with a vascular cartridge passed again through port A (Fig. 6). Hemostatic sutures of Prolene 5/0 or 6/0 (Ethicon, New Brunswick, NJ, USA) are placed on

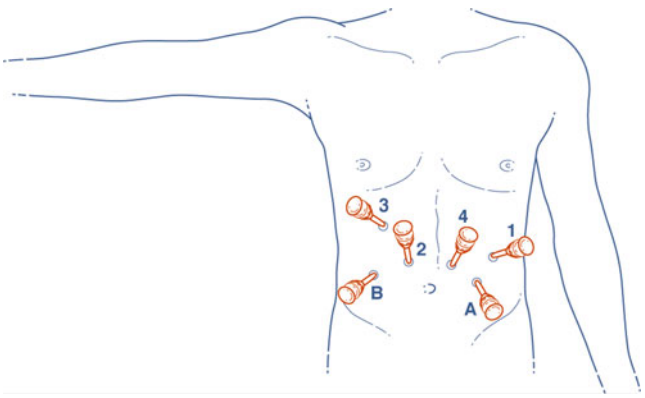

Fig. 2 Port setup for robotic distal spleenopancreatectomy. *1* and *2* 8-mm working ports for robot arms nos. *1* and *2*. *3* 8-mm port for robot arm no. 3 used for retraction. *4* 12-mm optical port. *A* 12-mm working port for assistant. *B* 12-mm port for assistant (optional for retractor). The *numbers* correspond to the robot arm used.

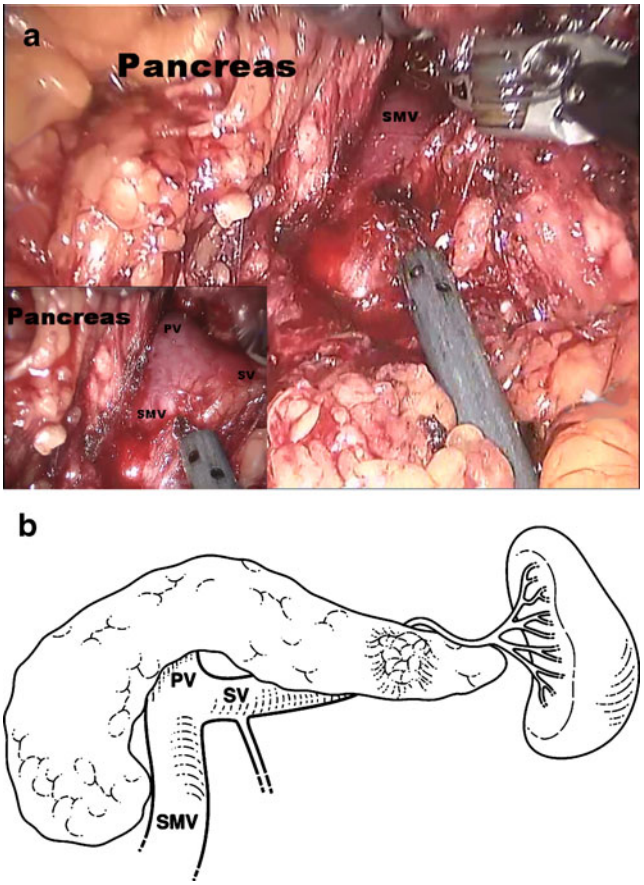

Fig. 3 **a** Retropancreatic tunnel and superior mesenteric vein dissection Inlay: superior mesenteric vein and splenic vein confluence dissected. **b** black and white line drawing *SV* splenic vein, *SMV* superior mesenteric vein, *PV* portal vein.

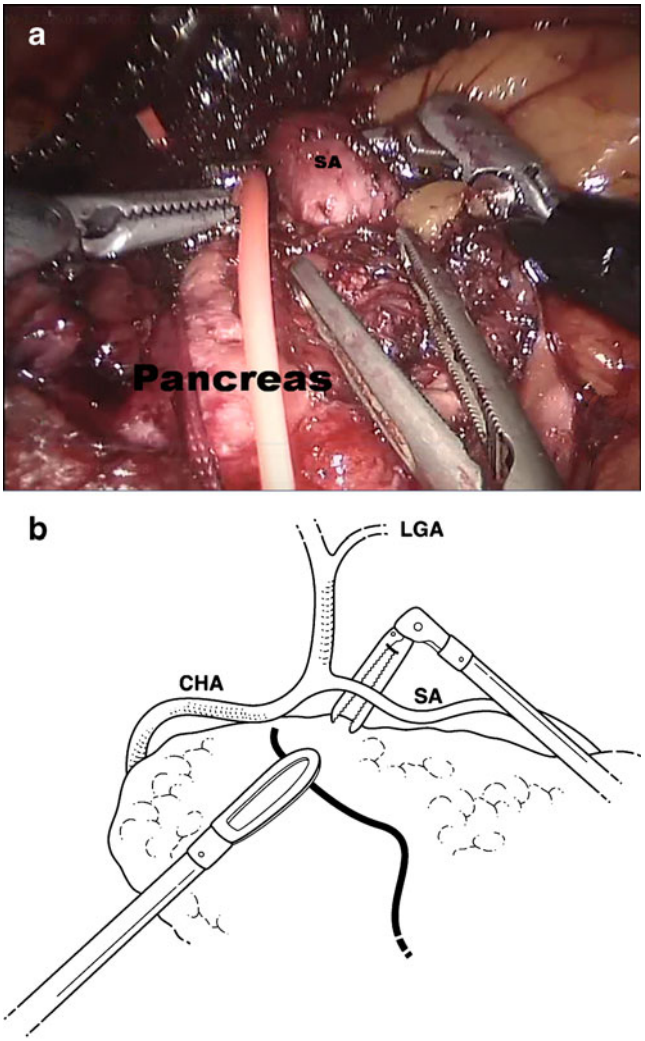

Fig. 5 **a** Splenic artery in vessel loop. The pancreas is retracted caudally. **b** black and white line drawing *SA* splenic artery, *CHA* commun hepatic artery, *LGA* left gastric artery.

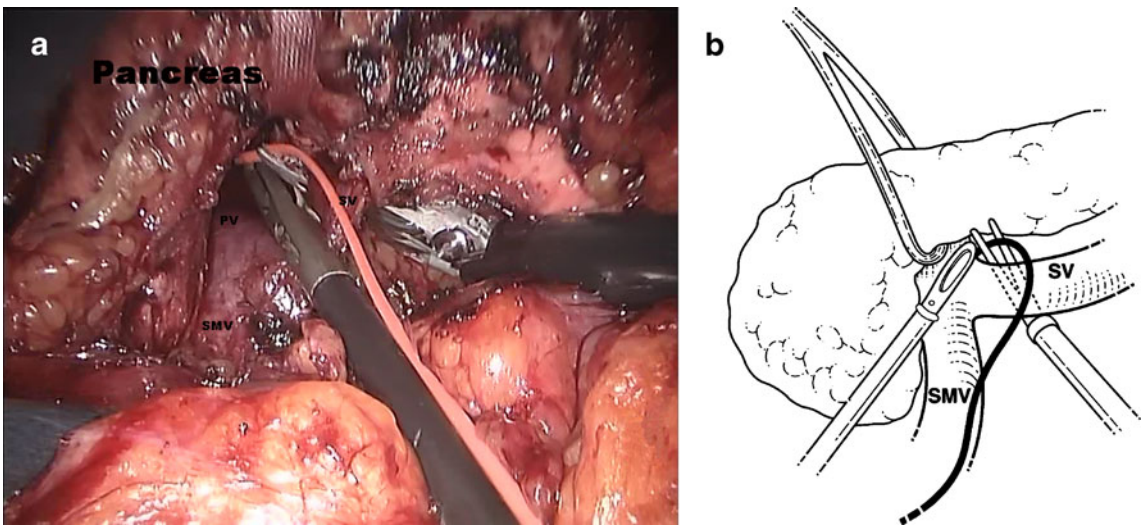

Fig. 4 **a** Splenic vein in vessel loop. The pancreas is retracted cephalad by a white tape. **b** black and white line drawing *SV* splenic vein, *SMV* superior mesenteric vein, *PV* portal vein.

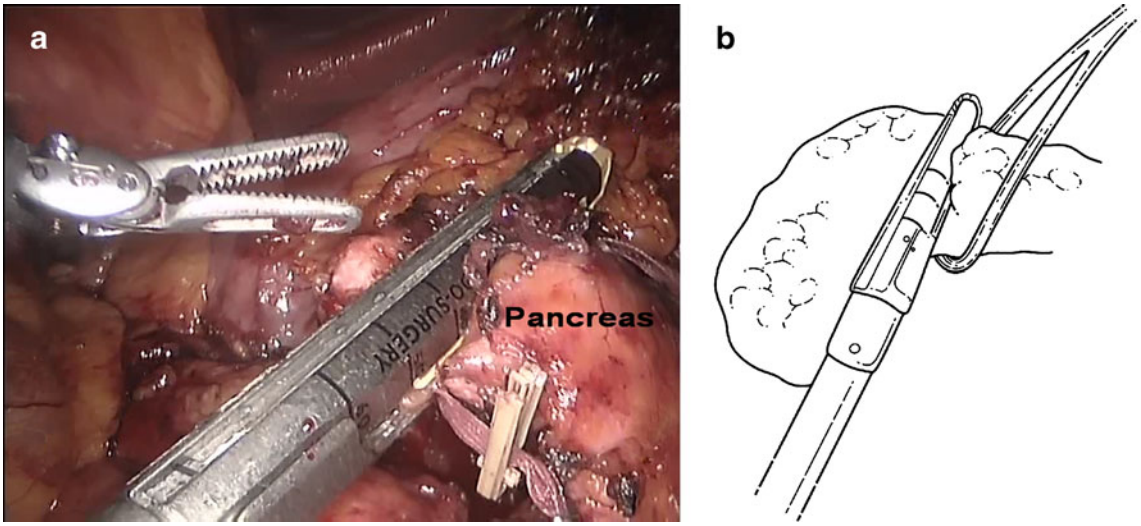

Fig. 6 **a** Transection of the pancreatic body by a vascular stapler. **b** black and white line drawing.

the pancreatic stump if necessary to secure the pancreatic duct as well as to control minor bleeding.

The body and the tail of the pancreas are dissected free with the ultrasonic shears on its inferior border from the neck towards the tail. The first assistant retracts the transected distal pancreas upwards facilitating exposure. During this step the inferior mesenteric vein is encountered and transected after coagulation. Care must be taken to keep the dissection plane anteriorly of the renal fascia (Gerota's fascia) and the left adrenal. The gastrosplenic ligament and the short gastric vessels are controlled with metal clips proximally and then coagulated and divided with the harmonic scalpel distally. With the aid of a laparoscopic fan retractor the spleen is raised upwards, exposing the splenorenal and splenophrenic ligaments that are dissected by the ultrasound shears. The specimen is placed in a 15-mm laparoscopic endo-bag and extracted through a small Pfannestiel incision. Finally, after hemostasis, a silastic drain is placed in the lesser sac at the proximity of the pancreatic stump.

Results

This technique has been used in two selected patients with pancreatic body and tail tumors. The first patient was a 58-year-old male with a history of multiple sclerosis in whom was incidentally discovered at computed tomography (CT) scan a 3.5-cm hypodense lesion of the pancreatic tail with central calcifications. The diagnosis of a non-secreting nonmetastatic pancreatic endocrine tumor was made by a magnetic resonance imaging (MRI), an Octreoscan Scintigraphy and serum chromogranin-A level. The patient underwent Robotic DSP. The operative time was 6 h with an estimated blood loss of 350 ml. The postoperative period was uneventful except for a motor deficit of the lower limbs, attributed to the multiple sclerosis, requiring physiotherapy. He was discharged on postoperative day 14. The histological finding showed a well-differentiated pancreatic endocrine tumor with a low proliferation index (Ki-67=1%) without lymph node involvement (0/12).

The second patient was a 28-year-old female with a history of hyperthyroidism due to a multinodular goiter. A CT scan, performed because of a persistent high C-reactive protein level following cesarean section, discovered a 7-cm cystic lesion of the tail of the pancreas. The MRI scan showed a macrocystic lesion with internal septa, in contact with the spleen hilum. The tumor marker Ca 19-9 was normal. The patient underwent Robotic DSP. The operative time was 5 h with an estimated blood loss of 250 ml. The postoperative period was uneventful and she was discharged on postoperative day 8. The histological finding revealed a solid pseudopapillary tumor of the pancreas (pT2N0 (0/14)).

In both patients, there was no evidence of clinical, biological, and radiological pancreatic fistula (a control CT scan on postoperative day 8 did not show any abdominal fluid collection).

Discussion

A search of literature yields many articles describing the laparoscopic approach to distal pancreatectomy.[5, 6] The technique has been well described, and all authors agree that it is a technically demanding operation, requiring advanced laparoscopic skills. Nevertheless, some controversy exists concerning its indications, as for some authors its safety concerning long-term oncologic outcome has not been demonstrated.[1, 7]

Concerning the technical aspects, robotic DSP offers many benefits in comparison to the laparoscopic approach. Primarily, hand–eye coordination is greatly facilitated by the 3D stereoscopic vision. The incorporated motion scaling and muscle tremor filters enhance the capability to perform delicate tasks such as vascular dissection even at lengthy procedures, blunting the kinetic effects of the surgeon's fatigue.[8] Furthermore, the operator, who is not sterile, is comfortably seated in front of the console with his arms and head fully supported. It should be noted that the 6° of motion that the Da Vinci S robotic arms offer, make possible the execution of both blunt and sharp dissection in a fluid manner similar to that of an open surgical procedure.[9] Interestingly, the surgeon to robot interface resembles in many ways the hand and wrist movements performed during open surgery. In our experience, both the dissection of the splenic vein and artery as well as the creation of the retropancreatic tunnel were more easily feasible with the Da Vinci system in comparison to laparoscopy. Similar advantages are evident during the handling of suture needles and the practice of endocorporeal knots. Finally, the control of the robotic camera by the surgeon guarantees image stability and handling of the image field.

But as with all newly methods, several pitfalls may also be encountered during the procedure. Primarily, the setup of the robotic arms necessitates good spatial planning and is time consuming, especially at the beginning of the learning curve.[9] The placement of the robot on the upper left side of the patient necessitates displacement of the anesthesiology equipment towards the patient's upper left side. This impedes the anesthesiologist's access to the patient's head and left arm. Similarly uneasy is the position of the first assistant; his arms are between the robotic arms, requiring constant counterpoise to their movement. The lack of haptic and force feedback is evident but for the most part is counterbalanced by the beneficial effect of the stereoscopic vision.[8] Unfortunately, not all of the robot appendages offer 6° of motion. The ultrasonic shears works with only 3° of motion making its application ungainly in the mobilization of the splenic ligaments due to the extreme angle of dissection. Moreover, in order to change the operating table tilt to a steeper right anti-Trenelenburg position to facilitate the exposure of the splenic ligaments the robot arms have to be retracted and placed anew. As such the robot setup partly negates the possibility to achieve intestinal shift by constant by tilting the patient; a trick commonly used in laparoscopic surgery. Finally, utilization of the console interface requires superior psychomotor skills, as the surgeon has in his disposition two master controls operated by the thumb and index finger of each hand and five foot pedals. The above drawbacks compiled, make this procedure rather demanding, with a steep learning curve.

In conclusion, the use of robotic-assisted systems in pancreatic surgery is promising, even though its long-term oncologic results is yet to be investigated.[10, 11] In our experience it offers many advantages in the context of distal splenopancreatectomy even though it has a difficult learning curve. If haptic and force feedback interfaces become available in the future, robotic-assisted surgery will stride many steps closer to the open surgical experience. For now, the development of this technology along with the training of surgeons in its use will most likely yield important benefits for the minimally invasive pancreatic surgery.

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REVIEW ARTICLE

Adequate Length of the Distal Resection Margin in Rectal Cancer: From the Oncological Point of View

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Abstract
Introduction The distal resection margin (DRM) has been considered an important factor for the oncological outcome of rectal cancer surgery. However, the optimal distal margins required to achieve safe oncological outcome remains to be controversial.
Material and methods More recently, as circumferential resection margin or mesorectal margin has been additionally reported to be more important factors predicting patient outcome than the distal mucosal margin, a re-evaluation of the impact of DRM on patient outcome is needed.
Results The extent of distal tumor spread is known to be influenced by a variety of factors such as tumor location, lymph node metastasis, and tumor size. DRM might affect survival more than a local recurrence. Because distal intramural tumor spread rarely exceeds 1 to 2 cm in most rectal cancers, and local control and survival do not seem to be compromised by shorter distal resection margins, the generally accepted practice is to aim for a 2-cm DRM. However, in the recent trend of curative resection after preoperative chemoradiotherapy, with an otherwise favorable tumor such as well-differentiated tumor and no lymph node metastasis, a DRM at ≤1 cm does not necessarily portend a poor prognosis. In cases with preoperative chemoradiotherapy, distal resection margins need to be evaluated individually.
Discussion It has been suggested that down-staging of low-lying rectal cancers after preoperative radiation might well include the pathological clearance of distal intramural microscopic spread. Moreover, the measurement of DRM varies with respective study, making it difficult to compare.
Conclusion We need an applicable intraoperative method to accurately measure distal resection margin, enabling comparative outcome.

Keywords Distal resection margin · Local recurrence · Survival · Rectal cancer

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Introduction

The factors affecting the oncological outcome after rectal cancer operation have been investigated with progression of treatment modalities. Local recurrence has been the most common and major morbidity in patients with rectal cancer. Several authors have reported on the relationship between the distal resection margin (DRM) and local recurrence.[1–4]

Standard recommendation requires at least 5 cm of DRM as therapeutic goal.[5] Recently on the other hand, common application of sphincter-saving resection has led to somewhat generous acceptance of distal margin at various limit, which resulted in inconsistent outcome.[2,6–9] Although the distal margin at 5 cm has been advocated in the past, more recent data suggest that 2 cm margin is enough.[2–4,8,10,11]

Paty et al. found no increase in pelvic recurrence when the DRM was <2 cm compared with ≥2 cm.[11] Other investigations have even suggested that the DRM less than 2 cm did not increase local recurrence or compromise survival.[2,8] The current National Comprehensive Cancer Network guidelines recommend DRM of 4–5 cm for partial mesorectal excision and 1–2 cm for total mesorectal excision (TME), in patients with low rectal cancer.[12]

The status of the resection margin is one of the most important factors determining local recurrence after surgery.[13,14] The optimal DRM required to achieve an adequate oncological outcome remains controversial. Moreover, while the focus has previously been concentrated on distal mucosal margin, the circumferential or mesorectal margin has gained importance in rectal cancer operation more recently. The circumferential resection margin has been reported to be more important by some investigators than the distal margin.[14,15] In a Dutch study dealing with 1,861 patients, 17% of patients with positive circumferential margin had a 2-year local recurrence rate as 13% compared to 4% with negative margin.[16]

In addition, tumor spread beyond the distal tumor burden has been more frequently detected in advanced disease. Some studies have found that the length of DRM was not related to the local failure rate.[8,17] The optimal length for the DRM further remains to be determined with increment of preoperative chemoradiotherapy. The optimal length of the DRM and its importance has been thereby challenged in terms of safe oncological outcome. I performed a systematic literature search of National Library of Medicine (PubMed; January 1980 to September 2009). The following medical subject headings were used: rectal cancer, distal resection margin, recurrence, local recurrence, outcome, and survival. Literatures written in English were included. The bibliographies and all potentially relevant articles were then retrieved.

Extent of Intramural and Mesorectal Spread Reported in the Literature

Distal spread of rectal cancer has been reported as either intra- or extramural tumor extension to the anal side. The extent of distal spread is extremely important in deciding the DRM from the tumor when performing sphincter-preserving operations increased with the recent development of circular stapling devices and techniques such as the hand-sewn colo-anal anastomosis.[18,19]

Since Heald et al.[20] first reported the presence of microscopic deposits in the mesorectum and subsequently proposed a new surgical procedure for rectal cancer, TME, many investigators have described the pathological features of cancer spread in the mesorectum (Table 1). Heald et al. reported distal mesorectal spread to be as 4 cm from the distal tumor edge.[20] Scott et al. described discontinuous mesorectal deposits that were present 5 cm below the tumor mass.[21] The length of 5 cm is the greatest microscopic extent reported to date. Several investigators found that patients with distal spread to the lymphatics of the mesorectum have worse outcome at 4 years compared with patients without it.[20–22] Zhao et al. reported a 22.2% rate of distal intramural and/or mesorectal spread among 45 patients with lower rectal cancer who underwent curative surgery, of a maximum of 1.2 and 3.6 cm, respectively, in his prospective study.[23] According to their report, distal mesorectal spread is significantly correlated with a mesorectal lymph node involvement. They suggested 1.5 cm for the rectal wall and 4 cm for the distal mesorectum for an adequate oncologic resection. Shirouzu et al. analyzed retrospectively and pathologically 610 consecutive rectal cancer specimens to examine distal rectal spread of rectal cancer.[3] They reported a 7.2% rate of distal mesorectal spread, with most not exceeding 2 cm from distal tumor margin.[3] Fewer than 10% of the patients had cancer spread

Table 1 Extent of Distal Spread of Rectal Cancer

Author	Year	No. of patients	Rate of distal spread (%)	Extent of distal spread	Recommended distal resection margin
Williams NS	1983	50	6	1.3 cm	<5 cm
Shirouzu K	1995	610	7.2	2 cm	1 cm
Scott N	1995	20	20	3 cm	3–5 cm
Reynold JV	1996	50	24	5 cm	TME
Hida J	1997	198	20.2	Upper rectum, 4 cm; lower rectum 3 cm	5 cm
Tocci A	2001	53	35.1	–	TME
Ono C	2002	40	7.5	2.4 cm	3 cm
Wang Z	2006	62	6.5	<3 cm (almost stage III)	4 cm
Guillem JG	2007	109	–	2.1 cm (intramural spread, 0.95 cm)	1 cm

beyond 2 cm. Whenever distal intramural or extra rectal spread is present, it is limited to within 2 cm in 95% of all patients.[8,24]

The length of distal spread in rectal cancer is different, depending on various tumor factors. The extent of distal intramural or extramural spread is influenced by tumor location, lymph node metastasis, and tumor size. Andreola et al. reported that distal intramural spread appears to be strictly related to the tumor size, infiltration of the perirectal adipose tissue, multiple positive lymph nodes, presence of neoplastic emboli in the intramural lymphatic vessels, and neoplastic invasion of the nerve branches,[1] in their patients with lower third rectal cancer. They showed that the extent of distal intramural spread differed with tumor stages, and it did not exceed 1 cm in most cases. Hida et al. noted that in patients with pT3 and pT4 rectal cancer, the extent of distal mesorectal spread was related to the tumor location.[25] The longest distance to metastatic lymph node was 2 cm for rectosigmoid cancer, 4 cm for upper rectal cancer, and 3 cm for lower rectal cancer. Therefore, they concluded that the mesorectal margin of at least 5 cm is required in locally advanced rectal cancer. Shirouzu et al.[3] showed that distal spread was different for different tumor stages. The rate of distal spread was 0% in stage I, 1.2% in stage II, and 5.1% in stage III disease. In most cases, the spread was limited within 1 cm from distal tumor margin. Guillem et al.[26] assessed the extent of distal spread after preoperative chemoradiotherapy in 109 patients with mid to low rectal cancer. Distal resection margins were clear in all patients, and distal intramural spread of cancer was shown to be up to 0.95 cm from distal tumor margin

These findings showed that distal rectal cancer spread was confined to within 2 cm from the tumor. A recent series on patients with rectal cancer that received preoperative chemoradiotherapy suggested a shorter distal margin for oncologic safety. It remains as yet unclear whether preoperative chemoradiotherapy leaves distal tumor spread to a shorter distance or not. Even in cases without preoperative chemoradiotherapy, some authors suggest that the distal resection margin length at 1 cm is sufficient.[13,27] Randomized-controlled trial (RCT)-based further studies are needed in terms of known spreading factors, namely, tumor stage, size, and location.

Influence of Distal Resection Margin on Oncologic Outcome Reported in the Literature

A correlation between the length of the DRM and oncologic outcome has been evaluated by several investigators (Table 2).[2–4,8] Karanjia et al. compared patients that underwent anterior resections with TME that had ≤1- and >1-cm distal margins.[28] They found no difference between the two groups regarding local recurrence or survival. Pollet et al. retrospectively assessed the oncologic outcomes in 334 patients with rectal cancer who underwent curative surgery.[8] They categorized patient into three groups regarding length of distal resection margin: ≤2, >2, and <5 cm, and ≥5 cm. Three groups did not show difference for local recurrence and overall survival. They showed a similar result that local recurrence and overall survival did not differ in patients with more than 2 cm of DRM and those with the less margin. In the other study using 128 patients, including 55 patients with preoperative chemoradiotherapy, there was no significant difference in local recurrence between the two groups (one recurrence in the two groups: 11% for ≤2 cm vs. 6% for >2 cm of DRM, P=0.42).[11]

By contrast, Leo et al. investigated the prognostic role of the distal clearance margin in 203 lower rectal cancer surgery.[29] They classified distal clearance margin as positive or negative one using 1-cm cutoff. The 5-year survival rates with positive, <1 cm negative, and ≥1 cm negative distal margin were 51%, 81%, and 69%, respectively (P=0.018).

Table 2 Comparisons of Oncological Outcomes Regarding Length of Distal Resection Margin

Author	Year	Case	Study type	Local recurrence			5-Year overall survival			Distal resection margin (cm)
				Negative (%)	Positive (%)	*P*	Negative (%)	Positive (%)	*P*	
Pollett WG	1983	334	Retrospective	7	7.3	NS[a]	69.1	68.4	NS[a]	2
Vernava AM III	1992	243	Prospective	10.5	30	0.01	67.5	49.3	0.01	1
Paty PB	1994	128	Retrospective	6	11	0.42	–	–	–	2
Shiouzu K	1995	610	Retrospective	–	–	–	66.1	37.7	0.03[b]	1
Kwok SPY	1996	55	Prospective	–	–	–	88	70	0.19	2
Leo E	2009	203	Retrospective	8	30.3	0.006	81.1	51.	0.031	Clear

[a] Non-specific

[b] Only patients with stage III

The difference between the positive and negative distal margins was significant (P=0.031), but not between the negative margins <1 and ≥1 cm (P=0.106). The local and distant 5-year recurrence rates of the three groups were 30%, 8%, and 8% (P=0.006) and 38%, 26%, and 19% (P=0.857), respectively. The differences were only significant when the positive and negative margins were compared.

Vernava et al. found a decreased 5-year survival and increased anastomotic recurrence rate when the DRM were ≤ 8 mm[4] in 243 patients who underwent curative anterior resection for rectal cancer. Shirouzu showed that most of the 61 patients with distal spread died due to distant metastasis rather than local recurrence in their 610 patients between 1982 and 1994.[3] Williams et al. reported similar findings, suggesting that distal spread should be regarded as a more systemic spread rather than regional one.[2] Indeed, the impact on survival of rectal cancer with distal spread has also been studied.[2,4,10,30,31] Penfold reported that no patient with intramural extension of more than 1 cm survived more than 5 years.[31] Recently, Vernava et al. prospectively evaluated the DRM length, and found that the recurrence and 5-year survival rates were not improved even if the resection length was greater than 1 cm.[4] Similar results were reported by other investigators.[2,10,30] Most patients with distal spread had a lower survival rate and died of distant metastasis rather than of local recurrence, even though they had undergone a successful curative operation. Concurrently in cases with distal tumor spread, a longer DRM could not provide survival benefit. However, it could not be conclusive on the impact of length of DRM on oncologic outcome because the studies were retrospective and lack the number of patients included in studies though it is difficult to perform prospective study regarding this issue.

Distal Resection Margin in Patients with Preoperative Chemoradiotherapy

Recent studies consistently demonstrate considerable regression of rectal carcinomas with preoperative chemoradiotherapy, and this may offer an important adjunct for sphincter preservation.[32–36] A sphincter-saving resection was traditionally determined by the distance between tumor and the anal sphincters, considering the potential risk for microscopic tumor in the rectal wall below the tumor. Therefore, at least 5 cm of distal margin was required until the 1980s, and hence, 2 cm was considered adequate.[2,8] When we consider the median length of the anal canal to be the 3–4 cm,[37,38] the rectal tumors located below 5 cm from the anal verge are practically not considered for sphincter-saving procedures. Indeed, it is not technically feasible to obtain a 2-cm distal margin by conventional techniques.

Distal intramural spread of tumors rarely exceeds 1 to 2 cm in most rectal cancers[1,2,39], and local control and survival do not seem to be compromised by shorter DRM.[2,4,8,11,28,40,41] Consequently, the 2-cm distal margin is generally indicated for rectal cancer operation. However, because the likelihood of distal spread beyond 1 cm increases with the tumor stage,[3] this policy may not be justified in locally advanced rectal cancers requiring preoperative chemoradiotherapy. Factors associated with distal tumor spread beyond 1 cm include an advanced disease stage at diagnosis, histologically aggressive poorly differentiated or undifferentiated tumor,[8] and lymphovascular and perineural invasion.[42] These factors are also known as poor prognostic factors regardless of DRM. On the other hand, patients with microscopically clear resection margin after preoperative chemoradiotherapy may be adequate candidates for shorter than 2-cm distal margin in cases with histologically favorable tumor[42] (Table 3). The down-staging of low-lying rectal cancers probably achieves the pathological clearance of distal tumor spread after preoperative radiation.[43,44]

Guillem et al. analyzed distal intramural spread in 109 patients with rectal cancer after preoperative chemoradiotherapy and TME.[26] They used a comprehensive whole-mount pathology technique. Only two patients (1.8%) had intramural extension beyond the tumor edge measuring less than 0.95 cm. They concluded that resection margins of 1 cm are appropriate for patients treated with preoperative chemoradiation. These results were supported by others who noted no adverse effects with DRM less than 1 cm on local recurrence and the disease-free survival rates.[43,44]

Table 3 Influence of Distal Resection Margin on Oncological Outcome in Patients with Preoperative Chemoradiotherapy

Author	Year	Case	Study type	Local recurrence			5-Year overall survival			Distal resection margin (cm)
				Negative (%)	Positive (%)	P	Negative (%)	Positive (%)	P	
Kuvshinoff B	2001	28	Prospective	0	10	NS[a]	–	–	0.06	1
Moore HG	2003	94	Retrospective	9	12	0.99	85	82	0.8	1
Rullier E	2005	92	Prospective	0	2	NS[a]	–	–	–	Clear

[a] Non-specific

Kuvshinoff et al. addressed whether sphincter-sparing techniques with distal margins ≤1 cm adversely influenced the oncological outcome in patients received preoperative chemoradiotherapy.[43] They showed that a distal margin clearance <1 cm did not adversely affect pelvic recurrence or disease-free survival. Despite margin as short as 1 mm, there was only one pelvic recurrence in 28 patients (3.6%) that underwent sphincter preservation surgery. In their study, only limited radial margins (≤3 mm) were associated with increased disease recurrence. Moore et al. compared recurrence-free survival and local recurrence at 3 years between ≤1 and >1 cm and between ≤2 and >2 cm groups in 94 patients with preoperative chemoradiotherapy, without showing significant differences between them.[44] They concluded that for patients with locally advanced rectal cancer undergoing resection after preoperative chemoradiotherapy, distal margin at ≤1 cm do not compromise the oncological outcome. Some investigators have reported that clear resection margin is sufficient for oncologic safety.[45,46]

Distal intramural spread after preoperative chemoradiotherapy for rectal cancer has not been well studied. Mezhir et al. studied specimens from 20 patients after preoperative chemoradiotherapy and total mesorectal excision for rectal cancer, and analyzed the extent of the distal intramural spread.[47] In their study, the vast majority of patients with rectal cancer after preoperative chemoradiotherapy had no tumor cells beyond 1 cm from the residual tumor. Although a RCT-based guideline is not presently determined in terms of local recurrence or survival and DRM in patients with preoperative chemoradiotherapy, an adequate length of the DRM appears to be shorter than that in patients without preoperative chemoradiotherapy. Although some investigators have even suggested less than 1 cm distal resection margin for non-irradiated tumors, the outcome for shorter distal resection margins in patients with preoperative chemoradiotherapy requires further study.[3,4]

The landmark for adequate resection margin varies due to preoperative treatment. Preoperative chemoradiotherapy often shrinks tumor contour, altering the location landmark in many cases.[48–50] With this alteration of the established landmark, the required distance from the distal edge of the gross tumor that is necessary to perform a safe resection must be carefully assessed.

Measurement of the Distal Resection Margin

Although the length of the distal margin is widely cited as an important prognostic factor, it is usually not specified how it is measured.[17,51] Some pathologists measure on a fixed unpinned stretched specimen[20,52,53]; others measure on the specimen after fixation but not pinned in a stretched-out state,[2,20,30] and others measure the fresh specimen[7] (Table 4). When tissue was fixated, it was shrinked some degree; therefore, it is not appropriated to compare two methods. About pinning, when we pinned the specimen, its mucosa was stretched more than unpinned status. Therefore, if we used unfixed pinned specimen, measurement of distal margin was quite different from fixed unpinned specimen.

Sondenna K et al. compared DRM based on the measurement method used.[54] They measured the distal margin prospectively in five different ways in 20 patients; their results showed differences in the measurements based on the methods used. The margin was significantly less in the unpinned compared to the pinned specimens. There was poor correlation between the in situ measurement and the measurement determined by the pathologist for both the unpinned and pinned specimens. There was no significant difference before and after fixation if the specimen had been pinned, but there was significant shrinkage with fixation when the specimen was not pinned. In addition to the tumor bulk, these variations in measurement affect the final result.

In a prospective study reported by Weese et al., it was shown that the method of measurement of the distal margin requires specific definition, as different techniques provide different results.[55] The tissue may shrink by 10–30% with formaldehyde fixation[56,57] and the value of results obtained from fixed specimens remains questionable. In fact, Heald[28,58] and others[17] advocated that the anus could be preserved, provided that it was not palpably invaded by tumor, and a clamp could be placed across the bowel distal to the tumor. Unfortunately, most studies do not identify how the length of the distal margin was determined. It indicates that results obtained from studies using fixed specimens may not be appropriate to determine distal resection margin during operation. Comparison of various measurement methods are urgently needed to determine oncologically safe DRM.

Table 4 Measurement Method of Distal Resection Margin Reported in the Literature

Author	Year	Case	Measurement method	Recommended distal resection margin (cm)
Kuvshinoff B	2001	28	Fixed	1
Moore HG	2003	94	Fixed unpinned	1
Pollett WG	1983	334	Fixed pinned	2–3
Paty PB	1994	128	Unfixed unpinned	2
Mezhir JJ	2005	20	Fixed pinned	2
Vernava AM III	1992	243	Unfixed unpinned	1

Conclusion

Although obtaining a negative DRM remains an important goal in rectal cancer resection, there continues to be a debate about the optimal length of the DRM. Distal intramural spread of rectal carcinoma beyond the gross tumor edge occurs in approximately 25% of patients. As only 10% of cases demonstrate the tumor spread beyond 10 mm and these patients are likely to die from metastatic disease, a wide distal resection margin does not appear to alter patients' survival. These results sufficiently indicate to recommend a minimum of 2 cm or even less DRM during the sphincter-preserving operation for rectal carcinoma. The value of the DRM for the oncological outcome thereby appears to be somewhat exaggerated. The circumferential resection margin and the mesorectal margin are currently considered to be more important for assessing prognosis. Presently, the impact of DRM on the oncological outcome is re-evaluated to establish the optimal DRM, especially lower rectal cancers with rare mesorectal tissues.

On the basis of historical clinical experience along with studies on detailed pathological measurements of intramural tumor spread, use of at least a 2 cm DRM in non-irradiated tumors has been widely accepted. Moreover, there has been no relationship found between the distal clearance margin and subsequent local recurrence and survival; therefore, a 2-cm distal margin has been considered acceptable. However, in the setting of a patient with curative resection (negative circumferential and distal margins) status post-preoperative chemoradiotherapy with an otherwise favorable tumor, a DRM $\leq$1 cm does not necessarily portend a poor prognosis. In addition, for patients given preoperative chemoradiotherapy, the extent of distal tumor spread might be shorter than for those with non-irradiated tumors. Therefore, the required DRM appears to be different for patients that received preoperative chemoradiotherapy. The length of the DRM varies depending on the measurement method used. Therefore, RCT-based studies are urgently needed to specifically define intraoperative measurement to determine adequate DRM in terms of prognosis.

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GI IMAGE

Inferior Vena Cava Gas, Portal Venous System Gas, and Pneumatosis Intestinalis

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Abstract Necrotizing enterocolitis is typically found in premature neonates, but it also has been described in adults, particularly those with cirrhosis and immunocompromised. Radiographic findings of diffuse pneumatosis intestinalis and portal venous gas are characteristic of necrotizing enterocolitis. Here, we provide radiographic findings of a serious adult case of necrotizing enterocolitis. To the best of our knowledge, a wide range of portal vein and vena cava gas has never been reported.

Keywords Inferior vena cava gas · Portal venous system gas · Pneumatosis intestinalis

A 50-year-old man was admitted to our hospital for an unknown fever, which had lasted for 1 month. The fever was intermittent with the body temperature up to 39°C daily at night and down to 37°C in the morning. The man had undergone mesenteric lymph node dissection for hyperplastic lymphadenitis in the past 14 years. After admission, B ultrasound detected the hepatosplenomegaly and retroperitoneal lymphadenectasis. An elevated blood concentration of C-reactive protein and a higher erythrocyte sedimentation rate were also found. No evidence of infection by virus, bacteria, tuberculosis, or hematological tumor was found. Retroperitoneal lymph node puncture biopsy revealed proliferation of small lymphocytes, T-cell-based. The body temperature maintained in the normal range after administration of glucocorticoid, methylprednisolone, 60 mg per day, for 3 days. On the tenth day, the patient suffered a sudden lower gastrointestinal bleeding with the appearance of continuous dark red bloody stool. Emergency digital subtraction angiography did not reveal any obvious arterial bleeding in gastrointestinal system, while computed tomography imaging of the abdomen showed gas throughout the inferior vena cava system, portal venous system, and diffuse pneumatosis intestinalis in the colon. Despite positive saving, the patient had an unrelenting downhill course and died 12 h thereafter. Autopsy prompted necrotizing enterocolitis (Fig. 1).

Radiographic findings of inferior vena cava gas, portal venous system gas, and pneumatosis intestinalis, such as those seen in this patient, are characteristic of necrotizing enterocolitis.[1] Although it has been reported occasionally,[2] to the best of our knowledge, a wide range of portal vein and vena cava gas has never been reported. Similar radiographic findings indicate that the patient can hardly be saved.

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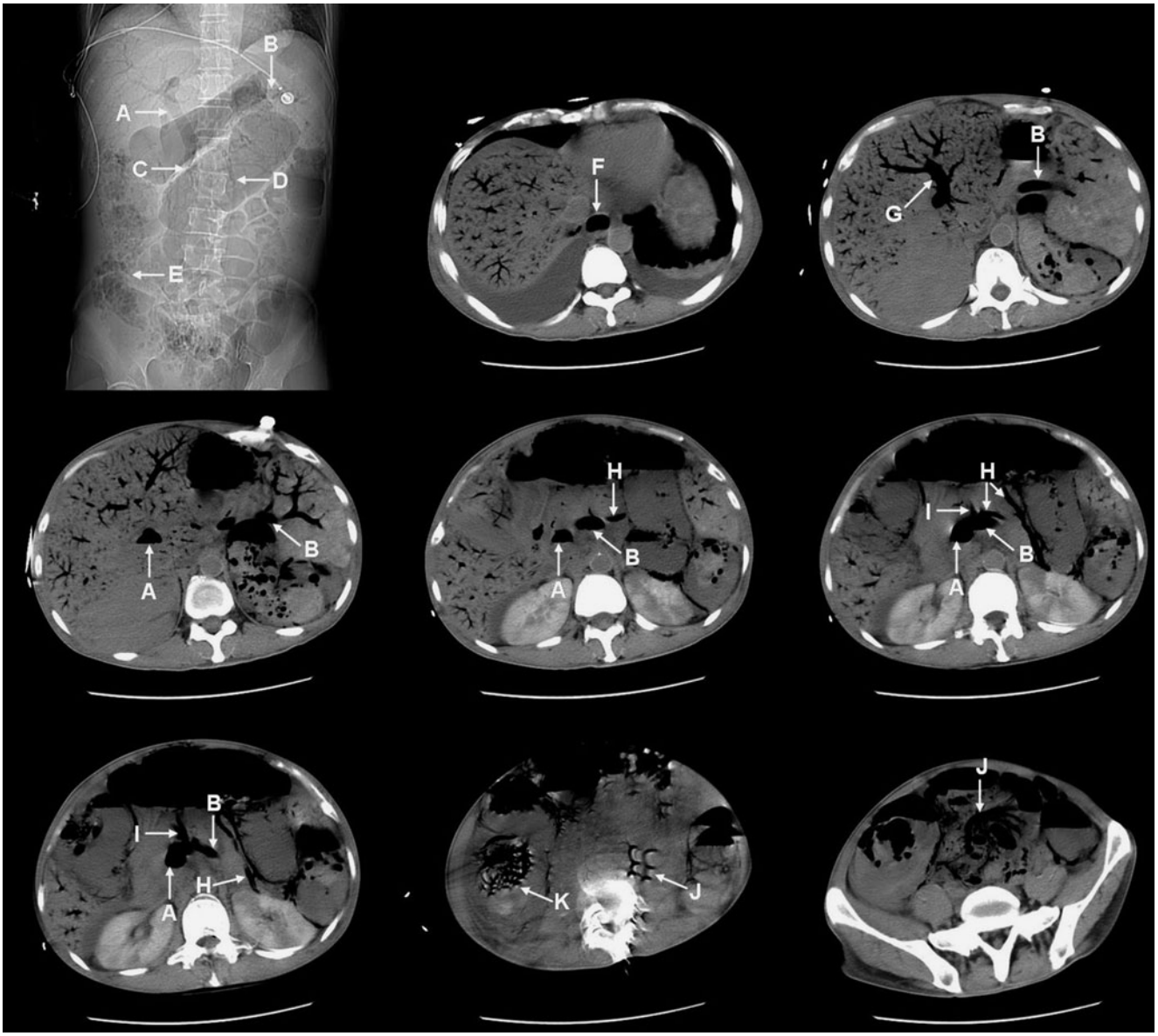

Figure 1 Gas in inferior vena cava, portal venous system, and pneumatosis intestinalis. Radiography of the abdomen reveals gas in portal vein trunk (*A*), splenic vein (*B*), superior mesenteric vein (*C*), inferior mesenteric vein (*D*), and diffuse intestinalis pneumatosis (*E*). Computed tomography shows frazil slush pattern change in liver, gas in inferior vena cava (*F*), portal vein trunk (*A*), portal vein branches (*G*), splenic vein (*B*), vena gastroepiploica (*H*), middle colic vein (*I*), and mesenteric veins (*J*). Computed tomography imaging also reveals mesenteric edema, diffuse intestinalis pneumatosis in the colon (*K*).

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GI IMAGE

Gastrointestinal Images: Complete Tubular Duplication of the Oesophagus in an Adult

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Abstract
Introduction Duplication of the oesophagus is a rare congenital abnormality that usually presents in children. Presentation in adults is extremely rare.
Case History We report a case of a19-year-old gentleman presenting with general gastrointestinal symptoms of pain and dysphagia. Diagnosis was made with CT and contrast studies, demonstrating complete tubular duplication of the oesophagus with communications at both ends of the duplication. The patient was managed conservatively.
Discussion We describe the difficulty in diagnosing these rare congenital abnormalities. We recommend that with a multidisciplinary approach, conservative management can be considered.

Keywords Oesophagus · Oesophageal duplication · Alimentary tract duplication

Introduction

Gastrointestinal duplications (GD) are rare congenital malformations that can occur at any site from mouth to anus. The oesophagus is the second most common site of duplication, accounting for 10% of cases.[1,2] Duplications can be cystic or tubular. Cystic duplications are more common, with tubular duplications being very rare. Presentation of duplication of the oesophagus usually occurs in children.[3,4] We present a late presentation of complete tubular duplication occurring in an adult.

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Case History

A 19-year-old gentleman presented with a 2-year history of chest pain and dysphagia. He described intermittent episodes of severe, burning retro-sternal pain. This was associated with dysphagia to solids, but not liquids. During this time, he felt nauseous, but was unable to vomit. These episodes were self-limiting and lasted 2–3 days before subsiding. The episodes occurred every 3–4 weeks for a period of 18 months. Between episodes, he was asymptomatic and was able to tolerate a full diet. He had no other medical history and took no regular medications. He was a non-smoker and refrained from drinking alcohol. Clinical examination was unremarkable.

High-resolution CT scanning was performed which showed evidence of a duplication of the oesophagus. Appearances on the CT scan suggested communication between the duplication and true oesophagus at both the proximal and distal end of the duplication. The patient proceeded to have a contrast swallow. This confirmed oesophageal duplication with contrast passing freely through both lumens and draining into the distal oesophagus. Upper

gastrointestinal endoscopy was performed showing a septum in the oesophagus at 25 cm from the incisors (Fig. 1). Further endoscopic assessment was performed using a smaller Olympus 5-mm endoscope normally reserved for paediatric or nasopharyngeal endoscopy. The narrow scope allowed passage through both the true oesophagus and the duplication. The endoscope was also able to traverse the distal defect to re-enter the true oesophagus, thus confirming a complete tubular duplication of the oesophagus. Biopsies of the duplication lumen (Fig. 2) were shown to be stratified squamous epithelium, confirming it to be a true duplication of the oesophagus. No dysplasia or evidence of malignancy was detected.

Due to the young age of the patient, careful planning of his management was undertaken with a multi-disciplinary team approach and full discussion with the patient. The patient was not keen to undergo surgery due the infrequency of the symptoms. Subsequent to this admission, he has been followed up regularly at 6-month intervals since the initial diagnosis. Currently, there are no plans to perform surgery, although he has been given the option of a thorascopic approach if he were to develop any complications or increase in symptom severity in the future.

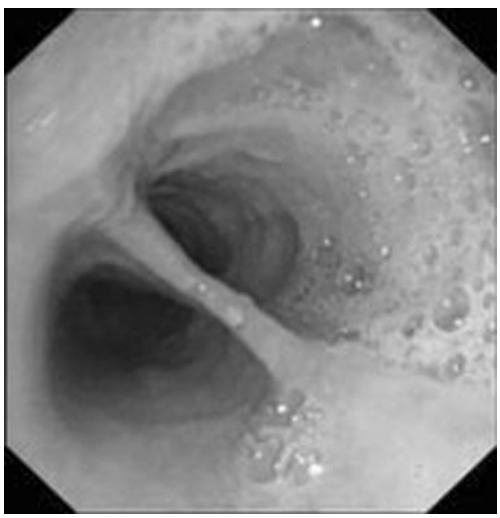

Figure 1 Septum at 25 cm.

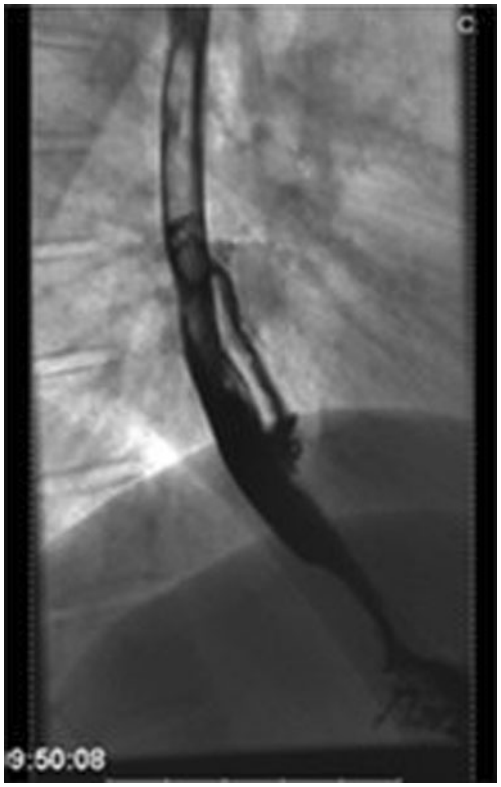

Figure 2 Contrast study demonstrating duplication of the oesophagus.

Discussion

GD can occur anywhere in the alimentary canal, from mouth to anus.[1] They are rare congenital abnormalities and are reported to have an incidence of 1 in 4,500 autopsies.[5] They are defined as structures that lie within or adjacent to the wall of part of the gastrointestinal tract.[6] They contain smooth muscle and are lined by mucosa that can be from anywhere in the alimentary tract, not necessarily by mucosa of the structure it is adjacent to.[6,7] Paediatric GDs have been reported frequently,[8–10] however, they present less commonly in adults.[2–4,11]

Duplications of the oesophagus account for 10% of all GDs, second only to the ileum.[2] GDs can be characterised as cystic or tubular. Cystic duplications are far more common,[2–4] with tubular duplications accounting for less than 10% of cases.[1,3] Duplications that are classified as tubular usually occur in the small or large bowel and are rare in the oesophagus.[6] A review of GDs showed a series of 55 duplications of the oesophagus, with all of them being a spherical shaped and of cystic nature, not communicating with the native oesophagus.[6] Although tubular duplications communicate with the oesophagus more commonly than cystic duplications, it is extremely uncommon to have proximal and distal communications between the duplication and the oesophagus.[6] Therefore, as with this case report, a complete tubular duplication of the oesophagus with both proximal and distal communication in an adult is an extremely rare occurrence.

Classically, these patients suffer from gastrointestinal symptoms with nausea, vomiting, dysphagia, odynophagia, gastro-oesophageal reflux, retro-sternal chest pain, and bleeding. Respiratory symptoms can also occur.[2,3,8,10] More rarely, respiratory distress,[2,12] and Horner's Syndrome have been reported[13]

Diagnosis can be made by radiography, with a number of duplications discovered incidentally on chest X-ray.[6] Con-

trast swallow is more specific, which demonstrates a double oesophagus with contrast flowing in both lumens.[7] High-resolution CT can be useful in making a diagnosis. It usually shows a second tubular structure adjacent to the oesophagus. However, this can sometimes be mistaken for the appearances of oesophageal perforation.[3,7] MRI scanning of the thorax has also been demonstrated as a useful tool in making a diagnosis and differentiating between perforation and duplication.[3,7] It allows visualisation of the entire length of the duplication and any communication that occurs.[3] In this case report, direct vision with upper GI endoscopy made the diagnosis possible. However, this is not possible if the duplication is non-communicating or if the communications are too small for passage of the endoscope.

Management of this condition is dependent on the type and size of duplication and the frequency and severity of symptoms.[10,12] Treatment can be conservative or surgical.[11,12] It is well-documented that surgery is a viable option for duplication of the oesophagus. Many authors recommend surgery if the patient demonstrates significant symptoms with video-assisted thoracoscopy being the first-line approach.[14] Laparoscopic resection has been successful in a number of distal oesophageal and gastric duplications.[15] A staged approach may also be necessary.[12]

Conclusion

Tubular duplication of the oesophagus is a rare congenital abnormality. Presentation in adulthood is unusual, and a high index of suspicion is needed to make a diagnosis. Contrast studies and upper gastrointestinal endoscopy are useful to confirm the diagnosis. Although surgery remains a treatment option, conservative management may also be considered in patients with mild symptoms without any complications. However, it is advisable to take a multi-disciplinary approach to confirm diagnosis and to plan management of these rare entities.

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