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2010 SSAT PLENARY PRESENTATION

Repair of Symptomatic Giant Paraesophageal Hernias in Elderly (>70 Years) Patients Results in Improved Quality of Life

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Abstract
Introduction Giant paraesophageal hernias (PEH) involve herniation ofstomach and/or other viscera into the mediastinum. These are usually symptomatic and commonly occur in the elderly. The benefits and risks of operating on elderly patients with giant PEH have not been clearly elucidated.
Materials and Methods We performed a retrospective chart review of consecutive patients aged 70 or greater with giant PEHs undergoing repair.Quality of life data were gathered using QOLRAD, GERD-HRQL and adysphagia severity score.
Results Fifty-eight patients (34 females), median 78 years old, presented for repair. Nine patients presented urgently. There was no 30-day mortality. Major morbidity was 15.5%. At mean follow-up of 1.3 years, 81% were symptom free compared to baseline ($p<0.0001$). Both short-term ($p<0.001$) and long term QOLRAD ($p<0.001$) scores improved significantly, as did GERD HRQL scores ($p<0.001$). Dysphagia scores worsened in the short term but returned to baseline at long term follow up.
Conclusions Symptomatic giant PEH in this elderly population can be repaired with symptomatic improvement, minimal morbidity and mortality in both the elective and urgent setting. The decision to operate should be made by a physician experienced in managing this complex patient population.

Keywords Elderly · Quality of life · Paraesophageal hernia repair · Laparoscopic · Surgery

Introduction

Giant paraesophageal hernias (PEH) involve herniation of a substantial portion of the stomach and/or other viscera into the posterior mediastinum. When these hernias are discovered, they are usually symptomatic and occur more commonly in the elderly, particularly women.[1,2] Surgical repair of symptomatic hernias is generally recommended[3] and largely results in relief of symptoms and improvement in quality of life (QOL).[4] Even though the elderly are more likely to suffer from a symptomatic PEH and experience diminished quality of life, clinicians may be reluctant to seek surgical consultation secondary to fear of increased morbidity and mortality and a perceived lack of symptomatic benefit.

The laparoscopic approach has gained favor to manage PEHs because of reported excellent results, low morbidity, and very low mortality. The approach is ideally suited for elderly patients, but few reports have examined the results of repair solely in the elderly. We sought to review our clinical and quality of life outcomes with giant PEH repairs in patients 70 years and older.

Materials and Methods

We performed a retrospective chart review of consecutive patients with age greater than or equal to 70 years with symptomatic giant PEHs undergoing repair from October 2003 to October 2009. In this series, a giant PEH was defined as greater than 5 cm from the endoscopic gastroesophageal

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junction to the diaphragmatic impressions with a paraesophageal component and/or having a paraesophageal configuration defined as type II–IV.[5] Patients admitted to the hospital with symptoms of incarceration or obstruction that necessitated early endoscopy, nasogastric decompression, and repair during the same admission were included in this analysis. We excluded all patients with sliding hiatal hernias as well as those requiring emergent operative intervention for strangulated PEH or incarceration that did not respond to decompression.

Preoperative evaluation of each patient included a detailed history and physical examination, an upper gastrointestinal videoesophagogram, upper endoscopy, and high-resolution manometry when possible. Wireless pH analysis was done at the discretion of the attending surgeon. Other imaging such as computed tomography, pulmonary function tests, and gastric emptying tests were obtained as needed on an individual basis.

Operative Techniques

All procedures were performed by a team including an attending surgeon (R.A. or B.L.) with a senior resident or MIS fellow. The laparoscopic approach is performed in low lithotomy with five small incisions following principles previously described.[1] Esophageal lengthening procedures were not employed in any of our patients. Crural reconstruction was performed with simple non-absorbable, braided "0" sutures (polyethylene terephthalate coated with polybutilate, Ethicon-Johnson and Johnson, Cincinnati, OH). Bioabsorbable mesh reinforcement was used liberally after the trial by Oelschlager was published.[6]

An open approach was used sparingly. A transabdominal approach was performed when the PEH was concomitantly repaired with another intra-abdominal procedure. A transthoracic approach was used if the abdomen was hostile and inaccessible to either laparoscopic or transabdominal approaches. Mesh reinforcement was not used in the transthoracic repair.

An anti-reflux procedure was routinely performed after crural reconstruction. Three procedures were used at the discretion of the operating surgeon. When a Nissen fundoplication was created, it was performed over a 60-Fr bougie and fashioned to 2 cm in length. When a Hill repair was created, it was done according to the principles described by Aye.[7] A special 43-Fr bougie with an open tip to allow for a water perfused manometry catheter to be advanced was utilized to perform intraoperative manometry. The last anti-reflux procedure was a hybrid procedure combining the Nissen fundoplication and the Hill repair.[8] At our center, we have surgeons experienced in both the Nissen and Hill procedures. This hybrid operation was conceived and evaluated in an institutional review board approved pilot study where the Nissen fundoplication is performed over two Hill gastroplasty sutures placed through the collar sling fibers of the gastrointestinal junction and secured to the pre-aortic fascia in hopes of mitigating axial tension and cephalad displacement.

Quality of Life Instruments

Quality of life data were gathered using three disease specific instruments: the Quality of Life in Reflux and Dyspepsia Questionnaire (QOLRAD), GERD-HRQL, and a dysphagia score. These instruments were completed by the patient at the first office consultation and postoperatively for short-term follow-up at 4 to 6 weeks and long-term follow-up at 6 and 12 months and yearly thereafter.

The QOLRAD is a validated 25-item questionnaire designed for self-administration by patients with upper gastrointestinal symptoms with a maximum score of 7.[9] Each item asks the patients to reflect on the impact of GERD or esophageal problems over the past week and to rate it on a 7-point scale. A higher score represents an improved quality of life. Although it is a disease specific questionnaire focusing on GERD and dyspepsia, it has been broadly applied across many upper intestinal disorders as an overall QOL instrument. The 25 questions attempt to ascertain GI health in terms of emotional distress (six items), sleep disturbance (five items), food/drink problems (six items), physical/social functioning (five items), and vitality (three items).

The GERD-HRQL is a disease specific quality of life instrument that has been validated to measure symptom severity in gastroesophageal reflux.[10] It has been used and validated to assess response to medications, endoscopic procedures, and surgery. The self-administered instrument consists of ten questions and a separate global satisfaction question. Likert-type responses are possible, with 0 representing no symptoms to 5 reflecting incapacitating symptoms and unable to do daily activities. The scores can range from 54 to 0 with a lower overall score equating to better quality of life.

To assess the symptom of dysphagia, we used the validated dysphagia score as described by Dakkak.[11] This instrument was designed to be used with a standardized meal eaten within 7 days of completing the questionnaire and combined with a blinded observer documenting the actual food ingestion. Patients are asked about the ease of ingesting certain textures of foods and defined amounts. A score of 0 reflects that no food was ingested, whereas a maximal score of 45 represents the ability to ingest the entire meal. To simplify the use of the instrument, patients were asked about their ability to ingest each of the nine foods and points are awarded according to the standardized weighting system. If a patient related that no difficulty was encountered full points were awarded. Conversely, if the patient admitted difficulty in ingesting a certain food, no points were awarded. Half of the points were awarded if moderate difficulty was encountered.

Table 1 Patient demographics and hernia characteristics

	N=58
Characteristics	
Median age (years)	78 (70–91)
Female	34 (59%)
Urgent presentation	9 (16%)
Size of hernia (cm from diaphragm to top of fundus)	10 (5–20)
Type of hernia	
II—paraesophageal	3 (5%)
III—combined	45 (78%)
IV—mixed	10 (17%)

Data and Statistical Analysis

Demographic, operative, clinical, and quality of life data were collected from the clinic chart and hospital medical record. Long-term quality of life analysis was conducted by phone interview by one of the attending surgeons in patients who were not able to travel to the clinic. Statistical analysis was performed using SPSS 18. Continuous variables were analyzed using Student's *t* test. Categorical variables were analyzed using chi-squared. Symptoms were analyzed by McNemar's test. The institutional review board approved this study.

Results

A total of 58 patients were assessed and underwent surgical repair of a symptomatic giant PEH. Median age was 78 years. Their baseline demographics and hernia characteristics are outlined in Table 1. The most common PEH was a type III (78%), with an average size of 10 cm as measured from diaphragmatic hiatus to the top of the gastric fundus on imaging. Nine patients presented urgently with symptoms of incarceration. There was no 30-day mortality. Three patients died in follow-up: one from lung cancer and two from natural causes.

The three different repairs used in this series were evenly applied with 18 Nissen fundoplications, 19 Hill procedures, and 20 combined Hill–Nissen repairs. One patient had an Allison repair with PEG. A bioabsorbable mesh was placed in 38% of cases. A laparoscopic repair was attempted in 55 patients and successfully completed in 53 with two cases (3.4%) converted to open laparotomy. One conversion was for an intraoperative esophageal perforation during a Hill repair and the other for poor visualization while attempting to reduce a large complex type IV hernia. Three cases utilized an open incision from the outset. Two were done in this fashion because a concurrent abdominal procedure was also planned. One was performed via a left thoracotomy because the patient had significant previous abdominal surgery.

Thirteen patients experienced morbidity (Table 2). There were five (8%) minor morbidities including two patients requiring mechanical ventilation for less than 24 h. One required intubation overnight for hypercarbia after repair of a large type IV hernia, and one was re-intubated briefly after developing re-expansion pulmonary edema after repair of large type III hernia compressing the left lower lobe. There were nine (16%) major morbidities. Four patients required readmission for dehydration. There were two esophageal perforations during passage of the bougie for intraoperative manometry. One was repaired laparoscopically and the other converted to an open procedure and then repaired. Both patients were discharged without further complications or interventions.

At a mean follow-up 1.3 years (6 months–5.5 years), 81% of patients were entirely symptom-free compared to baseline ($p<0.001$). When pre- and postoperative symptoms are compared, heartburn, chest pain, shortness of breath, regurgitation, and aspiration are significantly improved after PEH repair (Table 3).

Short-term quality of life was measured at a median of 47 days postoperatively. Paired scores were completed in

Table 2 Minor and major morbidities

	N
Minor morbidity	
Arrhythmia	2
Mechanical ventilation (<24 h)	2
Port site hernia	1
TOTAL	5 (8.6%)
Major morbidity	
Readmission for dehydration/nausea	4
Esophageal perforation	2
Pulmonary embolism	2
Esophageal obstruction POD#1—revision of anti-reflux procedure	1
TOTAL	9 (15.5%)

Table 3 Pre- and postoperative symptom resolution

Preoperative Symptoms	Present postoperatively?		p value[a]
	No	Yes	
Heartburn			
No	22	2	<0.001
Yes	30	4	
Chest pain			
No	20	0	<0.001
Yes	37	1	
Shortness of breath			
No	43	1	=0.001
Yes	14	0	
Dysphagia			
No	41	4	=0.118
Yes	11	2	
Anemia			
No	49	1	=0.125
Yes	6	2	
Regurgitation			
No	23	1	<0.001
Yes	34	0	
Aspiration			
No	43	0	<0.001
Yes	14	1	

[a] Related samples McNemar's chi-squared change test

57% of patients. When compared to baseline preoperative scores, the QOLRAD improved from 5.0 to 6.1 ($p<0.001$) and GERD-HRQL improved from 14.7 to 7.6 ($p=0.01$; Figs. 1 and 2). Short-term dysphagia scores worsened from 40.5 to 32.5 ($p<0.001$; Fig. 3).

Long-term quality of life was measured at a median of 1.3 years of follow-up. Paired scores were completed in 68% of patients. When compared to preoperative baseline scores, QOLRAD improved from 4.8 to 6.6 ($p<0.001$) and

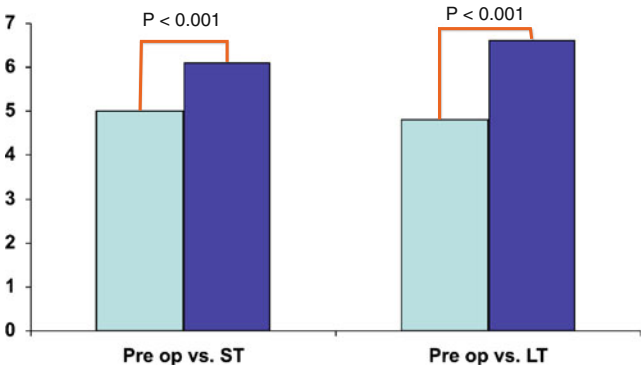

Fig. 1 Short- and long-term quality of life in reflux and dyspepsia (QOLRAD) score. Compared to baseline, short-term (ST) QOLRAD scores increased from 5.0 to 6.1 ($p<0.001$). Long-term (LT) QOLRAD scores are maintained when compared to baseline where scores increased from 4.8 to 6.6 ($p<0.001$)

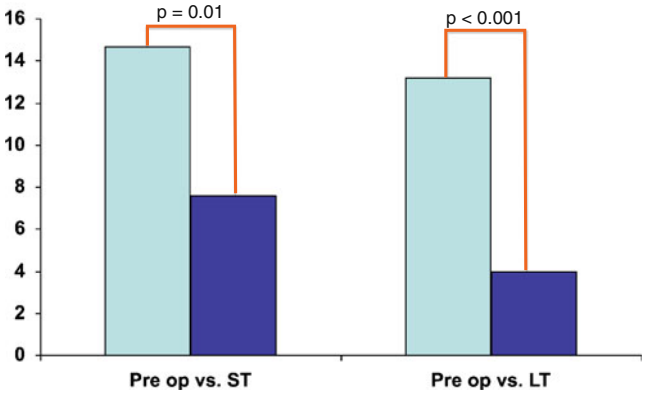

Fig. 2 Short- and long-term GERD-HRQL scores. Compared to baseline, short-term (ST) GERD-HRQL scores decreased from 14.7 to 7.6 ($p<0.001$). Long-term (LT) GERD-HRQL scores are maintained when compared to baseline where scores decreased from 13.2 to 4.0 ($p<0.001$)

GERD-HRQL scores improved from 13.2 to 4.0 ($p<0.001$; Figs. 1 and 2). Dysphagia scores returned to near normal in long-term follow-up (Fig. 3).

There were nine patients who presented urgently. When these patients were compared to the elective group of patients, the urgent group was 4 years older ($p=0.06$). The most likely presenting complaint was chest pain (8/9). All underwent successful laparoscopic repair. The only morbidity was one patient who required overnight ventilation for hypercarbia. Long-term quality of life measures in this group were compared to the elective group. The QOLRAD was 6.8 (vs 6.7; $p=0.6$), GERD-HRQL was 3.6 (vs 3.7; $p=0.9$), and dysphagia was 40.8 (vs 41.1; $p=0.9$).

There were six (10%) recurrences identified by barium swallow and/or endoscopy. The average size was 3 cm (2–4 cm). One recurrence occurred in the urgent repair group. Recurrences were distributed between the types of repairs as follows: three in the Nissen group, one in the Hill, and two in the hybrid group. None of the patients

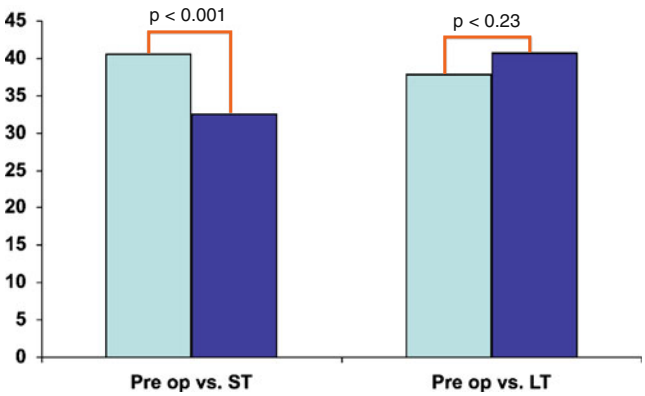

Fig. 3 Dysphagia score. Compared to baseline, short-term (ST) dysphagia scores decreased from 40.5 to 32.5 ($p<0.001$) suggesting worsening swallowing. Long term (LT) dysphagia scores returned to baseline with scores of 37.8 to 40.7 ($p=0.23$)

required re-operation. Mesh was placed in three of the recurrences. Mesh was not placed in one patient at the discretion of the surgeon and the other patients had surgery before mesh was popularized. Despite the presence of recurrence, the median long-term QOLRAD score was 6.8, GERD-HRQL was 3.5, and the dysphagia score was 39. These were not statistically different from the patients without recurrence.

Discussion

Herniation of the stomach into the chest in elderly patients (>70 years of age) is a dilemma to many physicians. These patients often have associated comorbid disease that delays referral to surgical specialists because of concerns about the prohibitive risks of surgery. Secondly, the presenting complaints such as chest pain or shortness of breath often direct the physician to consider a cardiac or pulmonary etiology for their symptoms. Accordingly, these systems are evaluated. The PEH is often discovered incidentally on imaging. Even when it is determined that the PEH is the cause of their symptoms, both patients and physicians are reluctant to seek surgical consultation for fear of increased morbidity and mortality and a perception that surgical intervention will not resolve their symptoms or improve their QOL.

However, in this series as well as others published previously,[1,2] it has been demonstrated that surgical reduction of the hernia and its sac, crural reconstruction with bioabsorbable mesh, and an anti-reflux repair in an elderly patient population can be performed safely, with a very low mortality rate and acceptable morbidity. Even though no mortalities were reported in this series and major morbidity was 16%, it remains important to carefully evaluate the elderly patient since age greater than 70, BMI greater than 35 kg/m^2, and multiple comorbidities have been identified as factors that may increase the chances of an adverse outcome.[1]

The symptoms derived from giant PEHs are often secondary to both acid reflux and mechanical factors.[5] Although the acid-related symptoms may be partially or less often totally resolved with proton pump inhibitors, the mechanical or obstructive symptoms such as chest pain, aspiration, or shortness of breath are not relieved by medical therapy. Repair of the giant PEH offers patients the opportunity to successfully control both the acid reflux and mechanical symptoms in most cases. Patients appear to experience early benefit from repair and these benefits appear to improve further in longer-term follow-up.

It is not surprising that control of symptoms also translates into an improvement in QOL. Many studies have focused on QOL as a primary outcome. Previous studies have used a generic quality of life instrument (SF-36) combined with a disease specific score such as GERD HQRL[1] or a disease specific instrument such as QOLRAD[2] alone to determine quality of life outcome measures. In our opinion, neither of these instruments adequately assesses health in patients with PEH where overall status is impacted by acid reflux, mechanical symptoms, and difficulty with swallowing. Dysphagia is one particular area where QOLRAD and GERD-HRQL are insufficient. Therefore, we have adopted both the QOLRAD and GERD-HRQL to assess more completely aspects of health and the disease state. We added the dysphagia score to directly evaluate swallowing.

The QOLRAD and GERD-HRQL have clearly demonstrated an improvement in quality of life both in the short and long term compared to their respective preoperative baseline. However, unlike prior studies, the dysphagia score has allowed us to quantify dysphagia and demonstrate that in short-term follow-up dysphagia is worse, likely due to ongoing healing and the reconstruction of the hiatus with its attendant edema. Dysphagia scores did return to baseline in long-term follow-up confirming that fear of dysphagia should not preclude patients from undergoing repair. This knowledge allows us to prepare and educate our patients before and early after surgery, counseling them that in most cases dysphagia if present improves with time.

Observation has been proposed as a reasonable alternative in elderly patients with a minimally symptomatic PEH.[12] Using this paradigm, urgent or emergent surgery may be required if the patient develops rapidly worsening symptoms or acutely incarcerates with or without the presence of a gastric volvulus. The nine urgent patients in our series who were stabilized with nasogastric decompression and early endoscopy to rule out strangulation went on to successful laparoscopic repair with minimal morbidity and restoration of quality of life. While the ability to help these elderly patients in an urgent setting is possible with acceptable results, we believe that decision should be made after surgical consultation with physicians familiar with treating and managing PEH.

The argument against observation is based on other series of PEH repairs from respectable centers that have reported an increased mortality and morbidity rate in patients presenting urgently and undergoing repair.[1,3] While not presented in this study, we did exclude from this analysis all emergent operations for strangulated PEH, which carries an inherently higher morbidity and mortality risk. Our goal should be to avoid observing a patient until they present in extremis and require emergent repair. Lastly, the ability to do an appropriate workup for PEH in a stable, elective fashion is far more likely to be successful than after an urgent admission to the hospital, when tests like manometry are more difficult to obtain.

The radiographic recurrence rate of 10% compares favorably with other series in the literature.[6,13] The recurrences occurred evenly among our three repair groups suggesting that

it is likely not the anti-reflux procedure that is central to developing a recurrence. It is more likely that adequate mediastinal mobilization of the esophagus and the crural closure are central to the outcome of the repair and likely whether or not a recurrence will develop. Collis gastroplasty has been proposed as a method to reduce axial tension after mediastinal mobilization. Even with more liberal use of the Collis gastroplasty, the observed recurrence rates are similar to reports where a Collis was used more selectively. In general, our group advocates using an esophageal lengthening procedure selectively. The additional staple lines add increased risk for postoperative leak and complications in this frail population, but lengthening can be very important if short esophagus is truly found and there is a need to reduce axial tension on the repair.[1,14]

Although this study did not focus on the use of bioabsorbable mesh as an adjunct to the crural repair, we observed that 50% of our recurrences did not have mesh placed. Since the report by Oelschlager,[6] we have changed our practice to reinforce the diaphragm reconstruction with bioabsorbable mesh. There remains some controversy about the utility of mesh[1,15] particularly when an esophageal lengthening procedure is performed. These two adjuncts to PEH surgery address different physiologic components of the pathologic process, namely axial tension (gastroplasty) and radial hiatal tension (mesh). The primary principle in all types of hernia surgery has been to avoid tension. Certainly both adjuncts may be important for an optimal outcome, as long as the principle of a tension free repair is the foundation upon which those adjuncts are utilized.

The standard anti-reflux repair associated with PEH repair in North America has been the Nissen fundoplication. However, a variety of repairs have been used in reconstruction of the gastroesophageal junction after hiatal closure including partial fundoplication,[2,16] Hill repair,[17] and the Belsey operation.[18] Since both Nissen fundoplication and the Hill repair are performed at our center, we have observed distinct advantages and disadvantages to both these operations. To capitalize on the advantages of both operations (reflux control in the Nissen and axial maintenance in the Hill), we combined aspects of these procedures to see if a hybrid anti-reflux repair would confer distinct advantages over the traditional repairs (in preparation).[8]

This study has several strengths and limitations. We believe the use of three different QOL instruments better assesses the quality of life in PEH patients who may have symptoms of GERD, mechanical symptoms and/or dysphagia. This study details consecutive patients 70 years and older undergoing primarily laparoscopic repair but also includes urgent and open cases. One of the limitations is that dysphagia score used was not used in the manner that it was validated. This limits the conclusions we can draw using this score. However, we have found it to be an important part of our quality of life assessment. Lastly, our median follow-up 1.3 years is short compared to others even though the range extends out to 5.5 years. We hope to be able to report on the long-term QOL of this cohort to demonstrate durability of the repair in the future.

Conclusions

These data support repair of symptomatic giant paraesophageal hernias in patients aged 70 years or greater. These hernias can be repaired in the elderly with minimal surgical mortality and acceptable morbidity in both the elective and urgent setting. A significant number of patients undergoing repair can expect resolution of the symptoms they suffered from preoperatively. Similarly, patients should expect improvements in both short- and long-term quality of life measures including patients who presented urgently or have small recurrent herniation.

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Discussant

Dr. Piero Marco Fisichella (Maywood, IL): You show that with an operation of this high-risk group of patients that can be treated, can achieve good results in terms of quality of life.

However, based on your results, one may think that the operation is still safe. Still, you had a 10% recurrence rate and two perforations. Moreover, the overall complication rate was 24% if you combine minor and major complications. That means that more or less one patient out of four will have some sort of complication.

I have three questions.

First, I am interested in the surgical technique. Based on your experience, what are the technical elements that can allow you to achieve good results?

You briefly mention in the paper the dissection of the sac, the posterior mediastinal dissection. You also mentioned lengthening procedures. Although, you did not use any lengthening procedures, in the discussion, you say that you used these selectively. In addition, you also said that you used three different techniques.

In summary, could you tell us what is the right approach that you would use for these patients?

Second. When did recurrence occur? Is there a specific time that you saw the recurrence coming? Basically, is there a threshold in the follow-up beyond which patients may be safe from recurrence?

Third. Do you know if mesh plays a role in the recurrence or not?

Last question. You had roughly 25 to 35 patients with short-term quality of life data, results before and after surgery. And you have 68% of patients with long-term results. Do you have any idea what is the complication rate in these patients?

Closing discussant

Dr. Brian E. Louie: To address your first question around our technique or what we think is important, I think we are like most laparoscopic surgeons, we prefer entire sac reduction. We believe bringing the sac down is important and detaching it circumferentially around the esophageal hiatus. We spend a considerable amount of time in the operation, probably two thirds of the time mobilizing the intrathoracic esophagus. And our general goal has been to reestablish at least 2 to 3 cm of intra-abdominal esophagus once we're satisfied about tension.

And if that means taking the dissection up above the inferior pulmonary veins, that generally means doing so. So we spend an inordinate amount of time doing that. And I think that esophageal mobilization is probably the key to the whole operation. And I think, regardless of which anti-reflux procedure you add on to mobilization of the esophagus, at least in our series, it doesn't seem to make much difference whether we used a Nissen, a Hill, or a hybrid procedure; I think mobilization is key.

To answer your third question about the complications and the quality of life and recurrences, the recurrences for us, when we follow these patients, they are generally studied at 6 months and 12 months with the barium swallow and/or other tests, so the recurrences generally occur between that 6 and 12 month interval. We have seen a couple out later than that, but I don't have a definite time frame for that.

In terms of quality of life for that group, we didn't pull that specifically out for the paper, but the patients that did have the perforations or did get readmission, their general quality of life in this group is generally very good and very similar to the elective group.

And then your other question was recurrence of mesh. So early in the series, we used no mesh until the report by Dr. Oeschlager and colleagues saying that mesh reduced the hernia rate, then we began to use mesh much more liberally. I'm not sure.

We looked at the data one way and said, you know, we probably should be using mesh because of the six recurrences, three didn't have mesh. But the other way to look at it is 60% of our patients didn't have mesh and we still had the same recurrence rates. And I know Dr. Luketich's group said the need for mesh is not as great as everybody thinks it is. I think that is very controversial. For now, I think we are going to continue to use mesh.

Discussant

Dr. Nathaniel Soper (Chicago, IL): This is something that we all struggle with. What do you do with the old patient who has a paraesophageal hernia, because there is a significant morbidity and mortality?

First of all, you state all of these patients had symptoms, so you do not operate on asymptomatic patients who have paraesophageal hernias; is that correct?

Dr. Brian E. Louie: That would not be quite correct because I would think we have operated on them. They might not have been over 70, but in this group they were all symptomatic that were—in the consecutive series, that they all happened to have symptoms.

Discussant

Dr. Nathaniel Soper (Chicago, IL): You said you did not include the emergency operations that were done for strangulation. Just to give us a perspective, in this same period of time, how many of those were there in your medical center?

Closing Discussant

Dr. Brian E. Louie: In the medical center, we had about a dozen over the five-year period that the two senior surgeons have counted that came in for strangulation and went to the operating room the same night for endoscopic findings of strangulation, so 12.

Discussant

Dr. Nathaniel Soper (Chicago, IL): And so it's so hard to know what the denominator is total in any of this series.

Last but not least, you had a 10% recurrence rate, but your mean follow-up was only about 1.3 years. Do you routinely perform anatomical tests to really assess what your true recurrence rate is, or were these symptomatic patients who happened to get studied?

Closing discussant

Dr. Brian E. Louie: Our follow-up protocol is generally to get a barium swallow at about a year. And then if the patients are willing, we will undergo full foregut evaluation with endoscopy, pH analysis, and manometry. We did not include that in this series because we have not gotten some of the patients out that far yet. But if we follow them long enough, I think we'll continue to have objective data on recurrences down the road.

But it is our protocol generally to get some imaging study, whether it's upper GI esophagogram or an endoscopy.

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2010 SSAT PLENARY PRESENTATION

Preoperative Infliximab is not Associated with an Increased Risk of Short-Term Postoperative Complications After Restorative Proctocolectomy and Ileal Pouch-Anal Anastomosis

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Abstract
Introduction Considerable controversy exists over whether the preoperative use of infliximab (IFX) for refractory ulcerative colitis (UC) increases the risk for surgical complications after restorative proctocolectomy and ileal pouch-anal anastomosis (IPAA). The aim of this study was to assess the association between preoperative IFX use and short-term surgical complications in a single-surgeon cohort at a tertiary care academic center.
Methods UC patients who underwent IPAA from September 2005 through May 2009 were retrospectively identified. Twenty-nine patients treated with IFX within 12 weeks of surgery and 52 non-IFX control subjects were identified. Short-term postoperative outcomes were compared between groups occurring within 30 days of loop ileostomy closure.
Results Patients were similar with respect to demographics, co-morbidities, rate of emergency surgery, hand-sewn anastomosis, and preoperative use of cyclosporine, azathioprine, and high-dose steroids. IFX patients were more likely to have received a laparoscopic hand-assisted IPAA, low-, medium-, and any-dose steroids, 6-mercaptopurine (6-MP), methotrexate, and to have failed medical therapy. There was no short-term mortality. Overall postoperative and infectious complications were similar between IFX and non-IFX groups. Multivariate regression models revealed no independent predictors for postoperative complications when including IFX [odds ratio (OR) 0.78, $p=0.67$], laparoscopic hand-assisted IPAA, 6-MP, methotrexate, steroids, failure of medical therapy, and body mass index.
Conclusions Preoperative IFX use was not associated with an increased risk of short-term postoperative complications after IPAA.

Keywords Infliximab · Ulcerative colitis · Ileal pouch-anal anastomosis · Short-term complications

This study was presented, in part, at the 51st Annual Meeting of the Society for Surgery of the Alimentary Tract in New Orleans, LA on May 5, 2010 and published in abstract form in *Gastroenterology* May 2010; 138(5):S-867.

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Introduction

Ulcerative colitis (UC) is a disease of the colonic mucosa characterized by recurrent inflammatory episodes. The treatment of UC is to a large extent medical, using such agents as 5-aminosalicylic acid (5-ASA), corticosteroids, and the immunomodulators 6-mercaptopurine (6-MP) and azathioprine. For those patients unresponsive to the aforementioned medications, rescue therapies such as cyclosporine and tumor necrosis factor alpha (TNF-α) inhibitors are available.

Approximately one half of chronic UC patients receiving medical treatment relapse per year. Nearly one fifth of UC

patients experience an acute severe colitis episode requiring hospitalization. Of those, around 60% will respond to intravenous corticosteroids within 72 to 96 h. An additional 15–20% may improve following rescue medical therapy. Ultimately, however, 30% require surgical management within 1 year and 80% will undergo colectomy by 10 years.[1–4] Proctocolectomy with ileal pouch-anal anastomosis (IPAA) remains the surgical procedure of choice for UC patients refractory to medical therapy. IPAA offers cure for the intestinal manifestations of the disease while eliminating the risk for colonic malignancy. Recent data from large volume institutions suggest improved health-related quality of life following IPAA with reliable functional outcomes.[4]

Infliximab (IFX) is a chimeric IgG1 monoclonal antibody that targets TNF-α, an important regulator of many chronic inflammatory diseases such as UC.[5] IFX received FDA approval in September 2005 for use in induction and maintenance therapy for moderate to severe UC. Although IFX holds several boxed warnings, including the increased risk of malignancy and opportunistic infections such as disseminated fungal infections and tuberculosis, its use is considered safe and effective. Recent studies, however, have shown that between 30% and 50% of patients treated with IFX still fail rescue therapy and proceed to colectomy.[4] Considerable controversy exists in the literature on whether such preoperative IFX use increases short-term postoperative complications for these patients after proctocolectomy with IPAA.

As summarized in Table 1, a study by Selvasekar et al. at the Mayo Clinic found that IFX use in UC patients within 2 months of IPAA significantly increased the risk for anastomotic leak, pouch-specific, and infectious complications.[6] Similarly, Mor et al. from the Cleveland Clinic reported significantly increased rates of anastomotic leak, pouchitis, abscess, and overall complications in UC and indeterminate colitis patients with preoperative IFX exposure.[7] Conversely, Kunitake et al. at Massachusetts General Hospital found similar rates of pouch-specific, surgery-related, and infectious complications between preoperative IFX and non-IFX groups.[8] These results were supported by Ferrante et al. who found no differences in anastomotic leak, pelvic abscess, pouch-related or infectious complications in their study population.[9] A recent meta-analysis by Yang et al. further confounds the literature by reporting an association between IFX exposure and overall postoperative complications but no association individually between preoperative IFX use and infectious or non-infectious complications for UC patients.[10] These studies demonstrate that the surgical community is still unclear as to whether patients who undergo medical rescue therapy with IFX prior to an IPAA can expect a safe and functional outcome.

Table 1 Literature-based comparison of postoperative complication risk associated with preoperative use of infliximab

Authors	Overall complications OR (95% CI)	Infectious complications OR (95% CI)
Selvasekar et al.[6]	1.7 (0.9–3.2)	2.7 (1.1–6.7)
Mor et al.[7]	3.5 (1.5–8.3)	13.8 (1.8–105)
Kunitake et al.[8]	1.1 (0.6–2.0)	0.5 (0.2–1.4)
Ferrante et al.[9]	Not available	0.3 (0.07–1.4)
Yang et al.[10]	1.8 (1.1–2.9)	2.2 (0.6–8.0)

Therefore, the aim of our study was to assess the association between preoperative IFX use and short-term surgical complications in a single-surgeon cohort at our tertiary care academic referral center.

Methods

Data were collected from an IRB-approved IPAA Registry at Boston University Medical Center. We identified 81 consecutive UC patients who underwent IPAA between September 2005 and May 2009 by a single surgeon (J.M.B.). Of the 81 subjects, 29 had received IFX treatment within 12 weeks of the first stage of their IPAA surgery. Fifty-two control subjects remained as the non-IFX group. Short-term postoperative outcomes were compared between the two groups as described below.

Inclusion and Exclusion Criteria

Patients with a diagnosis of UC registered in the IPAA database who underwent IPAA at Boston University Medical Center between September 2005 and May 2009 were included in this study. Patients with other pre- or postoperative diagnoses such as Crohn's disease, familial adenomatous polyposis, or indeterminate colitis were excluded.

Clinical Variables

Medical records of all included subjects were retrospectively reviewed. Data abstracted from the medical records included the following patient demographics: age, gender, body mass index (BMI), smoking status, American Society of Anesthesiologists (ASA) class, and co-morbidities. Information regarding medications used 12 weeks prior to surgery included IFX, cyclosporine, methotrexate, 6-MP, azathioprine, oral low-dose steroids (<20 mg/day), medium-dose steroids (20–40 mg/day), and high-dose steroids (>40 mg/day). Surgical factors evaluated included indication for surgery, type of procedure (two- versus three-stage), modality (open versus laparoscopic hand-assisted), and ileal pouch-anal anastomosis technique (stapled versus hand-sewn).

Outcome Measures

Our primary outcome was the rate of overall short-term postoperative complications between the IFX and non-IFX groups. Secondary outcomes included the rate of short-term infectious and non-infectious complications. Short-term postoperative complications were defined as having occurred between the first-stage IPAA surgery until up to within 30 days after the last-stage IPAA surgery, the closure of the diverting loop ileostomy. Complications were defined as pouch/anastomotic leak, pelvic/intraabdominal abscess, pouch-related complications, wound infection, and other. "Other" complications included thrombosis (pulmonary embolus, portal vein thrombosis, and deep venous thrombosis), small bowel obstruction, ileus, and one episode of wound dehiscence. Pouch-related complications included one episode of pouch dehiscence and one episode of a fistula originating from the pouch. Pouch or anastomotic leaks were defined as contrast extravasations seen on computed tomography or loop-o-gram studies. Wound infections occurring in the midline, port-site, or ostomy-site locations were included in this study.

Statistical Analysis

Categorical variables were reported as frequencies and percentages. Continuous variables were reported as mean and standard deviation (SD). Student's *t* test for continuous variables and chi-square or Fisher's exact tests for categorical variables were used as appropriate in evaluating the associations between IFX use and patient factors such as demographics, medications, and IPAA data. Logistic regression analysis was used to assess multivariable associations between potential risk factors and the following outcomes: overall postoperative complications, infectious and non-infectious complications, and wound infection. Results are presented as odds ratios (OR) with 95% confidence intervals (CI).

A p value of <0.05 was considered statistically significant. Statistical analyses were conducted using SAS version 9.1 (SAS Institute).

Results

Demographics

A total of 81 patients meeting inclusion criteria underwent IPAA during the study period. Twenty-nine of those were treated with IFX within the predetermined 12-week period prior to surgery and 52 remained as non-IFX control subjects. Patients were similar between the two groups with respect to age, gender, BMI, smoking status, ASA score, and co-morbidities (Table 2).

IPAA

There were no statistical differences between the IFX and non-IFX groups with respect to the rate of emergency first-stage procedures and the number of patients receiving a two-staged IPAA. All 81 patients underwent a mucosal proctectomy and hand-sewn ileal pouch-anal anastomosis. IFX patients were, however, more likely to have undergone IPAA due to failure of medical therapy as opposed to other indications for surgery such as dysplasia or perforated viscus. Patients in the IFX group were also more likely to have undergone laparoscopic hand-assisted surgeries (Table 2).

Preoperative Medications

No differences in the rates of preoperative cyclosporine, azathioprine, and high-dose steroid (>40 mg/day) use were observed (Table 2). Infliximab patients, however, were more likely to have received 6-MP, methotrexate, low-dose steroids (<20 mg/day), medium-dose steroids (20–40 mg/day), and any-dose steroids.

Postoperative Complications

There were no short-term mortalities. Overall short-term postoperative complications were similar between the IFX and non-IFX groups (44.8% vs. 44.2%, $p=0.96$) (Table 3).

Infectious Versus Non-infectious Complications

Infectious complications, defined as pelvic/intraabdominal abscess or wound infection, were similar between the IFX and non-IFX groups (17.2% vs. 26.9%, $p=0.32$) (Table 3). There was no difference in the rate of pelvic/intraabdominal abscess between groups (13.8% vs. 13.5%, $p=1.00$). There was also no significant difference in the rate of wound infection between groups (3.5% vs. 19.2%, $p=0.09$), although a trend towards *lower* rates of wound infection in the IFX group was observed.

Non-infectious complications, defined as pouch/anastomotic leak, pouch-related or other complications, were similar between the IFX and non-IFX groups (41.4% vs. 30.8%, $p=0.34$) (Table 3). No statistical differences were observed between groups when comparing the categories of pouch/anastomotic leak, pouch-related or other complications separately.

Logistic Regression Analysis

Logistic regression models revealed no independent predictors for overall postoperative complications when including IFX, failure of medical therapy, laparoscopic hand-

Table 2 Patient characteristics compared between infliximab and non-infliximab groups

Demographics	IFX (n=29)	Non-IFX (n=52)	p value
Patient factors			
Age, years[a]	36.2±12.6	42.0±12.7	0.06
BMI[a]	27.0±7.0	27.6±5.9	0.68
Gender, male	11 (37.9%)	22 (42.3%)	0.70
ASA score ≤2	21 (72.4%)	44 (84.6%)	0.68
Smoker	5 (17.2%)	18 (34.6%)	0.10
Co-morbidities			
Diabetes mellitus	2 (6.9%)	1 (1.9%)	0.29
Hypertension	6 (20.7%)	4 (7.7%)	0.16
Cardiac	4 (13.8%)	1 (1.9%)	0.06
Pulmonary	2 (6.9%)	4 (7.7%)	1.00
Renal	0 (0%)	3 (5.8%)	0.55
Surgical factors			
Failed medical therapy	26 (89.7%)	36 (69.2%)	0.04
Emergent/urgent first stage	3 (10.3%)	5 (9.6%)	1.00
2-stage IPAA	28 (96.6%)	47 (90.4%)	0.41
Laparoscopic colectomy	13 (44.8%)	4 (7.7%)	<0.001
Hand-sewn anastomosis	29 (100%)	52 (100%)	NA
Medication use			
Cyclosporine	0 (0%)	1 (1.9%)	1.00
Methotrexate	4 (13.8%)	0 (0%)	0.02
Azathioprine	5 (17.2%)	14 (26.9%)	0.32
6-MP	15 (51.7%)	13 (25.0%)	0.02
Steroid, any	27 (93.1%)	36 (69.2%)	0.02
Steroid, ≤20 mg/day	9 (31.0%)	10 (19.2%)	0.03
Steroid, 20–40 mg/day	11 (37.9%)	12 (23.1%)	0.03
Steroid, ≥40 mg/day	7 (24.1%)	14 (26.9%)	0.20

[a] Reported as mean ± standard deviation. All other values reported as frequency (percent)

assisted IPAA, BMI, and use of any-dose steroid, 6-MP, or methotrexate (Table 4). Logistic regression models also revealed no independent predictors of infectious or non-infectious complications when including these same factors (Table 4). On multivariate logistic regression, patients were more likely to develop wound infections with higher BMIs (OR 0.88, CI 0.78–0.99, p=0.049) (Table 5). IFX, any-dose steroids, 6-MP, failure of medical therapy, and laparoscopic hand-assisted procedures were not found to be predictors of wound infection (Table 5).

Subgroup Analysis

In a subgroup analysis in which all urgent/emergency and three-stage IPAA surgery patients were excluded, the results remained very similar. Logistic regression models continued to reveal no independent predictors of overall, infectious, or non-infectious complications when including IFX. On multivariate logistic regression, however, BMI no longer predicted the development of wound infections (OR 0.89, CI 0.78–1.01, p=0.06).

Table 3 Short-term complication rates compared between infliximab and non-infliximab groups

Complication	IFX (n=29)	Non-IFX (n=52)	p value
Overall	13 (44.8%)	23 (44.2%)	0.96
Infectious	5 (17.2%)	14 (26.9%)	0.32
Pelvic/intraabdominal abscess	4 (13.8%)	7 (13.5%)	1.00
Wound infection	1 (3.5%)	10 (19.2%)	0.09
Non-infectious	12 (41.4%)	16 (30.8%)	0.34
Pouch/anastomotic leak	1 (3.5%)	5 (9.6%)	0.41
Pouch-related	0 (0.0%)	2 (3.9%)	0.53
Other	12 (41.4%)	13 (25.0%)	0.13

Table 4 Multivariate logistic regression analysis of factors associated with postoperative complications after IPAA

Covariate	Overall complication	Infectious complication	Non-infectious complication
IFX[a]	0.78 (0.26–2.38) p=0.67	1.87 (0.46–7.57) p=0.38	0.59 (0.19–1.87) p=0.37
Steroid, any	1.29 (0.32–5.29)	2.41 (0.46–12.7)	1.02 (0.23–4.46)
6-MP	1.05 (0.38–2.89)	1.02 (0.30–3.54)	0.81 (0.28–2.33)
Methotrexate	2.43 (0.20–30.1)	NA[b]	1.79 (0.14–23.0)
Failed medical therapy	0.94 (0.24–3.61)	0.57 (0.11–3.03)	1.41 (0.35–5.65)
Laparoscopic colectomy	1.25 (0.30–5.10)	0.31 (0.06–1.72)	1.13 (0.27–4.82)
BMI	1.02 (0.94–1.10)	0.93 (0.85–1.03)	1.04 (0.95–1.14)

Results are expressed such that OR <1.0 predicts the outcome of interest and OR >1.0 predicts the absence of the outcome

[a] Results expressed as odds ratio, confidence interval, and p value. All other results expressed as odds ratio and confidence interval

[b] Due to the presence of zero cells, logistic regression for methotrexate is not valid

Discussion

Our study indicates that preoperative IFX use 12 weeks prior to undergoing IPAA for UC is not associated with an increased risk of overall short-term postoperative complications. Moreover, no differences were observed in infectious or non-infectious complications between IFX- and non-IFX-treated patients. These findings suggest that for UC patients refractory to medical therapy, a rescue trial of IFX will not affect the short-term postoperative outcomes for those who subsequently require restorative proctocolectomy and IPAA. To our knowledge, we are the first study to examine only patients who had received IFX after its FDA approval in September 2005 for use in moderate to severe UC. Prior studies have included patients who received IFX during off-label usage, which can make results difficult to interpret as these patients may have been in poorer condition prior to surgery.

Infliximab patients were more likely to have failed medical therapy and to have received methotrexate, 6-MP, and low-, medium-, and any-dose steroids. These observations were not surprising since IFX use is generally reserved for those patients failing other medical therapies. Ultimately our data show no increased risk among IFX exposed patients for overall, infectious, or non-infectious complications. Of note, the proportion of ASA scores ≤2 at first-stage IPAA were similar among patients with an overall complication and those without any complication (p=1.00, data not shown). We therefore do not believe that overall health status is confounding the likelihood of developing a complication after surgery. In our study population, IFX patients were more likely to have undergone laparoscopic hand-assisted IPAA. This is an unusual association not previously reported in the literature. Our institution does not have any preset selection criteria for laparoscopic hand-assisted procedures, which makes this finding difficult to reconcile. Incidentally, a recent study suggests preoperative IFX treatment does not affect outcomes after laparoscopic restorative proctocolectomy with IPAA.[11]

Interestingly, our data demonstrated a trend toward fewer wound infections in the IFX treated group, however, this was not statistically significant. Regression analysis revealed higher BMIs to be predictive of developing wound infections, which is widely supported by the literature.[12–15] Logistic regression did not show laparoscopic hand-assisted proctocolectomy to be protective against wound infection in our study population despite reports in the literature suggesting the contrary.[16,17]

Our findings are incongruent from those of Mor et al.[7] and Selvasekar et al.,[6] who reported increased risk of postoperative complications after IFX use. In the Mor et

Table 5 Multivariate logistic regression analysis of factors associated with wound infection after IPAA

Covariate	Odds ratio	95% Confidence interval	p value
IFX	9.49	0.71–126.6	0.09
Steroid, any	9.47	0.93–96.2	0.06
6-MP	0.36	0.06–1.98	0.24
Methotrexate[a]	NA	NA	NA
Failed medical therapy	0.21	0.02–2.42	0.21
Laparoscopic colectomy	0.31	0.02–4.73	0.40
BMI	0.88	0.78–0.99	0.049

Results are expressed such that OR <1.0 predicts the outcome of interest and OR >1.0 predicts the absence of the outcome

[a] Due to the presence of zero cells, logistic regression for methotrexate is not valid

al.[7] study, immunomodulators were more frequently used among the IFX-treated group. Immunomodulator use was one of the factors adjusted for on multivariate analysis, which greatly minimizes but can never completely eliminate its influence on study results. They also looked at patients with any preoperative exposure to IFX, with a 37-week upper interquartile range. Upon subset analysis, the authors reported that whether patients received IFX within 16 weeks of their surgery or after did not change the fact that sepsis was significantly greater in the IFX group. The duration of infliximab's biological activity is not known, but with a half-life of 7 to 12 days and onset of action of approximately 2 weeks, it would seem unlikely that IFX alone could be responsible for this increased risk after 16 weeks. In the Selvasekar study,[6] IFX patients were more likely to be on high-dose steroids, 5-ASA, and azathioprine, which were also adjusted for on multivariate analysis.

In the studies by Kunitake et al.[8] and Ferrante et al.,[9] IFX was not found to increase the risk of postoperative complications. Kunitake et al.[8] investigated UC, Crohn's disease, and indeterminate colitis patients undergoing any abdominal surgery. The results of a mixed IBD cohort may be difficult to interpret since Crohn's patients do not seem to be at increased risk for postoperative complications from IFX.[18–20] In the study by Ferrante et al.,[9] IFX-exposed patients were younger, had shorter disease duration prior to surgery, and lower C-reactive protein levels. Although these factors were examined on univariate analysis, multivariate analysis was not performed. One could argue that these patients were healthier than their control counterparts, and perhaps less likely to develop complications. The Ferrante et al.[9] study population included patients who underwent a single-stage IPAA without an ileostomy. It is therefore difficult to reconcile results with those at our institution, where two- or three-stage procedures with diverting loop ileostomies are consistently performed. Furthermore, the authors reported IFX-exposed patients were more likely to receive an IPAA with ileostomy than controls. And the patient cohort without ileostomies was found at increased risk for complications, further confounding the data.

Beyond UC, infliximab has been used in several other preoperative clinical settings. There seems to be consensus in the literature regarding Crohn's disease, as several studies have shown no increased postoperative risk associated with IFX use.[18–20] Controversy is, however, apparent in orthopedic literature. Giles et al. reported increased risk of infectious complications following orthopedic procedures in rheumatoid arthritis patients on IFX.[21] Conversely, others have found no such increased risk among IFX-exposed rheumatoid arthritis patients after orthopedic surgery.[22,23]

Our study is not without its limitations. It is retrospectively designed, examines a single center study population operated on by a single surgeon, and has a small sample size. It is possible that our study is not powered enough to detect small differences between the IFX and non-IFX groups. Laparoscopic hand-assisted IPAA, failure of medical therapy, low-, medium-, and any-dose steroid use, methotrexate use, and 6-MP use were unequally distributed among the groups. The effects of these differences were minimized by inclusion in multivariate analysis models but never eliminated. Furthermore, there may be other potential confounders we were unable to assess such as UC disease severity, malnutrition, duration of colitis prior to surgery, and total number of IFX infusions received. Other factors associated with septic complications following IPAA have recently been reported by the Cleveland Clinic including BMI, blood transfusion, and individual surgeon. This study was unable to find an association between IFX use and septic outcomes.[24]

No study should be taken in isolation. The need for a multi-centered prospective study or a collaborative retrospective study from multiple registries with well-defined variables echoed in the literature deserves to be re-mentioned. An end to the controversy regarding IFX use in UC and postoperative complications is unlikely to be found without such an undertaking.

Financial disclosures Dr. Farraye sits on the Advisory Board for Centocor and recently resigned from Centocor's Speakers Bureau 8/2010.

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Discussant

Dr. Amy L. Halverson (Chicago, IL): I congratulate you on taking on this controversial topic. And really one of the most important issues is what are the baseline patient characteristics in terms of impacting outcome versus what is the impact of the infliximab. That is, and is the use of infliximab just a marker for more severe disease?

I want to focus on two questions.

The first question involves looking at the patients that underwent the initial ileal pouch operation versus those patients that were so sick they were deemed to undergo just the colectomy and then have an interval ileal pouch operation. Now, I noticed that in your control group, there were some patients that underwent just a colectomy and a subsequent ileal pouch. In contrast, with the infliximab group, they all underwent the ileal pouch initially.

So can you talk a little bit about how you decide who gets the pouch and who gets a colectomy and then a pouch, and then how you think that those patients that underwent the colectomy and then the subsequent pouch affect the outcomes? Do you think that maybe they are at increased risk for complications because they were sicker, or do you think that they are at decreased risk because they had their pouch surgery long after the other morbidity related to the ulcerative colitis was sort of out of the picture and they had a more elective pouch operation?

My second question relates to this laparoscopic surgery. Can you give a little insight into how you think that there is such a difference in the infliximab versus the non-infliximab group and the role that laparoscopic surgery plays with that?

Closing Discussant

Dr. Melanie L. Gainsbury: To address the first question, certainly patients who underwent an emergency first-stage procedure, causing them to have a three-staged IPAA, were very different from their two-staged counterparts. Those requiring emergency surgery are certainly much sicker and unable to tolerate the pouch creation at the time of their first surgery. Whether inclusion of these patients would impact the data because they are at increased risk for complications or perhaps at less risk because they were staged was difficult to tell.

But we were able to actually run a subset analysis where we eliminated those patients who were emergency surgeries, and we did not find significant changes in the data. Essentially, the rate of overall complications, infectious complications, and non-infectious complications between the infliximab and non-infliximab groups continued to be insignificantly different.

In terms of the second question, regarding the laparoscopic colectomies, it was rather surprising to us at first when we discovered that the rate of laparoscopic colectomies was significantly different between the infliximab and non-infliximab groups. We did not have any preset criteria at our institution for selecting patients for laparoscopic colectomy, so it was rather difficult for us to reconcile this difference. We included it as a factor in all of our multivariate analyses to try to help offset some of that influence on the data.

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SSAT/AHPBA Joint Symposium: Today's Approaches to Colorectal Cancer (CRC) Liver Metastases

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Each year in the USA, approximately 150,000 patients are diagnosed with colorectal cancer with an associated 55,000 attributable deaths.[1] Colorectal cancer is the second most common cause of cancer-related death in the USA, with most patients dying of metastatic disease. Up to 40–50% of patients with colorectal cancer will develop metastasis,[2] with about 10–20% presenting with liver metastasis (CRLM) at the time of diagnosis[3,4] and another 20–25% developing metachronous liver metastasis some time later.[5,6] Despite this high incidence of developing metastatic disease, the median survival of patients with CRLM has increased substantially over the past 10 years; patients are living with persistent or recurrent metastatic disease longer and with better quality of life than those treated in the 1980s and 1990s.[7,8] This improvement in outcome is related to improved patient selection, newer and more aggressive surgical techniques,[9,10] and more effective chemotherapy agents and regimens.[11] Five-year survival following curative intent surgery of CRLM now approaches 45–60%[12–15] and is as high as 20% at 10 years in select individuals.[16]

Patients with extensive metastatic liver disease previously thought to be unresectable can now be rendered free of disease after receiving multimodality therapy that includes both systemic and liver-directed therapies. Modern liver-directed therapy may include simple or radical liver resection, and second- and third-stage hepatectomies as well as a variety of nonresection therapies including ablation (radiofrequency, microwave, cryoablation, electroporation), radiotherapy (external beam or transvascular), and hepatic artery infusion chemotherapy.[17] The later approaches can be employed singularly or in combination with resection and systemic therapy to reduce and/or control the magnitude of metastatic disease and render many patients completely free of disease.

Despite these advances, many patients with CRLM who could potentially benefit from multidisciplinary liver-directed therapy alone or in combination with neoadjuvant and adjuvant therapy are not seen by clinicians who are knowledgeable and experienced with CRLM and hence are not offered this opportunity for improved survival. The lack of understanding by medical oncologists, gastroenterologists, and many surgeons on how liver-directed therapies fit in with modern chemo and biological regimens prompted the American Hepatopancreatobiliary Association (AHPBA), the Society of Surgical Oncology (SSO), and the Society for Surgery of the Alimentary Tract (SSAT) to host a consensus conference on this topic in 2006 during the Annual Meeting of the American Society of Clinical Oncology (ASCO) meeting.[18,19] Since 2006, additional experience has been accrued with more widespread aggressive multimodality therapy to convert patients from unresectable to resectable status. Kopetz and colleagues published a review in *The Lancet* that discussed this new paradigm for patients with both resectable and initially unresectable disease (see Fig. 1).[20] Despite these efforts of knowledge dissemination, the rapid evolution of so many new approaches and therapies has confused many practitioners and patients. The rapid evolution of therapy and paucity of adequately powered randomized clinical trials to define best therapy have led patients and physicians to ask a

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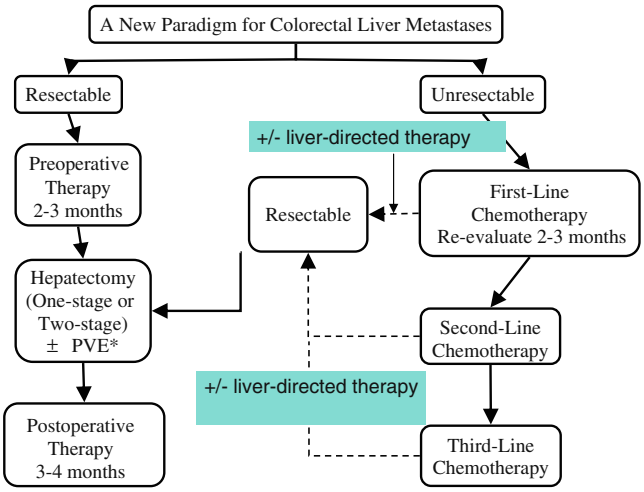

Fig. 1 A new paradigm for colorectal liver metastases. *Portal Vein Embolization

number of questions surrounding therapeutic choices. These include but are not limited to:

- Can we currently identify individuals who will benefit from specific therapies and what factors, if any, are reliable predictors of survival?
- With so many therapies, which one is best for which patient?
- If chemotherapy is so much better today for CRLM, should everyone receive it, regardless of having resectable liver tumor(s) upon presentation? If so, should they receive chemotherapy before or after liver resection? For those who receive chemotherapy before liver resection, does this increase subsequent surgical morbidity or even preclude surgery?
- For patients who refuse or are ineligible for liver resection, which other therapies are most effective or reasonable?
- What are the limits of liver resection today and how much residual liver reserve is necessary for survival?
- How do we balance the benefits and risks of resection against other less invasive therapies for a given patient?

In an effort to address these questions, the scientific and program committees of the AHPBA and SSAT agreed that it would be worthwhile to host a symposium on the management of CRLM during Digestive Disease Week in the Spring of 2010. The goals of this symposium were to increase knowledge of surgeons and gastroenterologists about recommended best practice based upon current evidence for treating patients with CRLM and to bring clarity to many of the questions above. Four experienced surgical oncologist from four prominent cancer centers delivered outstanding talks on this subject. The AHPBA and SSAT are extremely pleased and appreciative of the fact that they agreed to publish their comments in the *Journal of Gastrointestinal Surgery*. With this effort, our two associations hope to advance the care and improve the outcomes of patients with CRLM.

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Prognostic Markers and Staging Systems for Patients with Colorectal Liver Metastases

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Keywords Colorectal cancer · Hepatic metastasis · Chemotherapy · Clinical risk score · Biomarkers

Abbreviations

CRLM	Colorectal liver metastases
CEA	Carcinoembryonic antigen
CRS	Clinical risk score

Introduction

The treatment of colorectal liver metastasis has evolved over the last 20 years. What was previously thought to be a contraindication to surgery, metastatic disease in the liver has been demonstrated to be amenable to locoregional therapy. Up to 70% of recurrences after resection of primary colorectal cancer occur in the liver and up to 40% of these patients present with liver-only disease. These patients are amenable to potential cure following metastasectomy as demonstrated by 10 year actual survival of nearly 20% in selected patients.[1]

The treatment of colorectal liver metastasis (CRLM) is multidisciplinary and often includes treatment with regional or systemic chemotherapy. The use of chemotherapy as an adjunct to surgical resection, however, is debatable and the therapeutic impact is relatively small. The optimal timing (before or after surgery) and duration of treatment is unknown. To help guide the clinician when evaluating patients with CRLM, numerous groups have developed prognostic scoring systems based on retrospective analyses.[2–5] These scoring systems include clinicopathologic factors that impact outcome such as nodal disease in the primary tumor, timing of the development of metastasis (synchronous vs. metachronous), size and number of metastasis in the liver, carcinoembryonic antigen (CEA) level, and the presence of extrahepatic disease.

Despite these efforts, currently there is no "ideal" predictor of outcome for patients with resectable CRLM. The ideal predictor of outcome would include the following characteristics: low cost and easy to measure, reproducible across institutions, and measurable both before and after treatment. Most importantly, this factor would predict major differences in outcome that significantly impact treatment (Fig. 1a). A clinical example of this paradigm is K-ras status as a predictor of response to therapy with cetuximab, a monoclonal antibody against the epidermal growth factor receptor.[6] In a prospective randomized controlled trial, patients with advanced colorectal cancer were randomized to treatment with or without cetuximab. When stratified for K-ras status, patients with wild type K-ras tumors demonstrated a significant survival advantage compared to those with mutated K-ras tumors, who derived no benefit from the chemotherapeutic agent (Fig. 1b). Therefore, patients with mutated K-ras do not receive cetuximab therapy and are spared the toxicity associated with a treatment with no proven benefit. To date, there is no specific clinical risk

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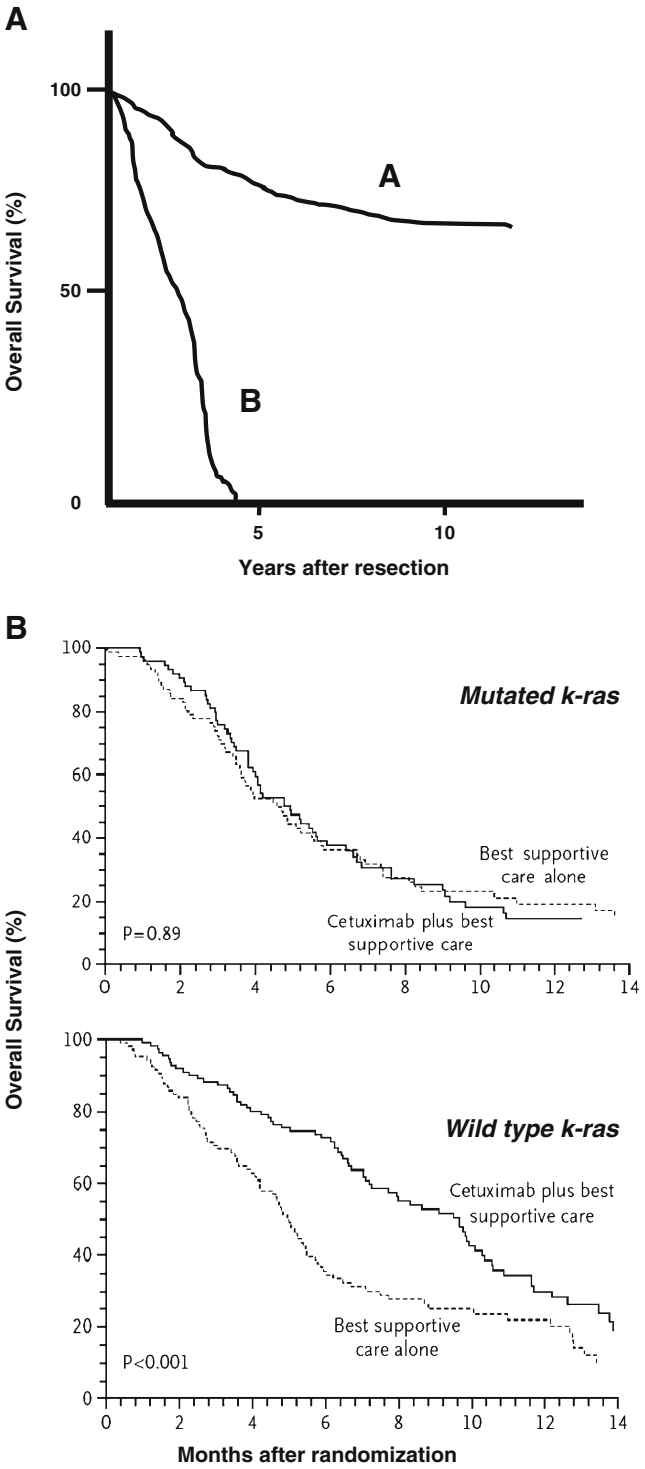

Fig. 1 Prognostic markers in cancer and disease. **a** Theoretical example of the "ideal" biomarker. The ultimate biomarker is a predictor of outcome that is simple, cheap, easy to measure, and predicts major differences in outcome in patients with a given disease. **b** Overall survival of patients with advanced colorectal cancer based on treatment with cetuximab and K-ras status. Patients with wild type K-ras derived a significant benefit from cetuximab while those with mutated K-ras had equivalent outcome. Adapted from Karapetis et al.[6] with permission

score or biomarker that specifically prognosticates or guides therapy for patients with resectable CRLM to this degree.

Clinical risk scoring systems are based on multivariate analyses of large clinical databases of patients selected for surgical resection. Multiple independent predictive factors are combined into a "score" that correlates with outcome. The Memorial Sloan Kettering Cancer Center clinical risk score (CRS) was established after review of 1,001 consecutive hepatic resections for CRLM.[3] The following preoperative factors were significant predictors of disease-free survival on multivariate analysis (1 point is earned for each factor): node-positive primary tumor, disease free interval <1 year, more than one liver tumor, largest liver tumor >5 cm, CEA >200 ng/mL. A score of 0 was associated with a 5-year recurrence-free survival (RFS) of 60% vs. a score of 5 which was associated with a 5-year RFS of 14%. However, high clinical risk score does not preclude 10-year survival in that patients with high scores still demonstrate actual 10-year survival of up to 16%.[1] Furthermore, with the exception of positive liver resection margin, there is not a single clinical or pathologic factor that precludes 10-year survival (Table 1). These types of scoring systems serve as a general guide for prognosis but are not ideal in that they do not specifically impact treatment decisions and do not predict universally good or universally bad outcomes.

Not surprisingly, some of the same clinical factors (i.e., node-positive primary tumor) are predictive of outcome at other institutions. Despite this, these scoring systems are not always prognostic across institutions. The Mayo Clinic devised a scoring system from their patient cohort that included factors such as positive hepatoduodenal lymph nodes, perioperative blood transfusion, node-positive primary tumor, disease-free interval, and size and number of metastatic lesions.[5] While validating their scoring system, they imported the data from their cohort into three other scoring systems, including the Memorial CRS.[5] Survival and recurrence were not stratified by any of the scoring systems from other institutions (concordance indexes for all systems approximated 0.55). Clearly, there is significant variability which highlights the complexity of developing a reliable and reproducible scoring system independent of surgeon and institutional biases.

There are numerous possibilities for why scoring systems are not generalizable, and one of them is clearly an overall selection bias. Surgeons are typically very good at choosing appropriate surgical candidates who have demonstrated good disease biology, which limits the ability to generalize to patients with very high or very low clinical risk scores. There is also variability in the selection bias in that at different institutions patient selection, referral patterns, and institutional

Table 1 Clinicopathologic variables from 612 patients treated at MSKCC with 10 year follow-up and impact on overall survival

Variable	<2-Year survival (%)	2–5-Year survival (%)	5–10-Year survival (%)	>10-Year survival (%)
Synchronous disease	13	11	5	7
Node-positive primary tumor	63	56	52	50
Preoperative CEA >200 ng/mL	16	11	8	7
Disease-free interval <12 months	51	46	36	36
Number of hepatic metastases >1	59	51	32	39
Size of hepatic metastases >5 cm	53	41	41	35
Positive resection margin (liver)	20	10	9	0
Resection > or = lobectomy	63	63	62	68
> or = 4 hepatic metastases	23	16	11	5

Adapted from Tomlinson et al.[1] with permission

CEA carcinoembryonic antigen

neoadjuvant and adjuvant paradigms differ. Another factor is tumor biology, which ultimately dictates outcome and is poorly understood. Taken together, selection bias and a poor understanding of tumor biology leads to the development of risk scores that are not generalizable and that do not impact treatment decisions. Overall, there is no scoring system to date that fits the paradigm demonstrated in Fig. 1 (the ideal prognosticator).

Nomograms have been increasingly developed and utilized as prognosticators in multiple malignancies. A nomogram for predicting disease-specific survival after resection of CRLM was recently published from our institution.[7] Ten factors were weighted and scored from an analysis of 1,477 patients treated from 1986 to 1999 (Fig. 2). The concordance index was 0.68 compared to 0.65 for the CRS. This nomogram has been recently validated at another institution and was found to be more predictive of outcome than the CRS.[8] Despite these positive findings, nomograms suffer from the same inherent biases as other scoring systems and are not proven to be clinically helpful in guiding treatment decisions.

Neoadjuvant chemotherapy is being utilized with increasing frequency and response to therapy is another potential biomarker of patients with CRLM. However, it is clear from prospective phase II and III trials that the rate of progression on neoadjuvant chemotherapy is less than 10%. Older studies suggest a poor outcome for patients that progress on systemic chemotherapy. However, newer studies in the era of modern chemotherapy

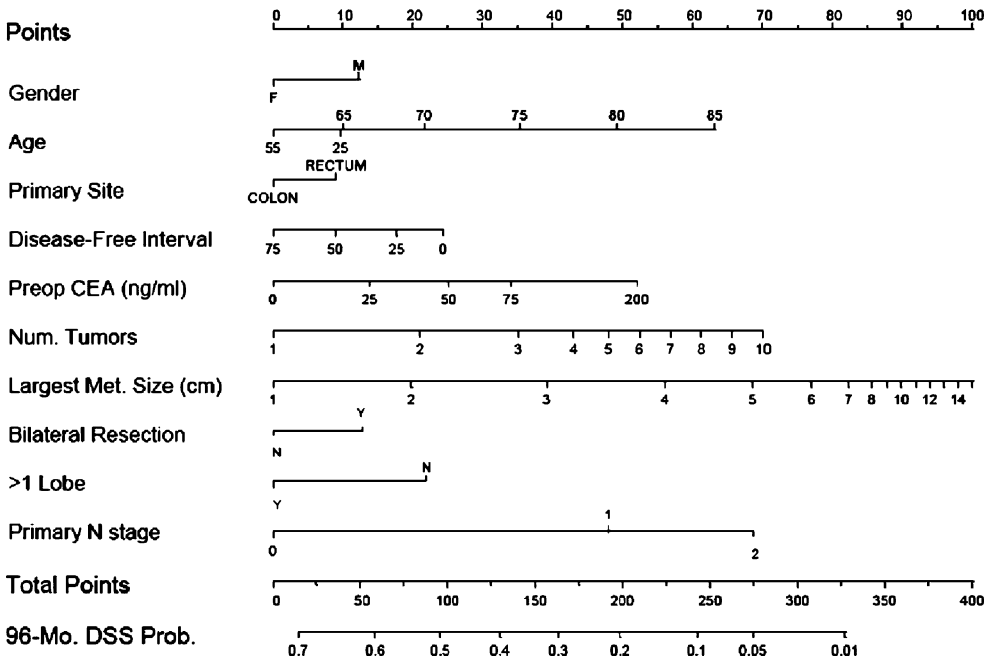

Fig. 2 Nomogram for predicting 96 months disease-specific survival. Draw a *straight line* for each patient variable up to the point axis. The cumulative number of points correlates to the disease-specific survival probability for a given patient. Taken from Kattan et al.[7] with permission

have shown that progression during neoadjuvant chemotherapy does not necessarily preclude a good outcome after resection.[9] Therefore, response to neoadjuvant chemotherapy alone is of low yield as a predictive marker and is not a reliable prognosticator independent of other clinical risk factors.

It is clear that we need something better that, in the era of modern chemotherapy, can help guide treatment decisions. The hope of an "ideal" prognostic marker will likely have to come from the benchtop where tumor biology can be predicted independent of the aforementioned selection biases. The answers likely reside in tissue and serum banks which, with new technologies and better understanding of tumor genetics, are amenable to future study. Tumor immunologic and inflammatory response, markers of sensitivity to certain chemotherapies (i.e., thymidylate synthetase), chemokines, and tissue microarray profiling have all demonstrated the ability to prognosticate tumor biology.[10] However, these studies have been small in number and at single institutions and need further validation.

In conclusion, better prognostic factors are needed to guide the treatment of patients with resectable CRLM. Despite the numerous staging and scoring systems that exist to stratify outcomes, they have limited utility in that they are not reproducible and do not define either universally good or bad outcomes. To improve risk stratification, we need to explore the prognostic factors related to tumor biology that are independent of the currently utilized clinical and pathologic variables.

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The Role of Peri-operative Chemotherapy for Resectable Colorectal Liver Metastasis: What Does the Evidence Support?

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Introduction

With improved patient selection, better surgical techniques, and more effective cytotoxic chemotherapy agents, 5-year survival following curative intent surgery of colorectal metastasis now approaches 45–60%.[1–4] While there have been significant advances in prolonging overall survival of patients with colorectal liver metastasis, many patients still develop recurrent disease. De Jong et al.[5] reported a contemporary experience in which the 5-year disease-free survival was only 30% following curative intent surgery for colorectal liver metastasis, with 60% of patients developing extrahepatic disease at 5 years. Tomlinson et al.[6] noted that approximately one third of actual 5-year survivors succumb to cancer-related death. Noting that the chance of "cure" after hepatectomy was roughly a one-in-six chance, the authors estimated a "maximal cure" rate of only about 25% for patients undergoing surgical resection of colorectal liver metastasis. Given the persistent high recurrence rates and the overall poor "true" long-term survival following surgical resection of colorectal liver metastasis, there has been great interest in the use of adjuvant chemotherapy for patients with resectable colorectal liver metastasis.

The role of adjuvant chemotherapy after resection of colorectal cancer liver metastasis has recently been reviewed by Power and Kemeny.[7] For both pedagogical and practical purposes, "peri-operative" chemotherapy for colorectal liver metastasis can be divided into three different treatment "strategies;" neoadjuvant, peri-operative, and adjuvant. We herein review each one of these peri-operative chemotherapy treatment strategies for resectable colorectal liver metastasis.

Adjuvant Chemotherapy

The use of adjuvant chemotherapy for colorectal cancer has been well studied in stage III disease. Specifically, multiple randomized clinical trials have noted a survival benefit associated with the use of adjuvant chemotherapy for patients with colorectal cancer and lymph node metastasis.[8–11] Sargent et al.[8] reported that surgery plus adjuvant 5-flurouracil (5-FU) versus surgery alone was associated with an overall survival benefit (8-year overall survival—surgery+5-FU-based chemotherapy, 53% versus surgery alone, 43%; $P<0.0001$). In the phase III NSABP C-07 trial that examined adjuvant FULV (5-fluorouracil plus leucovorin)+oxaliplatin, the FLOX regimen, in stage II or III colorectal cancer patients, there was noted to be a trend toward improved survival with the addition of oxaliplatin.[10] In the MOSAIC trail, the addition of oxaliplatin (FOLFOX4) was shown to provide an additional benefit over 5-FU monotherapy (LV5FU2) among patients with stage III disease (6-year overall survival—FOLFOX4, 73% versus LV5FU2, 69%; $P=0.023$).[11] Collectively, data from these studies have firmly established the beneficial role of

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adjuvant chemotherapy in the management of stage III colorectal cancer.

Multiple phase I and II studies have similarly shown improved efficacy of modern-era chemotherapy in the treatment of unresectable stage IV colorectal liver metastasis. While monotherapy with 5-FU previously resulted in response rates only in the range of 20–25%,[12] current regimens that include oxaliplatin or irinotecan have response rates in the range of 45–55%.[13–17] More effective cytotoxic chemotherapy has translated into a significant increase in the median survival of patients with unresectable colorectal liver metastasis from 6 months with best-supportive care to 12–15 months with monotherapy 5-FU to now 20–24 months with oxaliplatin- or irinotecan-based therapies. Additional advances have been associated with the addition of biologic agents, such as bevacizumab or cetuximab, to cytotoxic chemotherapy, as outlined in the BEAT[18] and CRYSTAL studies.[19]

Given the robust data on the role of systemic chemotherapy for both resected stage III and unresectable stage IV colorectal cancer, there has been interest in the potential use of adjuvant chemotherapy in the setting of resected stage IV colorectal liver metastasis. Unfortunately, data on the role of adjuvant chemotherapy for resected colorectal liver metastasis are scant.[20–23] Of the four randomized trials published to date, two were published only in abstract form and each had fewer than 52 patients analyzed.[20,22] Of the two other randomized trials,[21,23] both suffered from poor accrual and had fewer than 175 patients analyzed. In the Langer et al.[21] study, only 107 patients were analyzed and there was no noted difference in overall survival when daily bolus 5-FU was compared with observation alone following resection of colorectal liver metastasis. In the Portier et al.[23] trial, 173 patients were randomized to surgery alone versus surgery+5-FU. In this study, two patients were lost to follow-up, leaving 85 patients randomized to the surgery alone arm versus 86 patients to the surgery+5-FU arm. Among the patients randomized to adjuvant 5-FU, 94% of assigned patients received post-operative chemotherapy. No difference in overall survival was noted between the study arms (5-year overall survival—surgery alone, 42% versus surgery+5-FU, 51%; $P=0.13$). The authors did note, however, that adjuvant 5-FU conferred a disease-free survival benefit (5-year disease-free survival—surgery alone, 27% versus surgery+5-FU, 34%; $P=0.028$). Cox multivariate analysis confirmed a statistically significant beneficial effect of chemotherapy on disease-free survival (HR=0.66, 95% CI, 0.46–0.96).[23] While this study did show an improvement in disease-free survival, it failed to show an overall survival benefit with adjuvant therapy. The reasons for this lack of effect were undoubtedly multifactorial and included the relatively small study sample size and lack of statistical power. In an attempt to increase the overall number of patients available for analysis, Mitry et al.[24] performed a pooled analysis of the Langer et al.[21] and Portier et al.[23] studies. In this pooled analysis, a total of 278 patients were analyzed, 140 of whom had been randomized to surgery alone and 138 of whom had been randomized to surgery+5-FU. Among those patients randomized to adjuvant chemotherapy, 95% of assigned patients received chemotherapy. In this study, the authors again noted no difference in overall survival and a trend toward improved disease-free survival associated with adjuvant therapy. On multivariate analysis, after controlling for other competing risk factors, a marginal statistically significant associated benefit of adjuvant 5-FU chemotherapy was noted ($P=0.046$).

There have been several large retrospective, non-randomized studies that have also examined the issue of adjuvant chemotherapy for resectable colorectal liver metastasis.[25–27] Each of these studies have reported a survival benefit for adjuvant 5-FU versus surgery alone for patients with resectable colorectal liver metastasis. In general, these studies have noted a relative 25–60% decreased risk of disease-specific death associated with receipt of adjuvant 5-FU. Obviously, these retrospective studies have serious threats to validity including selection bias and treatment bias, not to mention issues with possible confounding. As such, any causal inferences drawn from such data need to be carefully considered.

There has been one study that has examined the use of "modern" era chemotherapy in the adjuvant setting for resected colorectal liver metastasis. Ychou et al.[28] reported a randomized phase III study comparing adjuvant 5-FU versus FOLFIRI among patients having undergone complete resection of liver metastases from colorectal cancer. In this study, 321 patients were randomized to receive either 5-FU alone or FOLFIRI. Of those patients assigned to FOLFIRI, 95% received the assigned post-operative chemotherapy. The authors noted no benefit for FOLFIRI compared with 5-FU with regards to either disease-free or overall survival. In fact, survival curves for the 5-FU versus FOLFIRI arms of the study were nearly super-imposable with a reported overall 5-year survival of 63% and 65%, respectively, and a disease-free survival of 37.5% in both groups. The lack of benefit for FOLFIRI compared with 5-FU as adjuvant therapy for stage IV disease was perhaps not surprising given the findings of the ACCORD 02[29] and CALGB 89803[30] trials, which had previously shown no difference in disease-free survival with the addition of irinotecan to 5-FU in the setting of resected stage III colorectal cancer.

Peri-operative Chemotherapy

By administering chemotherapy prior to surgery, peri-operative chemotherapy has the theoretical benefit of earlier

delivery of systemic treatment for stage IV disease. Peri-operative chemotherapy has been shown to have efficacy in the treatment of other solid gastrointestinal malignancies such as gastric cancer.[31] The use of peri-operative chemotherapy in the treatment of colorectal liver metastasis has recently been reported in a large multi-institutional trail.[32] The EORTC Intergroup trial 40983 randomized 364 patients to either surgery alone versus peri-operative chemotherapy with the FOLFOX4 regimen. Peri-operative chemotherapy consisted of six cycles (3 months) of FOLFOX4 followed by surgery and then an additional six cycles of FOLFOX4. Only patients with one to four liver metastases and no extrahepatic disease were eligible for the study. Of the 182 patients randomized to the surgery alone arm, 91.8% patients were taken to surgery and 81.9% underwent resection, for a non-therapeutic laparotomy incidence of about 10%. In contrast, of the 182 patients randomized to peri-operative chemotherapy, 86.8% were taken to surgery and 83.0% underwent resection, for a lower non-therapeutic rate of about 4%. Overall mortality was low in each study arm (surgery $n=2$ versus peri-operative chemotherapy $n=1$). While the overall complication rate was higher in the peri-operative chemotherapy group (25%) versus the surgery alone group (16%), most complications were minor. Regarding outcome, when all randomized patients were analyzed on an intention-to-treat analysis, the benefit of peri-operative chemotherapy was associated with a trend toward improved progression-free survival (HR 0.79, 95% CI 0.62–1.02; $P=0.058$). When only the eligible patients were considered in the analysis, peri-operative chemotherapy was associated with an overall 8% absolute improvement in progression-free survival at 3 years (surgery alone, 28.1% versus peri-operative FOLFOX, 36.2%) (HR 0.72, 95% CI 0.60–1.00; $P=0.041$). When only patients who underwent resection were considered, FOLFOX4 peri-operative chemotherapy was associated with an absolute increase in 3-year progression-free survival of 9.2%. The authors concluded that peri-operative chemotherapy with FOLFOX4 was compatible with major liver surgery and prolonged progression-free survival in eligible and resected patients. The results of the EORTC 40983 trial have, however, been somewhat difficult to interpret. Specifically, it is not clear why the observed benefit of chemotherapy in the setting of stage IV disease was less than that reported in adjuvant trials for stage III patients, although the disease biology is likely different in these groups. In addition, whereas the EORTC 40983 trial assessed peri-operative versus no chemotherapy for patients with resectable colorectal liver metastasis, most patients in the USA are routinely offered chemotherapy in conjunction with resection. As such, the EORTC 40983 trial did not address the specific issue that may be of most interest in the USA: how best to sequence peri-operative chemotherapy. Specifically, given that the overwhelming majority of patients with resectable metastasis receive some type of systemic chemotherapy, the question remains: is it better to give the entire systemic chemotherapy course in the adjuvant setting or should part of the systemic chemotherapy regimen be given in the neoadjuvant setting?

Neoadjuvant Chemotherapy

Neoadjuvant chemotherapy is the administration of chemotherapy in the pre-operative setting in patients with resectable disease. Neoadjuvant chemotherapy should be distinguished from conversion chemotherapy, which is also administered in the pre-operative setting but is administered to patients with initially unresectable disease, with the intention of downsizing the tumor burden, and, ultimately, considering resection. The administration of neoadjuvant chemotherapy to patients with initially resectable disease has a number of potential benefits and risks.

While already resectable, lesions treated with neoadjuvant chemotherapy may benefit from further downsizing to facilitate increased rates of margin negative as well as parenchymal sparing resections. The data on margin status and neoadjuvant chemotherapy, however, are conflicted and biased by the retrospective nature of most reports as well as the inclusion of some initially unresectable patients in these studies.[33–35] Another theoretical benefit of neoadjuvant chemotherapy is that administration of chemotherapy in the pre-operative setting will allow the biology of the disease to declare itself. Data from the EORTC 40983 trial would suggest that this is an infrequent occurrence.[32] Specifically, in the EORTC 40983 trial, only 14 patients (7.7%) experienced progressive disease while on peri-operative FOLFOX prior to surgery. Among these 14 patients, only seven patients (4%) were "saved" an operation due to progressive disease. As such, the routine administration of neoadjuvant chemotherapy as a means to define tumor biology appears to have a low yield. The administration of neoadjuvant chemotherapy does, however, provide an in vivo gauge of tumor response to a particular regimen, which may help tailor adjuvant therapy. In addition, response to neoadjuvant chemotherapy can be used as a powerful prognostic tool. Specifically, Adam et al.[36] reported that disease progression while on preoperative chemotherapy is an ominous prognostic factor. In fact, Adam et al.[36] noted that among patients with four or more liver metastases who had progressive disease while on neoadjuvant chemotherapy the 5-year survival following complete surgical resection was only 8%. In a separate study, Blazer et al.[37] reported that pathologic response to pre-operative chemotherapy was a potent predictor of long-term survival. Patients who had a complete or major pathologic response had a significantly

better long-term outcome compared with patients who had a minor response.

There has been some concern, however, that neoadjuvant chemotherapy may result in liver-associated injury. Injury to the liver from chemotherapy has been shown to be drug specific as well as related to the duration of chemotherapy.[38,39] Specifically, patients treated with irinotecan appear more likely to incur liver injury characterized by steatosis, while oxaliplatin is more typically associated with sinusoidal injury. The incidence of chemotherapy-associated steatohepatitis remains ill defined, with some centers[38] reporting an incidence of 8% while other institutions have reported a much lower rate of only 2%.[39] The impact of chemotherapy on outcome has also been somewhat controversial. In one study, the use of pre-operative chemotherapy was associated with an increased risk of peri-operative mortality,[38] while others have reported no increased risk of peri-operative mortality with its use.[39] It is important to note that in the study by Vauthey et al.[38] pre-operative chemotherapy was only associated with an increased risk of mortality among those patients with underlying steatohepatitis (steatosis with associated inflammation) who had undergone a major hepatic resection (extended hepatectomy or hemi-hepatectomy with contra-lateral ablation). Data from the EORTC 40983 trial, as well as retrospective reports,[40,41] strongly suggest that short-course pre-operative chemotherapy appears to be safe. One does, however, need to be mindful of the risk of chemotherapy-associated liver injury among those patients with high BMI and/or diabetes in whom a major hepatectomy is planned, as these patients are at a higher baseline risk of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis.

While the use of neoadjuvant chemotherapy may have some theoretical appeal, its routine use cannot currently be supported by any level 1 data. The NSABP C-11 trial will hopefully provide data to determine if neoadjuvant versus adjuvant chemotherapy for resectable colorectal metastasis is associated with a disease-free or overall survival benefit. The NSABP C-11 trial will evaluate the role of peri-operative/neoadjuvant chemotherapy in patients with potentially resectable hepatic colorectal metastasis. Patients with potentially resectable hepatic colorectal cancer metastasis will be randomized to liver resection followed by adjuvant chemotherapy versus neoadjuvant chemotherapy followed by liver resection and then consolidation adjuvant chemotherapy. The chemotherapy regimen will include either oxaliplatin or irinotecan and will be determined by the patient's previous exposure to oxaliplatin, and both adjuvant and neoadjuvant treatment arms will receive bevacizumab. Of note, kras mutational status will not be assessed, nor stratified for in this study, as no EGFR-directed antibody therapy has been included in the chemotherapy regimens. Eligibility is not restricted by the number of liver metastases, as patients with four or more lesions can be enrolled in the trial. The primary endpoint is recurrence-free survival, with overall survival, R0/R1 resection rate, peri-operative complication rates, and chemotherapy toxicity as secondary endpoints.

Conclusion

Liver resection is the ultimate treatment strategy for colorectal liver metastasis; however, recurrence is common. There is a sound rationale for the use of adjuvant chemotherapy based on robust level 1 data demonstrating its benefit among patients with resected stage III colorectal cancer. Unfortunately, level 1 randomized controlled data on the use of peri-operative systemic chemotherapy for resected colorectal liver metastasis are limited. Only a handful of randomized trials have been reported and each has suffered from poor accrual and being underpowered. The largest adjuvant trial examining adjuvant 5-FU did demonstrate a disease-free survival benefit. Similarly, the EORTC 40983 trial reported a disease-free benefit among eligible patients treated with peri-operative chemotherapy. The predominance of evidence suggests that peri-operative chemotherapy has a role in the treatment of patients with resectable colorectal liver metastasis. As such, it is not feasible to perform clinical trials of chemotherapy versus surgery alone for patients with resectable colorectal liver metastasis in the USA. However, the sequence of chemotherapy for resected colorectal liver metastasis remains ill defined. Short-course neoadjuvant chemotherapy appears to be safe with limited hepatotoxicity. While neoadjuvant chemotherapy has the theoretical benefit of being able to deliver treatment earlier for both measureable and microscopic stage IV disease, the true benefit of neoadjuvant therapy is not established. Hopefully, the upcoming NSABP C-11 trial will help characterize the relative benefit of neoadjuvant/peri-operative versus adjuvant chemotherapy in patients with resectable colorectal liver metastasis. Ultimately, rather than treating all patients with colorectal liver metastasis as a monolithic group, both the timing and type of chemotherapy will potentially need to be tailored in the future based on as yet unidentified factors and molecular markers.

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Resection of Colorectal Liver Metastases

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Abstract
Introduction The gold-standard treatment for colorectal liver metastases (CLM) is liver resection. Advances in staging, surgical technique, perioperative care and systemic chemotherapy have contributed to steady improvement in oncologic outcomes for patients following surgery in this subset of patients with stage IV colorectal cancer. The limits of resection continue to expand to include patients with more, larger and bilateral CLM, yet outcomes continue to improve with 5-year overall survival exceeding 50% following resection. Chemotherapy is an important element of treatment for patients with CLM, and chemotherapy can be combined safely with surgery to improve outcomes further.
Methods Tailored approaches to patients include major (anatomic) resection, minor (wedge) resection, liver volumetry, and preoperative enhancement of the volume and function of the planned future remnant liver using portal vein embolization.
Results Assessment of response to chemotherapy, analysis of liver remnant volume changes following portal vein embolization, and consideration of the surgical recovery following multistage surgical resection of bilateral CLM enable remarkable survival even among properly selected patients with extensive disease.
Conclusions Until laboratory, pathologic, biologic, or genetic studies can define which patients will benefit most from surgical and other treatments, careful application of proven diagnostic and therapeutic approaches to patients with advanced disease will continue to allow surgeons to direct tailored, patient-centered treatment as part of a multidisciplinary team.

Keywords Colorectal liver metastases · Liver resection · Hepatectomy · Portal vein embolization · Liver volumetry

Introduction

The gold-standard treatment for colorectal liver metastases (CLM) is resection, which leads to 58% 5-year overall survival in this minority of patients with stage IV colorectal cancer who are candidates for complete removal of all of their disease. This increase in overall survival (compared to the historical rate of about 36% 5-year overall survival) has occurred despite significant expansion of criteria for resectability, including patients with more, larger, and bilateral CLM. Framing the discussion of resection for CLM is the consensus definition of resectable disease, which focuses on complete resection of tumor-bearing liver sparing an adequate liver remnant volume,[1] with focus on the liver that will remain rather than the characteristics of the resected tumors to define which patients are considered for surgery. Actually tailoring treatment to the patient includes consideration of the patient's overall health, the condition of the underlying liver, and to the disease extent and "biology" of the patient's oncologic disease. This brief overview touches briefly on the practical approach to tailored resection of CLM.

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Chemotherapy

There is uniform agreement that chemotherapy, which converts patients with unresectable disease to resectable,

is of value, and that subsequently resected patients benefit from resection.[2] Although not all agree that chemotherapy should be given to patients before resection of *resectable* CLM, significant data support the utility of chemotherapy in this setting. Firstly, progression of extensive disease (four or more CLM) predicts poor outcome from liver resection even if resection remains feasible.[2] Furthermore, comparison of patients who underwent similar workup and imaging revealed a significantly reduced likelihood of nontherapeutic laparotomy because of unsuspected disease among those treated with chemotherapy prior to laparotomy vs. those who went directly to surgery.[3] Importantly, the EORTC Phase III prospective, randomized trial of perioperative FOLFOX (5-fluorouracil, leucovorin, oxaliplatin) demonstrated that the nontherapeutic laparotomy rate was only 5% in the group that received chemotherapy vs. 11% nontherapeutic laparotomy in the no chemotherapy group, proving this hypothesis.[4] Furthermore, the population studied in the prospective trial included patients with very limited disease—more than half had solitary CLM, two thirds had metachronous disease, and the tumors were small. Thus, it is important to understand that the population which benefits from preoperative chemotherapy (in terms of selection for resection), includes patients with limited disease.

Treatment of Patients with Extensive or Bilateral Disease

Discussion of patients with bilateral disease permits discussion of several key elements of liver surgery for CLM. Such patients generally require major resection of disease on one side of the liver, with minor (wedge) resection(s) on the contralateral side. Furthermore, those with extensive disease often require an approach to increase the volume and function of a small liver remnant. Thus, issues of wedge vs. anatomic resection, minimal margin resection, and liver enhancement (portal vein embolization) are addressed as these patients are considered.

Anatomic vs. Nonanatomic Resection and Resection Margins

Careful study of nonanatomic vs. anatomic resection for CLM shows that as long as the margin of resection is free of disease, the two different surgical approaches are oncologically equivalent.[5] No differences in overall survival, recurrence-free survival, recurrence at the cut edge of liver (marginal recurrence), intra- or extrahepatic recurrence can be shown. Similarly, when large cohorts are studied, it is clear that the width of the negative margin for CLM has no impact on overall survival, disease-free survival, recurrence at the cut edge of the liver, or overall recurrence patterns.[6] Patients with a positive margin (tumor within 1 mm of the cut edge of the specimen after resection) have an increased risk of local recurrence and decreased overall survival compared to those with a negative margin (>1 mm); however, those with a margin ≥10 mm do no better than those with a 1–4-mm margin. Thus, a negative margin is a negative margin, and wedge resection and anatomic resection are oncologically equivalent.

Portal Vein Embolization

The definition of resectable CLM focuses on complete resection leaving an adequate liver remnant. Consensus has been reached, based on objective data, as to the adequate remnant, specifically >20% in patients with normal liver, >30% in patients with liver damage, e.g., from very extensive chemotherapy, and >40% in patients with well-compensated cirrhosis[1] (Fig. 1). Prior to extensive resection (e.g., extended right hepatectomy), systematic liver volumetry is used to assess the volume of the liver remnant before resection, as this volume predicts postresection liver function. If that volume is inadequate, preoperative portal vein embolization (PVE) should be considered. PVE is generally performed percutaneously by the interventional radiologist who accesses the portal branches under ultrasonographic guidance, and then occludes the portal branches within the liver to be resected. As a result, portal blood flows solely to the future liver remnant (FLR, or liver that will remain after resection), inducing hypertrophy. This liver growth or degree of hypertrophy (DH) of the FLR in response to PVE has been shown to increase both the volume and function of the remnant, and to decrease the risk for major complications, hepatic insufficiency, and death from liver failure postresection.[7] Of importance is the volumetric response to PVE. In patients with normal liver who undergo resection leaving an FLR≤ 20% or with a DH≤5%, complications, liver insufficiency, and death are significantly more common than in patients with FLR>20% and DH>5%[7] (Table 1). Among patients with cirrhosis, the needed DH appears to be greater; in a small series, all patients with a DH≤10% died postresection vs. no deaths in those with DH>10%.[8] Thus, PVE directly increases the volume and function of the liver remnant, and analysis of volumetric data post-PVE allows preoperative estimation of postoperative risk for complications and death.

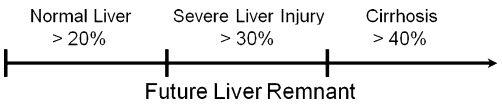

Fig. 1 Minimum FLR volume needed for safe hepatic resection in patients with normal liver, intermediate liver disease, or cirrhotic liver. Adapted from Zorzi D et al., Br J Surg 2007; 94: 274–286, with permission

Table 1 Short-term clinical outcome by standardized future liver remnant, degree of hypertrophy, and combined criteria following portal vein embolization and extended hepatectomy

Normal liver	FLR≤20% or DH≤5%	FLR>20% and DH>5%	*P*
Major complication (%)	47	14	0.01
Hepatic insufficiency (%)	20	1.9	0.03
Death within 90 days (%)	13	0	0.049

No deaths occurred when FLR volume target volume >20% was reached and DH after portal vein embolization was >5%. Modified from Ribero D et al., British Journal of Surgery 2007; 94: 1386–1394, with permission

Potential concerns regarding PVE in patients with CLM generally surround fear that embolization might induce growth not only of liver but also of tumors. Other concerns include the fear that chemotherapy, especially chemotherapy with agents such as bevacizumab, which block the action of vascular endothelial growth factor, might also impair liver regeneration after PVE. Both of these problems can be avoided. Firstly, if all tumor-bearing liver is embolized (e.g., in the case of the need for extended right hepatectomy, embolization of the right liver and segment IV), the median (and mean) change in tumor size pre-PVE vs. post-PVE is 0.0 cm.[7] Furthermore, it has been shown that there is no difference in DH post-PVE in patients with no chemotherapy vs. chemotherapy alone vs. chemotherapy with bevacizumab[9] (Fig. 2). Thus, attention to the details of embolization allows surgery, chemotherapy, and embolization to be combined effectively.

Surgery, Chemotherapy, and PVE

Combining all these data, a measured approach to patients with extensive disease, such as multiple, bilateral CLM can be devised. Such patients have advanced disease of uncertain biology, are likely to benefit from systemic chemotherapy, require extensive resection, and generally undergo a combination of wedge and anatomic resections as follows.[10] Following initial staging, chemotherapy is the first treatment step. Only patients with a decrease in tumor size and who do not develop new lesions on treatment at repeat staging are considered for the next therapeutic step, first-stage liver resection. First-stage resection includes laparotomy and wedge or minor resection clearing the FLR of metastatic disease. Typically, this includes wedge resections in the lateral liver, in preparation for future extended right hepatectomy, but may rather include wedges in posterior liver in preparation for extended left hepatectomy, or many combinations focused on developing a disease-free remnant. Recovery from the first-stage resection is an important assessment of the patient before moving to future steps. Patients with a now disease-free FLR are considered for PVE based on volumetry and consideration of the degree of underlying liver disease determined at first-stage surgery. Restaging typically occurs, further allowing tailored treatment (some will require chemotherapy because of progression of in situ disease between stages, and others will not). Those who undergo PVE are again restaged, and DH is assessed. If FLR volume, DH, staging, and recovery from first-stage surgery are acceptable, then second-stage major resection is performed to clear remaining disease. Patients with intact primary tumors generally undergo resection of the primary at the first stage, but emerging data suggest that the primary, which responds to chemotherapy, is rarely a problem and can be addressed after surgical treatment of the liver metastases with excellent oncologic outcomes.[11,12] Between two thirds and three quarters of patients who undergo first-stage resection will proceed to complete all stages of treatment, leading to a remarkable 86% 3-year overall survival in this cohort with extensive disease (median seven tumors per patient).[10] Thus, selection using chemotherapy, surgery, and when indicated, PVE, allows even patients with extensive disease to be selected for therapy enabling remarkable long-term survival.

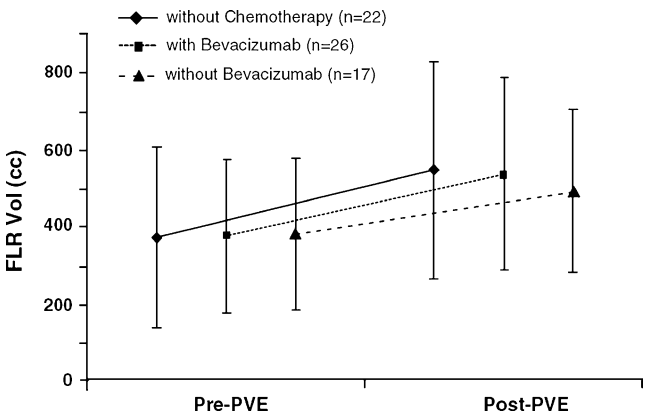

Fig. 2 Changes in absolute future liver remnant volume after portal vein embolization are not impacted by the use of chemotherapy with or without bevacizumab. *FLR Vol* future liver remnant volume, *PVE* portal vein embolization. Values are mean ± standard deviation. From Zorzi D et al., Ann Surg Oncol, 2008; Oct;15(10):2765–72

Conclusion

The limits of resection continue to expand, yet outcomes continue to improve. Chemotherapy is an important element, which improves survival in stage IV colorectal cancer, and can be combined with surgery to improve outcomes even further. Tailored approaches to patients include major (anatomic) resection, minor (wedge) resection, liver volumetry, and PVE with assessment of volume change after PVE (DH>5% in normal liver and DH>10%

in cirrhotic liver predict good outcome). Step-by-step approaches combining these elements allow even patients with extensive and bilateral disease to be properly selected for surgical therapy with excellent short- and long-term outcomes. Until laboratory, pathologic, biologic, or genetic studies can tell us which patients will benefit most from surgical and other treatments, careful application of proven diagnostic and therapeutic approaches to patients with advanced disease will continue to allow surgeons to deliver tailored, patient-centered treatment in a multidisciplinary way.

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What, How, and When to Offer Nonresectional Therapy for Colorectal Cancer Liver Metastases

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In recent years, there has been a philosophical paradigm shift in the characterization of treatment outcomes for the diagnosis of cancer. Rather than limit treatment outcome to cure versus failure, the concept of cancer as a "chronic disease" has come into general acceptance. This change has largely occurred due to the availability of various regional, targeted, or potentially life-extending therapies which enable continued "quality of life" while patients undergo cancer treatment.

For patients with colorectal liver metastases (CRLM), surgical resection remains the gold standard and is the only potentially curative approach; however, 75–90% of patients with CRLM are precluded from resection due to multifocal disease, anatomic limitations, inadequate functional liver reserve, extrahepatic metastasis, or other medical comorbidities. Consequently, several nonresectional strategies have been developed for unresectable CRLM which unfortunately defines the majority of patients. Broadly grouped, these strategies are: (1) systemic therapies (chemotherapy/biologics) with an intended goal of converting unresectable disease to resectable or to stabilize the disease and increase time to disease progression; (2) regional therapies such as regional transarterial drug/device delivery systems; and (3) local (chemical and thermal) ablative modalities.

It is crucial for physicians involved in the treatment of patients with CRLM to be familiar with these modalities and essential for surgeons to understand their potential role as adjuncts to surgical resection. Alternatively, for unresectable CRLM, these modalities can be used as "stand alone" approaches with well-documented response rates with maintained quality of life.

This paper briefly summarizes the most commonly employed regional modalities for treating CRLM: thermal ablation (cryoablation, radiofrequency ablation (RFA), and microwave therapy), hepatic arterial chemotherapy (HAIC and drug-eluting beads), and radioembolics (Y-90).

Ablative Therapies

Multiple ablative modalities have been applied to CRLM including thermal ablation using RFA, microwave therapy (MWT), cryoablation, and laser therapy. The most widely used of these modalities are RFA and MWT. Cryoablation was the first ablative therapy used to treat unresectable CRLM and was initially met with great fanfare and enthusiasm. However, substantial morbidity, occasional deaths, and high rates of recurrence led many to abandon cryotherapy in favor of radiofrequency and microwave ablation. RFA applies radiofrequency waves of about 500 kHz which is almost similar to surgical electrocautery but much less in frequency than microwave ablation antenna (2.5 MHz). These energy waves mobilize the ions of the surrounding tissue, resulting in a friction-induced heat and coagulative necrosis of tissue.

The reported 1-, 3-, and 5-year survival after RFA for patients with single tumors with a maximum diameter of 3 cm is 97%, 84%, and 40%, respectively.[1] These results approach those of surgical resection. However, technical and mechanical limitations of this technique are numerous.[2] First, the volume of tissue necrosis is limited as the real-time necrosis around the RFA electrode forms a mechanical barrier that reduces heat propagation. Second, ultrasound-

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guided RFA is limited in isoechoic lesions due to difficulty in targeting. Third, CT- or ultrasound-guided RFA is time-consuming, and a complete destruction of a 4-cm lesion can take up to 30 min when performed properly. As such, it is not practical to RFA more than three lesions in one setting. From an anatomical point of view, hepatic lesions in close proximity to large blood vessels are not thoroughly ablated by RFA due to convective heat loss from the cooling effect of local blood flow. Similarly, RFA of hepatic metastases in close proximity to a bile ducts can produce a biliary stricture. Finally, some lesions can be challenging to safely treat because of their location such as those in the dome of the liver or on the inferior surface of the liver adjacent to viscera. Evidence-based (level 2) recommendations for the use of RFA in the treatment of CRLM include:

- primary therapy for unresectable CRLM less than 3.0 cm in size
- combination treatment with surgery for unresectable CRLM
- combination treatment with HAIC for unresectable CRLM
- combination treatment with systemic chemotherapy for unresectable CRLM
- RFA may be feasible in the treatment of recurrent CRLM; however, limited or no data are available to assess outcome benefits.

Microwave coagulation therapy (MWT) is the most recent addition to the ablation armamentarium. Preliminary observations from prospective case series have shown MWT to be fast and effective at ablating CRLM up to 5 cm in size.[3] However, long-term treatment efficacy is not yet known. While it is expected that MWT will be equally effective as RFA at ablating CRLM, data in support of this modality are not yet available.

Regional Transarterial Therapies

Regional transarterial therapies are based on the principle that neoplastic hepatic lesions >3 mm in size receive the majority of their blood supply through the hepatic arterial system; thus, antineoplastic treatment through the hepatic artery can spare normal hepatic parenchyma which derives 80% of its main blood supply through the portal vein.

Hepatic arterial infusion (HAI) using a hepatic pump delivering chemotherapeutic agent to the hepatic artery territory has been demonstrated to be superior to systemic therapy by several randomized trials.[4,5] This modality requires surgical placement of a catheter into the gastroduodenal artery with its tip at the junction of common and proper hepatic artery. Various chemotherapeutic agents can be used; however, the most widely used has been floxuridine (FUDR). FUDR achieves higher hepatic concentrations and is nearly completely extracted on the first pass with limited systemic side effects.

The role of HAI in CRLM remains controversial. Although HAI has been demonstrated to produce a high response rate in both previously untreated (78%) and treated patients (52%), with 1-, 2-, and 5-year survival rates of 86%, 62%, and 12%, respectively, this technique is currently not commonly used. HAI-induced hepatic toxicity and pump-related complications are the main challenges for the use of this modality. Pump-related complications including catheter dislodgement and bleeding, poor arterial perfusion/arterial thrombosis, and pump pocket infection are all technical and related to surgeon experience with the technique. In a more recent analysis of pump complications, the incidence of pump failure was found to be 9% at 1 year and 16% at 2 years, and pump complications occurred in 22% of patients. Increased pump complication rates occurred in the setting of variant arterial anatomy, when the catheter was inserted into a vessel other than the gastroduodenal artery, and if the surgeon had performed fewer than 25 earlier procedures. Currently, the use of HAI for CRLM is limited to a few centers and is likely to be used even less in the future.

An alternative method of delivering high doses of chemotherapy transarterially to CRLM has been introduced by way of drug-eluting microscopic beads (DEBs). The DEBs are loaded with irenotecan in vitro and then injected into the hepatic artery and/or superselectively into tumor vessels. The DEBs are used to embolize the tumor vessels and then slowly release their chemotherapy over 2 weeks, thus diminishing systemic toxicity. Early results are promising, although follow-up is short.[6]

Selective Internal Radiation Therapy

In selective internal radiation therapy (SIRT), the pure beta-emitting isotope Y-90 is compounded onto millions of microspheres or thousands of glass spheres that are injected into the hepatic artery or one of its branches. Y-90 spheres deposit in the tumor vasculature with a resultant delivery of intense local radiation to the tumor but relative sparing of normal liver parenchyma.

The main advantages of SIRT are its selectivity, sparing peritumoral “normal” liver parenchyma often exposed to chemotherapy, and that it “burns no bridges” to other treatment modalities. On the other hand, inadvertent passage of the radioactive microspheres into the systemic circulation is associated with significant morbidities such as radiation pneumonitis or poorly healing gastroduodenal ulcers. A macroaggregated albumin (MAA) shunt test is mandatory when planning for SIRT, a shunt <13% is

considered low risk for radiation pneumonitis. Similarly, selective administration of SIRT can reduce the risk of gastroduodenal ulceration; alternatively, pretreatment ligation of the gastroduodenal artery and/or other aberrant arterial branches can eliminate this risk.

Two different Y-90 microsphere preparations are available in North America, the glass microspheres (Therasphere® (Nordion)) and resin microspheres (Sirs-sphere® (Sirtex)); however, only the resin type is available worldwide. In historical randomized controlled studies, SIRT used as adjuvant to HAI and to systemic chemotherapy increased the median time to disease progression to 15.9 months vs. 9.7 in patients receiving HAI[7] and to 18.6 months vs. 3.6 months for patients receiving only systemic therapy.[8] These favorable results are of limited value today because response rates from present day standard first-line chemotherapy and biologics are greater.

A multidisciplinary consensus conference in 2006 established the guidelines for SIRT use in CRLM. According to these guidelines, SIRT can be performed by physicians from different specialties including interventional radiology, nuclear medicine, and radiation oncology. Candidates for radioembolization are patients with unresectable primary or metastatic hepatic disease including bilobar disease with liver-dominant tumor burden and a life expectancy >3 months.

Another new radiation modality is the robotic/radiosurgery CyberKnife® (Accuray, Inc., Sunnyvale, CA, USA). Theoretically, the system delivers a substantial radiation dose to the liver metastases while avoiding wide radiation to the liver parenchyma known to cause hepatic toxicity. Limited data are available on its efficiency and on the hepatic sensitivity to localized radiation. Similarly, the cost of this advanced technology and its limited availability are main concerns.

In summary, there are numerous options for the treatment of unresectable CRLM. Several modalities have become obsolete due to limited efficacy or unacceptable morbidity, while newer approaches (microwave, DEBS, yttrium-90) appear more promising. However, the true benefits of recently introduced modalities will require more study and longer follow-up. In the interim, since the majority of patients with CRLM are not candidates for surgical resection, significant effort should be put forth to better define optimal treatment combinations and their sequence of use, and to define their indications and contraindications.

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ORIGINAL ARTICLE

Esophageal Achalasia in the Veneto Region: Epidemiology and Treatment

Epidemiology and Treatment of Achalasia

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Abstract
Introduction Achalasia is a rare esophageal motility disorder, incurable but amenable to palliative treatments to relieve dysphagia. Given the rarity of the disease, there is a paucity of data from population-based studies on incidence and outcome of the two treatments most commonly used in clinical practice, i.e., endoscopic pneumatic dilation (PD) and surgical myotomy (SM).
Materials and Methods A retrospective longitudinal study was conducted on the Veneto region, in north-eastern Italy. All patients with achalasia as their primary diagnosis between 2001 and 2005 were identified and their demographics and treatment details obtained.
Results The overall incidence of achalasia was 1.59 cases/100,000/year. Achalasia patients were mainly seen at University Hospitals. Fifty-five percent of the patients received treatment, 23.3% SM and 31.8% PD. The cumulative risk of any subsequent intervention for achalasia was 20% in treated patients (29.7% in patients treated primarily with PD and 4% in patients treated with SM first).
Discussion The epidemiology of achalasia in the Veneto Region is in line with the situation reported elsewhere and did not change between 2001 and 2005. Achalasia patients are mostly seen at University Hospitals. We observed a greater risk of subsequent intervention for patients previously treated with PD compared with SM.

Keywords Achalasia · Incidence · Heller's myotomy · Pneumatic dilation · Treatment failure

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Introduction

Achalasia is a primary motility disorder of the esophagus, characterized by a virtually absent peristalsis of the esophageal body and incomplete relaxation of the lower esophageal sphincter (LES).[1] Although the primary pathophysiological defect has been identified as a loss of the inhibitory ganglion cells and a persistence of cholinergic stimuli, the etiology of achalasia is not entirely clear.[2] It significantly impairs quality of life, with patients complaining of dysphagia, regurgitation, and chest pain, and sometimes suffering from significant weight loss. Its treatment remains controversial: all therapies are palliative and aim to reduce the LES resting pressure by stretching or disrupting the LES muscle fibers with endoscopic pneumatic dilations (PD) or surgical myotomy (SM). PD has been considered the first-line achalasia treatment for several decades, but in the last 15 years, the development of video-endoscopic methods has rekindled interest in the surgical management of this disease.

The incidence of achalasia has been reported around one case per 100,000 per year, but this figure comes from a handful of studies (Island, UK, Israel, USA), none of them from continental Europe. Despite its relative rarity, achalasia is the most commonly observed esophageal motor disorder, and—since there are no formal indications for case centralization—it is treated in tertiary referral centers (University Hospital or Teaching Hospitals) and in primary hospitals as well. Most of the data on achalasia treatment come from few highly specialized centers, which may not reflect the general approach to the disease's treatment. The purpose of this study was therefore to investigate the public health database on patients with achalasia in the Veneto Region, focusing particularly on: (1) the disease's incidence and (2) the treatment choices and their results, in order to obtain reliable information on the demographics and epidemiology of the disease and a more realistic picture of the current treatments for achalasia and their efficacy.

Materials and Methods

Study Design

The Veneto Region is one of the 21 Italian administrative regions; it is situated in the north-east of Italy and is considered fairly homogeneous in terms of welfare and healthcare services. In particular, the Veneto's healthcare services are perceived as being of high quality and virtually all the Region's residents are treated locally, along with many patients referred from other Italian regions of northern and southern Italy.

All discharge reports issued by public and private hospitals in the region are stored in the Veneto Region's Hospital Discharge Database (RVHDD) using a hospital discharge form (FHDF) containing information on patients' age, sex, home address, dates of hospital admission and discharge, up to six diagnoses on discharge, and up to six therapeutic or diagnostic procedures performed, classified according to the diagnosis-related groups (DRG) and coded according to the Ninth Revision, International Classification of Diseases, Clinical Modification (ICD9CM; Practice Management Information Corp., Los Angeles, CA 1993).

The survey was conducted by the Health and Social Agency of the Veneto Region, which has access to the longitudinal hospital care data: the data were crossed with information on the population of the Veneto Region (obtained by the Regional Statistics Service) for each year of the study. In the last year of the present study (2005), the Veneto Region had a population of 4,885,000 (2,493,000 females and 2,392,000 males), accounting for about 8% of the total Italian population.

Data Analysis

Patients with a "primary" discharge diagnosis of achalasia (based on the four-digit code 530.0 of the ICD9CM) between 2001 and 2005 were selected. To avoid misinterpreting the data, we also analyzed the hospital charts for 2000 (to rule out the possibility of a diagnosis of achalasia having already been established) and the charts for 2006–2008, to ensure that a diagnosis of cancer had not replaced the original diagnosis of achalasia and to assess the follow-up and any further intervention due to the failure of the initial treatment.

The data extracted from the RVHDD were stratified by categorical variables pertaining to patient demographics (age group, gender, etc.), and the characteristics of the hospital concerned (e.g., whether it was a University hospital or not).

1. Incidence of disease
   The numbers of resident patients admitted to hospital for achalasia by year and age group were divided by the corresponding number of Veneto residents in the same year and age group, and the rates were expressed per 100,000 persons. As mentioned above, only charts in which achalasia was the "primary" discharge diagnosis were considered.
2. Treatments
   Patients with a primary diagnosis of achalasia treated with endoscopic PD or SM between 2001 and 2004 were considered. Patients treated in 2005 were excluded, and the year 2005 was used only to ensure a sufficiently long follow-up (minimum 12 months) for further intervention due to the failure of initial therapy in patients treated in 2004.

Statistical Analysis

Data are expressed as medians with interquartile ranges in parentheses. We used the chi-square and Z-test to compare incidence and proportion. We used one-way analysis of variance and the Mann–Whitney, Kruskal–Wallis, or Wilcoxon tests to compare continuous variables. Survival estimates were calculated by the Kaplan–Meier method, and comparisons were drawn using the log-rank test. The Stata 9.1 statistical package (Stata Corporation, College Station, TX) was used to perform all analyses. A p value of less than 0.05 was considered significant.

Results

We identified 565 patients with achalasia as their "primary" discharge diagnosis between 2001 and 2005:

200 patients were non-residents referred from other regions and 365 were residents of the Veneto Region. Figure 1 shows the hospitalizations for achalasia distinguishing between residents and non-residents. Almost two thirds of achalasia patients (69%) were seen at University hospitals (Fig. 2).

Incidence

Only the 365 patients resident in the Veneto Region were considered to calculate the incidence of achalasia, the treatment chosen, and its outcome.

Table 1 shows the achalasia patients during the period 2001–2005 stratified by various demographic characteristics. The overall incidence was 1.59 cases/100,000/year and did not change over the 5-year period. No difference emerged between males and females. The highest incidence of achalasia was seen among patients >75 years old.

Treatments

In all, 127 patients (44.8%) with diagnosis of achalasia did not receive any treatment between 2001 and 2004. Of the other 156 patients (55.2%), 90 (57.7%) had PD, and 66 (42.3%) had SM. Figure 3 shows the time trends of the treatments for achalasia patients, distinguishing between teaching and non-teaching hospitals. Table 2 lists the patients treated with PD and SM according to various demographic characteristics and compared with patients given no treatment. The untreated patients were older, as reflected by a higher median age and a greater percentage of patients over 75 years.

One death occurred after a PD, while none were reported within 30 days of a SM during the period of time considered (p=0.37).

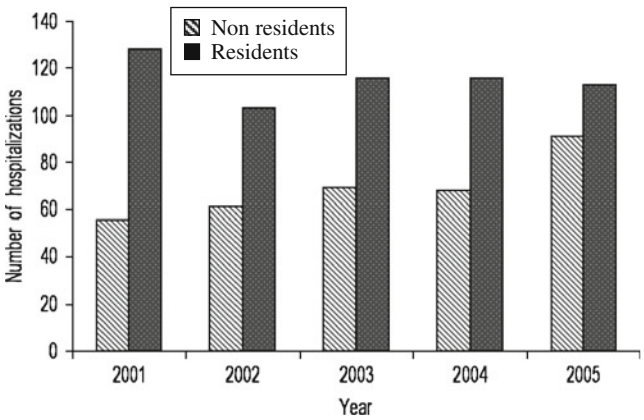

Fig. 1 Hospitalizations for achalasia distinguished for residents and non-residents (2001–2005)

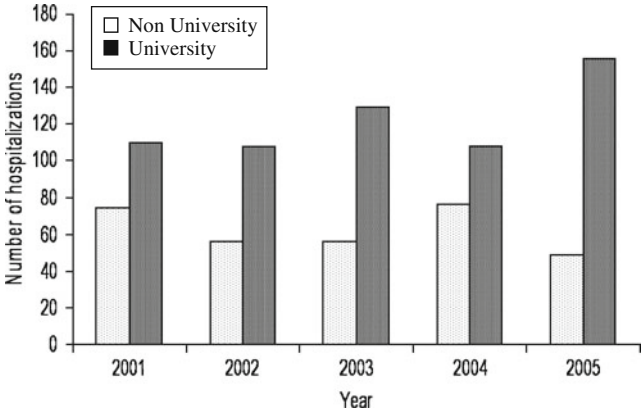

Fig. 2 Hospitalizations for achalasia distinguished by University vs. non-University hospitals (2001–2005)

Comparison of Endoscopic Pneumatic Dilation (PD) and Surgical Myotomy (SM)

Of the 90 patients who initially had PD, 26 (28.9%) received further treatment: 18 (20%) had one or more PDs, seven (7.7%) underwent SM (after an additional endoscopy in two cases) and one had esophago-gastric resection. Of the patients who initially underwent SM, three (4.5%) had further treatment, i.e., two (3%) had one or more PDs and one (1.5%) had a redo SM (p<0.01). Figure 4 shows the risk of having to have further treatment after an initial PD

Table 1 Incidence of achalasia as the "primary" discharge diagnosis among residents of the Veneto Region between 2001 and 2005

	No. of patients	Incidence	p value
Overall	365	1.59 (1.42–1.74)	–
Gender			
Male	179	1.59 (1.35–1.82)	p=0.93
Female	186	1.58 (1.35–1.80)	–
Age groups, years			
0–15	11	0.35 (0.14–0.55)	–
15–30	26	0.65 (0.40–0.90)	p=0.07
30–45	70	1.21 (0.93–1.50)	p<0.01
45–60	76	1.67 (1.29–2.04)	p<0.01
60–75	88	2.39 (1.89–2.89)	p<0.01
>75	94	4.81 (3.83–5.78)	p<0.01
Year			
2001	81	1.81 (0.42–2.21)	–
2002	60	1.30 (0.97–1.63)	p=0.07
2003	74	1.61 (1.24–1.98)	p=0.51
2004	68	1.46 (1.11–1.81)	p=0.21
2005	82	1.74 (1.37–2.12)	p=0.85

Data are expressed as no. patients/year/100,000 residents (95% confidence interval)

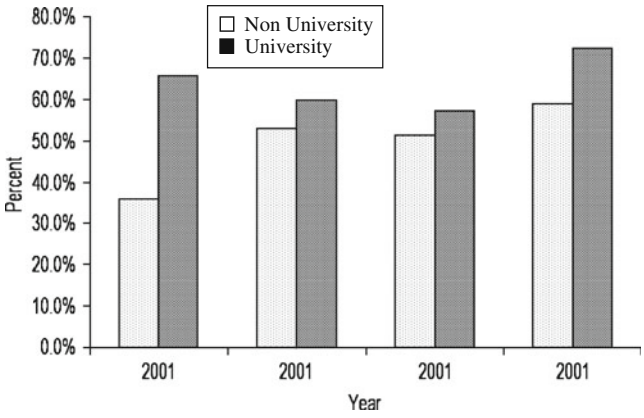

Fig. 3 Percentage of patients treated, by year and type of hospital (University vs. non-University)

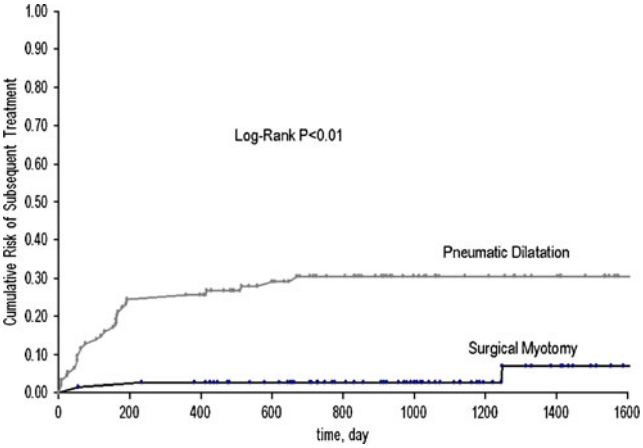

Fig. 4 Time to subsequent treatment after initial treatment during the study period

or SM during the study period. Patients initially treated with PD were significantly more likely to need further treatment than those treated with SM, OR of 10.18 (2.9–54.1), and the probability, adjusted for age and sex, of needing further intervention was also higher for patients treated initially with PD, OR of 9.8 (2.8–33.9).

Discussion

A few interesting observations emerge from this epidemiological study, which is the first to be conducted on the incidence of achalasia and its treatment in a large, homogeneous region in the north-east of Italy.

The rare nature of the disease explains why so few epidemiological studies have been published in Europe or America and why the number of patients is so limited in most of these series. To our knowledge, only four such studies have included more than 150 patients, i.e., two from the UK, one from Israel, and one from New Zealand.[3–5] In these series, the incidence varied between 0.8 and 1.2 cases/100,000/year, while in the Veneto region it was slightly higher, at 1.59 cases/100,000/year. Despite its retrospective nature, the strength of the present study lies in that all patients with achalasia were diagnosed and managed at about 20 hospitals in the region, two of them University hospitals, where all diagnostic discharge coding is done by physicians, which minimizes the risk of an erroneous code being indicated for such a rare condition as achalasia. To further reduce the risk of including patients without achalasia in this retrospective study (2001–2005), we only considered patients with a "primary" diagnosis of achalasia, and we checked the hospital charts for 2000 to rule out any diagnosis of achalasia having already been established (and giving rise to duplicate data).

We found a clearly age-related increasing incidence of achalasia up to the oldest age group (>75 years) and a similar incidence in men and women. The age-related pattern (with the lowest rate in the youngest and the highest in the oldest patients) had already been reported in other studies.[6–8] An age-related increase in neuron degeneration and loss of neuronal control has been suggested as a possible, partial explanation for this typical epidemiological pattern,[9] though it may be that other motility disorders not clearly identified in the ICD codes—such as diffuse esophageal spasm or presbyesophagus, or other non-specific esophageal motor abnormalities leading to functional obstruction—are classified under the same code as achalasia, especially in older patients unwilling to submit to physiological studies.

No significant changes were seen in the incidence of achalasia over the 5-year period in the Veneto region, as mentioned in long-term studies from the USA[10] and the UK.[6, 11] Although the short timeframe of this study prevents us from drawing any final conclusions on the temporal patterns of achalasia, similarly stable trends were reported in the past in incidence studies covering decades of analysis.[11]

Table 2 Demographics and clinical characteristics of patients undergoing pneumatic dilation or surgical myotomy or receiving no treatment between 2001 and 2004

	No treatment (*N*=127)	Pneumatic dilation (*N*=90)	Surgical myotomy (*N*=66)	*p* value
Gender, male	58 (45.7)	48 (53.3)	34 (51.5)	0.93
Age				
in years[a]	67 (50–80)	60 (43–75)	54 (34–64)	<0.01
in cases >75 years	47 (37)	20 (22)	8 (12)	<0.01
Mortality[b]	1 (0.8)	1 (1.1)	0 (0)	0.69

Data are expressed as *N* (%)

[a] Median (interquartile range)

[b] Including in-hospital deaths

Patients with achalasia were seen mostly at the two University hospitals involved in the study (Padova and Verona), which covered nearly 70% of cases. This was to be expected (similar data were reported by Sonnenberg in the USA), given that the diagnosis of achalasia relies on esophageal manometry, which is mostly available at university hospitals or gastroenterology centers dedicated to motility studies.

In addition to assessing the incidence of the disease, the second aim of this study was to investigate the treatments chosen and their outcome. Nearly half of the patients with a diagnosis of achalasia surprisingly received no treatment (at least during the period considered): these patients were generally older than those who were treated (37% of patients in the untreated group were over 75). Achalasia patients admitted at the University hospitals had a greater probability of receiving any treatment, than those admitted to the other hospitals (63.6% vs. 48.7%, $p=0.01$) and this difference was greater for 45- to 60-year-old patients (69.5% vs. 38.9%, $p=0.03$).

One explanation for so many untreated patients is that some may have been diagnosed with achalasia in our region and then treated elsewhere, though this is unlikely, given the high perceived quality of the Veneto Region's healthcare system (confirmed by the number of achalasia patients coming from other regions to be treated in the Veneto, whereas the numbers of achalasia patients "emigrating" from the Veneto would be minimal, if any). The most likely cause is a negative attitude on the part of physicians (especially in non-University hospitals) and patients (especially elderly people), who prefer to cope with this benign disease, rather than submit to any invasive treatment.

The majority of patients (156/283, 55.1%) did receive treatment, however. While the authors had expected SM to be the preferred therapy for achalasia (given their personal experience at one of the two teaching hospitals), on a regional basis, dilation was still the most often used option for achalasia treatment. A very small percentage of patients had botulinum toxin injections (BTI), which is consistent with the fact that PD and SM are really the only treatment options for achalasia, while BTI have only a transient effect and are usually reserved for patients unfit for surgery, or as a bridge to surgery.

The types of treatment were not evenly distributed in the Veneto Region and at the two University hospitals: in one (Padova) the preferred treatment was SM while in the other (Verona) it was PD; neither changed their approach to achalasia treatment during the study, which goes to show that the physician's expertise and personal preference were the main determinants of the choice of therapy with these two high-volume referral centers for achalasia.

Patients treated initially with PD were significantly more likely to have further treatment than those treated with SM first (20% vs. 4%, respectively). Although the limit of this study was the short follow-up, these data are nonetheless consistent with a similar study conducted in Canada on a larger sample and with a longer follow-up.[12] A large body of literature, including long-term follow-up studies, also suggests that symptoms recur after pneumatic dilation in up to 60% of patients,[13] whereas SM seems to achieve a permanent clinical and radiological improvement in up to 85–90% of cases.[14] These results of the two different types of treatment were also confirmed in the only prospective randomized study currently available comparing PD with SM.[15]

In conclusion, the incidence of achalasia seems to be slightly higher in north-east Italy than elsewhere, but remains a "rare" disease. The diagnosis of achalasia is three times more frequent in the elderly than in younger patients, and most older patients are not treated. This is an issue that should be further addressed by researchers and medical societies with a view to offering all achalasia patients the same treatment opportunities, whatever their age. The preferred treatment for achalasia in this first decade of the twenty-first century in the Veneto Region remains pneumatic dilation, except at one highly specialized unit where most of the regions' surgical myotomies were concentrated, despite the likelihood of patients needing a second treatment after PD being five times higher than after surgery.

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ORIGINAL ARTICLE

Mir-148a Improves Response to Chemotherapy in Sensitive and Resistant Oesophageal Adenocarcinoma and Squamous Cell Carcinoma Cells

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Abstract
Background Response to chemotherapy varies widely in patients with advanced oesophageal cancer. We investigated the impact of manipulating certain microRNAs on response to cisplatin and 5-fluorouracil (5-FU) in oesophageal cancer cells.
Methods Cisplatin-/5-fluorouracil-resistant oesophageal squamous cell carcinoma (SCC) and adenocarcinoma (EAC) cell lines were established, and the impact of ectopic upregulation of miR-106a and miR-148a on response to both drugs was assessed.
Results The impact of miR-106a-upregulation was inconsistent. Upregulation was followed by reduced sensitivity to cisplatin in chemotherapy-sensitive EAC cells (cell survival, +8.7 ± 0.8%; $p = 0.003$) and an improved response to 5-FU in cisplatin-resistant EAC cells (cell survival, −6.4 ± 2.5%; $p = 0.011$). MiR-148a upregulation significantly increased sensitivity to chemotherapy in seven out of ten cell lines, represented by a decrease in cell viability of 22.6 ± 7.9% to 50.5 ± 10.6% after cisplatin ($p \leq 0.014$) and 6.0 ± 0.8% to 15.0 ± 4.1% after 5-FU treatment ($p \leq 0.012$). The only cell lines in which miR-148a upregulation had no effect were cisplatin-resistant EAC exposed to cisplatin and 5-FU-sensitive and 5-FU-resistant SCC cells exposed to 5-FU.
Conclusion MiR-148a sensitized chemotherapy-sensitive oesophageal cancer cell lines to cisplatin and, to a lesser extent, to 5-flurouracil and attenuated resistance in chemotherapy-resistant variants. Further experimental and clinical studies to investigate the exact mechanisms involved are warranted.

Keywords miRNA · miR-148a · miR-106a · Chemotherapy · Resistance

Introduction

Oesophageal cancer is usually diagnosed at a locally advanced stage, and local lymph node metastases are common. Conse-

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quently, its prognosis is generally poor, and there has been considerable interest over recent decades in using chemotherapy, with or without radiotherapy, for the treatment of patients with oesophageal cancer, either before surgery or in the definitive treatment of patients in whom surgery is not appropriate. Most clinical studies report a variable response to chemotherapy, with some tumours disappearing completely, and others responding poorly to such treatment. More recent meta-analyses suggest that patients who undergo esophagectomy, and in whom neoadjuvant treatment has achieved a "complete" response, have a much better survival outcome.[1–3] Unfortunately, however, only 20–40% of patients have such a response to neoadjuvant therapy,[4,5] and the development of methods to improve the response to neoadjuvant therapies seems worthwhile.

In this context, we have been interested in the potential of microRNAs (miRNAs) to impact on chemotherapy. MiRNAs are small non-coding RNA molecules which regulate gene expression posttranscriptionally and control many fundamental cellular processes.[6] In various cancers, miRNAs have been demonstrated to regulate oncogenes or tumour suppressor genes.[7–10] In oesophageal carcinoma, different levels of miRNAs can be used to discriminate between benign and malignant oesophageal tissues,[11–21] and miRNAs are involved in cell proliferation, invasion, migration, apoptosis, cell cycle and tumorigenesis.[14–16,18,21,22] Most importantly, expression of miRNAs or RNASEN (enzyme in the biogenesis of miRNAs) correlates with the risk of lymph node metastasis, venous invasion,[21] and prognosis.[17,23–25] Interestingly, in other cancer types, some miRNAs have been shown to be associated with sensitivity to,[26–28] or modification of, response to chemotherapy agents such as cisplatin or 5-fluorouracil (5-FU).[29–31] However, no studies have investigated the potential effect of altered miRNA expression on response to chemotherapy treatment in oesophageal cancer.

Recently, we demonstrated that two miRNAs, miR-106a and miR-148a, are negatively associated with oesophageal cancer recurrence after surgical treatment in patients with advanced oesophageal squamous cell carcinoma, as well as the likelihood of tumour-related death. Furthermore, miR-148a expression was inversely correlated with adenocarcinoma differentiation grade.[32] As recurrent disease and poor tumour differentiation both suggest more aggressive malignancies, and aggressive tumours might be increasingly resistant to chemotherapy, we hypothesized that altered levels of these two miRNAs might have an impact on the outcome after chemotherapy for oesophageal cancer. As the standard chemotherapy treatment for both forms of oesophageal cancer is based on cisplatin and 5-fluorouracil, we investigated the potential for these two miRNAs to modulate the cellular response to either cisplatin or 5-fluorouracil in adenocarcinoma and squamous cell oesophageal carcinoma cell lines.

Material and Methods

Cell Lines and Cell Culture

The human squamous cell carcinoma cell line KYSE410 (obtained from the Microbiology Laboratory, University of Muenster, Germany) and the human adenocarcinoma cell line OE19 (obtained from the Department of Surgery, Flinders University Adelaide, Australia) were cultured using RPMI 1640 medium (GIBCO® Invitrogen, no. 11875) or Dulbecco's modified Eagle's medium (DMEM) high-glucose 1× medium (GIBCO® Invitrogen, no. 11995) respectively, supplemented with 10% foetal bovine serum (GIBCO® Invitrogen, no. 26140), 1% penicillin–streptomycin (GIBCO® Invitrogen, no. 15140; 10,000 U of penicillin and 10,000 μg of streptomycin per 1 mL) and 2‰ Normocin™ (InvivoGen, San Diego, USA, catalog no. ant-nr-1; 50 mg/mL) in a humidified atmosphere containing 5% CO_2 at 37°C. For drug sensitivity assays and transfection experiments, phenol red free medium (RPMI 1640: GIBCO® Invitrogen, no. 11835; DMEM/F12 1:1: GIBCO® Invitrogen, no. 11039) containing the same supplements was used. Drug-resistant variants of both cell lines were established using a repetitive pulsatile treatment with constant concentrations of cisplatin and 5-FU. Briefly, KYSE 410 cells were subjected to a 4-day exposure of 2 μM cisplatin (KYSE410/C2) or 5 μM 5-FU (KYSE410/5-FU5) and OE 19 cells were exposed to 5 μM cisplatin (OE19/C5) for 3 days; the medium was not changed during this period, providing a constant exposure to the drug. We were unable to establish a 5-FU-resistant variant of the OE19 cell line during this study. After removal of the chemotherapy agents, cells were allowed to recover and split when reaching approximately 70–80% confluency, followed by the next cycle of chemotherapy. Prior to transfection, the degree of chemotherapy resistance of the respective cell lines was assessed. All cell lines presented significant resistance to the corresponding chemotherapy agent (see Table 1).

In Vitro Drug Sensitivity Assay

Cells were seeded onto 96-well plates (2.5×10^3 and $5–6 \times 10^3$ viable cells/well for KYSE410 and OE19, respectively) and allowed to attach. After cellular adhesion, phenol red free medium containing cisplatin or 5-FU at distinct concentrations (5 μM cisplatin or 5 μM 5-FU for KYSE410 cell lines; 20 μM cisplatin or 100 μM 5-FU for OE19 cell lines) was freshly prepared and added to the corresponding cells. The concentration of drugs represented the approximate median lethal doses (LD_{50}) in the respective cell lines following 72 h of exposure to cisplatin and 5-FU. This was estimated in previous experiments in our laboratory which

Table 1 Relative cell survival after cisplatin and 5-FU treatment in different sensitive and resistant oesophageal squamous cell carcinoma and adenocarcinoma cell lines

	Sensitive SCC	5-FU-resistant SCC	Cisplatin-resistant SCC	Sensitive EAC	Cisplatin-resistant EAC
Cisplatin	53.4 ± 6.1	54.8 ± 10.8	68.9 ± 4.2 ($p = 0.035$)[a]	43.6 ± 6.3	64.1 ± 8.6 ($p = 0.028$)[a]
5-FU	62.5 ± 6.0	92.0 ± 3.3 ($p = 0.003$)[a]	57.0 ± 2.6	49.8 ± 8.7	57.6 ± 3.1

Data were presented as percentage of viable cells related to untreated controls and expressed as means ± standard deviation. Doses of chemotherapy agents used for the assay were as follows: cisplatin, 5 μM for SCC and 20 μM for EAC cell lines; 5-FU, 5 μM for SCC and 100 μM for EAC cell lines (see "Material and Methods")

[a] Statistical comparison to corresponding sensitive cell line

tested various drug concentrations over 24-, 48-, 72- and 96-h periods (data not shown). After 72 h, cell viability was assessed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), inner salt; Promega). Cells were washed with PBS. MTS reagent was prepared in fresh medium (100 μL phenol red free medium +20 μL MTS solution) and applied to the cells. The absorbance at 490 nm for each well was read on a spectrophotometer after 2 h, and the absorbance of the background (wells with medium and MTS solution) was subtracted from experimental wells to provide corrected absorbance readings. For the assessment of the effect of transfection on sensitivity to drug treatment, three independent experiments were performed with nine technical replicates each. Drug resistance was assessed in a minimum of two independent experiments.

Establishment of Transfection

Hsa-miR-106a mimic, hsa-miR-148a mimic and negative controls were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The negative control was designed to contain no homology to human gene sequences and miRNAs. Cells were transfected using Lipofectamine™ 2000 (Invitrogen, cat. no. 11668-019) according to a slightly modified manufacturer's protocol as follows: Cells were plated in 24-well plates in antibiotics containing phenol red free medium at a density of 1.9×10^4 KYSE410 cells/well or 5×10^4 OE19 cells/well and allowed to attach for 24 or 48 h, respectively. At a confluency of 15–20%, antibiotics containing phenol red free medium were changed and cells were transfected with 20 pmol oligonucleotides using Opti-MEM® I medium (GIBCO® Invitrogen, no. 31985) to prepare oligomer–Lipofectamine™ 2000 complexes. The medium was replaced 24 h after transfection, cells were harvested 48 h after transfection, and the lysate was stored at −20°C. Three independent experiments were performed in triplicate. RNA from the triplicates was pooled for the determination of miRNA levels.

Assessment of Effect of Transfection on Sensitivity to Anticancer Drug Treatment

Cells were plated in six-well plates in antibiotics containing phenol red free medium at a density of 9.5×10^4 or 2×10^5 cells/well and allowed to attach for 24 or 48 h (KYSE410 or OE19). Transfection was then performed as described above using the same miRNA mimics and negative controls and applying 100 pmol oligonucleotides to each well. Twenty-four hours after transfection, cells were seeded onto 96-well plates and allowed to attach overnight. Chemotherapy agents were applied 48 h after transfection, and in vitro drug sensitivity assays were then performed as described above. Cells in the remaining pellet after re-plating were harvested for confirmation of successful transfection.

RNA Harvest and Isolation

Just prior to harvest, cells were examined under the microscope to rule out contamination or other anomalies. RNA/cell harvest was then performed by applying TRIzol® (Invitrogen Life Technologies, NY, USA) either directly to the well/flask (transfection experiments: 500 μL per 24-well plate; resistant cell lines: 3 mL per T25 flask) or to the remaining pellet after re-plating experimental groups onto 96-well plates. The lysate was then transferred to 1.5-mL tubes and stored at −20°C until extraction of total RNA was performed according to the manufacturer's protocol. The concentration of RNA was quantified by UV spectrophotometry (NanoDrop® ND-8000 Spectrophotometer, Thermo Fisher Scientific, Wilmington, USA). RNA quality was determined by electrophoresis through a 1% agarose gel. All RNA samples were confirmed to be undegraded by visualization of distinct 28S and 18S rRNA species. The final RNA solution was stored at −20°C until required for cDNA synthesis.

RT-PCR and TaqMan® miRNA Assay

For the determination of miRNA levels, TaqMan® miRNA Assays (Applied Biosystems, Foster City, CA, USA) were

used. These assays detect only the mature form of the specific miRNAs. Assay IDs were as follows: hsa-miR-148a: ID 000470; hsa-miR-106a: ID 002169; RNU44: ID 001094. For each sample, 5 ng of total RNA was used for reverse transcription into cDNA. Following the manufacturer's protocol, we utilized 100 nM stem-loop RT primer, 100 mM dNTPs, 50 U/μL multiscribe reverse transcriptase, 20 U/μL RNase inhibitor, 1.5 μL 10× RT Buffer (all purchased from PE Applied Biosystems) and nuclease-free water. Incubation of reagents was performed in a thermocycler (Eppendorf Mastercycler, Eppendorf, North Ryde, NSW, Australia; protocol: 30 min at 16°C, 30 min at 42°C, 5 min at 85°C, then hold at 4°C). For real-time PCR, 5 μL of respective cDNA was mixed with 1 μL of gene-specific primers, 10 μL of Taqman® Universal PCR Mastermix (Applied Biosystems) and 4 μL of nuclease-free water. All samples were assayed in triplicate reactions using a Rotorgene 6000 thermocycler (Corbett Life Science, Sydney, NSW, Australia). Quantitative analysis was performed using Q-Gene software. MiRNA expression data were normalized to the expression levels of RNU44, which displayed comparable expression across the different groups (data not shown).

Statistical Analysis

The relative survival of resistant cell lines and mimic or negative control transfected cells, after treatment with anticancer drugs, was calculated by adjusting the mean corrected absorbance of the treated cells to the corresponding untreated controls (given in percent). For an assessment of the effect of transfection on sensitivity to chemotherapy drug treatment, the relative survival of the negative controls was then set to 0 and the effect of transfection was presented as relative survival of miRNA mimic-transfected groups compared to negative control-transfected groups (given in percent). Gene expression data for miR-106a and miR-148a were expressed as means of normalized expression with standard deviation. Data were assessed for statistical significance using one-way analysis of variance with post hoc testing/Student's t test for equal and unequal variances as appropriate. A value of $p < 0.05$ was considered to be statistically significant. All analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL).

Results

miRNA Expression in Sensitive and Resistant Variants

The expression of miR-106a and miR-148a in the different cell lines is summarized in Fig. 1a, b. MiR-106a was significantly downregulated in 5-FU-resistant but not in cisplatin-resistant SCC cells compared to sensitive controls (relative miR-106a expression in sensitive SCC, 0.88 ± 0.06; cisplatin-resistant SCC, 0.85 ± 0.24; 5-FU-resistant SCC cells, 0.47 ± 0.13), and there was no difference in miR-106a expression between cisplatin-resistant (relative expression, 0.47 ± 0.02) and sensitive (relative expression, 0.56 ± 0.06) EAC cells. The relative expression of miR-148a was very low in our samples, and there was no statistically significant difference in levels between sensitive and resistant cell line variants (relative miR-148a-expression in SCC cells: sensitive vs. cisplatin-resistant vs. 5-FU-resistant cells, 0.0009 ± 0.0001 vs. 0.003 ± 0.001 vs. 0.001 ± 0.0001; relative miR-148a-expression in EAC cells: sensitive vs. cisplatin-resistant cells, 0.026 ± 0.008 vs. 0.016 ± 0.002).

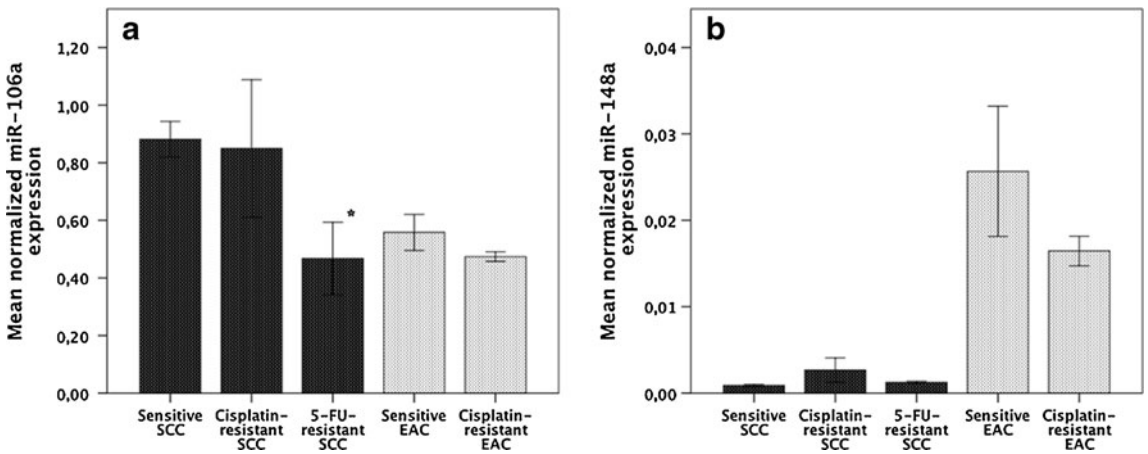

Fig. 1 Normalized expression of miR-106a (**a**) and miR-148a (**b**) in sensitive and resistant oesophageal squamous cell carcinoma and adenocarcinoma cell lines. SCC cell lines in *dark grey*, EAC cell lines in *light grey*. *Statistically significant compared to sensitive SCC ($p = 0.007$)

Transfection Experiments

Before testing the response to chemotherapy, pilot transfection experiments were performed to assess the level of overexpression of each miRNA after transfection. Forty-eight hours after transfection, PCR analysis demonstrated a successful increase in the levels of the transfected miRNAs (there were no significant differences in the increase of miRNA levels after transfection between the groups for either miRNA; Fig. 2a, b).

In the chemotherapy-sensitive maternal SCC and EAC cell lines, transfection with miR-106a did not affect chemotherapy treatment response, except in EAC cells where there was a slight increase in resistance to cisplatin (cell viability, compared to negative control, increased by 8.7 ± 0.8%, $p = 0.003$)). In contrast, miR-148a overexpression resulted in an improved response to 5-FU and cisplatin treatment in both maternal cell lines. Whilst effects of 5-FU on treatment were of relatively low magnitude (cell viability compared to negative control: SCC, −7.8 ± 9.0%, $p = 0.273$; EAC, −6.0 ± 0.8%, $p = 0.006$), miR-148a transfection led to a marked increase in sensitivity to cisplatin in both cell lines (cell viability compared to negative control: SCC, −50.5 ± 10.6%, $p = 0.014$; EAC, −22.6 ± 7.9%, $p = 0.008$; see Fig. 3).

In most chemotherapy-resistant cell lines, miR-106a upregulation had no significant effect on 5-FU or cisplatin treatment, although cisplatin-resistant EAC cells had a slightly greater sensitivity to 5-FU after transfection (cell viability compared to negative control, −6.4 ± 2.5%, $p = 0.011$; see Fig. 4).

In contrast to miR-106a transfection, miR-148a transfection was followed by an improved response to anticancer treatment in four of six resistant cell lines. Sensitivity to 5-FU was increased in cisplatin-resistant SCC (cell viability compared to negative control, −15.0 ± 4.1%, $p = 0.003$) and EAC cells (cell viability compared to negative control, −10.9 ± 2.1%, $p = 0.012$). Furthermore, the effect of increased miR-148a levels was again more pronounced with cisplatin treatment, with the decrease in cell viability being −25.0 ± 9.3% (cisplatin-resistant SCC: $p = 0.009$) and −30.6 ± 6.4% (5-FU-resistant SCC: $p = 0.014$) after treatment. MiR-148a did not have a sensitizing effect on cisplatin treatment in cisplatin-resistant EAC cells or on 5-FU treatment in 5-FU-resistant SCC cells (see Fig. 5).

Discussion

There is increasing evidence that the expression of miRNAs affects sensitivity to various chemotherapy agents across a broad variety of tumour types. Interestingly, so far, only a limited number of miRNAs (e.g. members of the let-7 family, miR-16, miR-21, or miR-451) had been confirmed to impact on more than one anticancer drug and/or to play a role in more than one tumour type, [33] and most importantly, little is known about synergies between these miRNAs in this context. Our study demonstrates, for the first time, an effect of miRNA modulation on sensitivity to anticancer treatment in oesophageal cancer. We have determined the effect of increasing the expression of miR-148a and miR-106a on sensitivity to cisplatin and 5-FU treatment in cisplatin- and 5-FU-sensitive and -resistant oesophageal cancer cell lines. In sensitive cells, transfection with miR-148a resulted in a marked increase in sensitivity to cisplatin in both oesophageal adenocarcinoma and squamous cell carcinoma cell lines, as well as a smaller but statistically significant increase in sensitivity to 5-FU treatment in

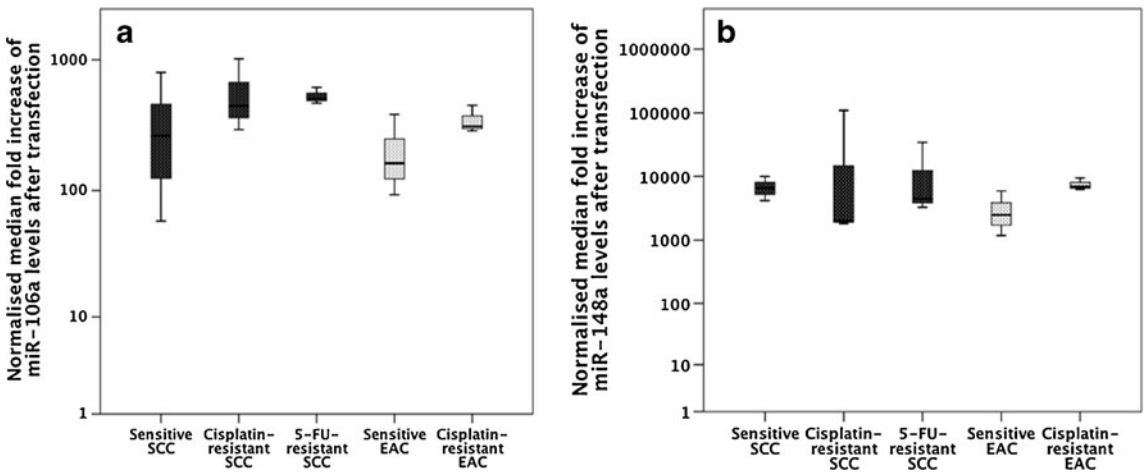

Fig. 2 Normalized median fold increase of miR-106a (**a**) and miR-148a levels (**b**) after transfection with the respective mimics. Scramble-transfected controls were set to 1 and the increase of miRNA levels of mimic-transfected groups was calculated as the ratio between expression in mimic and in scramble-transfected cells. SCC cell lines in *dark grey*; EAC cell lines in *light grey*

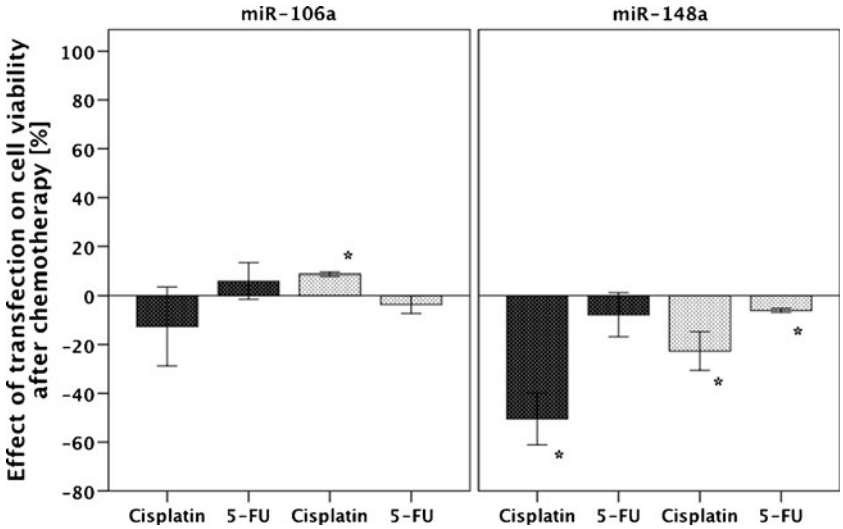

Fig. 3 Effect of transfection with miR-106a and miR-148a on sensitivity to 5-FU and cisplatin treatment in chemotherapy-sensitive squamous cell carcinoma and adenocarcinoma cell lines. Relative cell survival of negative control cells was set to 0 and the effect of transfection was presented as relative survival of transfected cells compared to negative control in percent. SCC cell lines in *dark grey*; EAC cell lines in *light grey*. *Statistically significant compared to respective negative controls (*p* values: see "Results")

oesophageal adenocarcinoma cells. The upregulation of miR-106a, on the other hand, did not impact on treatment, except for a slight increase in resistance to cisplatin in oesophageal adenocarcinoma cells. In general, these results were replicated in the chemotherapy-resistant variants of both cell lines, with upregulation of miR-148a improving the response to treatment with 5-FU in cisplatin-resistant squamous cell carcinoma and adenocarcinoma cells even more than the response observed in the sensitive cell lines. Only in the 5-FU-resistant squamous cell carcinoma cell line did the effect of miR-148a transfection fail to reach significance ($p = 0.117$). The distinct effect of miR-148a transfection on cisplatin treatment was also observed for cisplatin- and 5-FU-resistant variants of the squamous cell carcinoma cell line, but not in the cisplatin-resistant oesophageal adenocarcinoma cells. MiR-106a transfection showed, except a slight improvement of sensitivity to 5-FU in cisplatin-resistant EAC cells, no effect on cisplatin or 5-FU treatment in resistant variants.

Previous work investigating the role of miR-106a and its impact on sensitivity to anticancer medications has been conflicting. On the one hand, miR-106a expression has been shown to be reduced with increasing resistance to anticancer drug treatments in ovarian and multidrug-resistant gastric cancer cell lines.[27,28] Kovalchuk et al.[26], on the other hand, found an opposite effect, with miR-106a being upregulated in doxorubicin-resistant breast cancer cells. However, we could not confirm different expression patterns of miR-106a in most of the resistant cell lines we generated (except the 5-FU-resistant SCC variant) compared to chemotherapy-sensitive controls, and varying its expression had little impact on cisplatin and 5-FU chemotherapy treatment in the oesophageal cancer cell lines we evaluated. We are unable to explain why transfection resulted in lower levels of

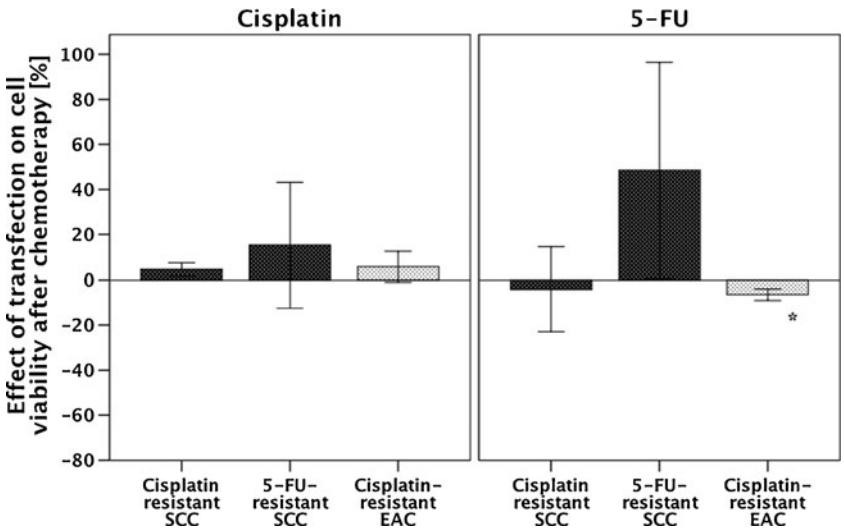

Fig. 4 Effect of transfection with miR-106a on sensitivity to chemotherapy treatment with cisplatin and 5-fluorouracil in resistant oesophageal squamous cell carcinoma and adenocarcinoma cell lines. Relative cell survival of negative control cells was set to 0 and the effect of transfection was presented as relative survival of transfected cells compared to negative controls in percent. SCC cell lines in *dark grey*; EAC cell lines in *light grey*. *Statistically significant compared to respective negative controls (*p* values: see "Results")

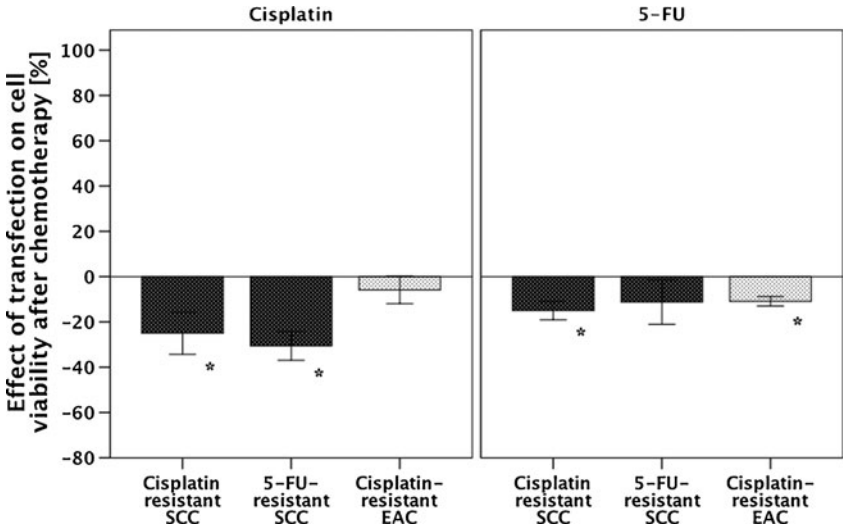

Fig. 5 Effect of transfection with miR-148a on sensitivity to chemotherapy treatment with cisplatin and 5-fluorouracil in chemotherapy-resistant oesophageal squamous cell carcinoma and adenocarcinoma cell lines. Relative cell survival of negative control cells was set to 0 and effect of transfection was presented as relative survival of transfected cells compared to negative controls in percent. SCC cell lines in *dark grey*; EAC cell lines in *light grey*. *Statistically significant compared to respective negative controls (*p* values: see "Results")

miR-106a expression compared with miR-148a expression, although this could be due to a difference in transfection efficiency between the two mimic molecules. In this context, however, the fact that miR-106a levels did not increase after transfection to the same extent as miR-148a levels (see Fig. 2a, b) is unlikely to affect the relevance of our findings for the following reason: miR-106a was found to be 2.3-fold upregulated in doxorubicin-resistant breast cancer cells,[26] more than 2-fold downregulated in multidrug-resistant gastric cancer cells,[28] and 1.9-fold downregulated in our own 5-FU-resistant SCC cell line. We observed a median increase of miR-106a levels of at least 162-fold, which is far higher than these reported pathological variations, and it is therefore reasonable to expect that the miR-106a levels obtained in our experimental regime are high enough to detect any possible effect on chemosensitivity.

In contrast, our results regarding miR-148a fit well with current knowledge about this miRNA. MiR-148a is considered an anti-oncogenic miRNA.[34,35] It has been shown that this miRNA is downregulated in a variety of human tumours and that its expression negatively affects tumour growth, cell motility, invasion, migration and metastasis.[36]. In accordance with these findings, previous array data from our lab suggest that miR-148a is downregulated in EAC compared to its precursor lesion, Barrett's oesophagus.[37] From the current data, however, we cannot conclude that miR-148a is further downregulated in our resistant variants when compared to the chemotherapy-sensitive tumour cell lines. Nevertheless, our experiments have shown a consistent improvement in response to cisplatin and 5-FU treatment in most chemotherapy-sensitive and -resistant oesophageal adenocarcinoma and squamous cell carcinoma cell lines after miR-148a overexpression.

The discrepancy between the sensitizing effect of miR-148a and the lack of a miR-106a effect in our study (or desensitizing effect in the case of cisplatin and the sensitive EAC cell line) is somewhat surprising. As we chose these miRNAs based on our previous findings of an inverse association between clinical signs of more aggressive tumours vs. miRNA expression, we expected a positive result for both miRNAs (at least in SCC). However, in our previous study, only miR-148a was also associated with tumour staging parameters in EAC. Furthermore, the literature supports roles for miR-106a both as a tumour-promoting miRNA and a tumour-suppressive miRNA, depending on the context.[32] It is also possible that other miRNAs, not assessed in this study, may impact upon the roles of miR-106a and miR-148a in resistance to chemotherapy and that these may differ between squamous carcinoma cell and adenocarcinoma cell lines. Overall, we conclude from our results that in this context, miR-148a is a more powerful tumour suppressor and affects anticancer treatment to a greater extent.

In a clinical context, our data suggest a possible application for miR-148a as a "supplement" to conventional chemotherapy. Applied together with cisplatin and 5-fluorouracil in patients with chemotherapy-sensitive tumours, miR-148a could allow a reduction of both agents whilst providing the same therapeutic effect. With this reduction, side effects of chemotherapy might be lowered. Furthermore, this might increase the overall response rate to chemotherapy, as in the case of therapy-resistant tumours, i.e. the effect of treatment could be restored by overcoming the resistance of the malignancy toward either of the drugs. However, our results are very preliminary and a lot more work will need to be done before any clinical application can be considered.

Our data are supported by recent work from Japan. Whilst preparing our paper for publication, Fujita et al.[36] published first evidence that miR-148a upregulation enhances sensitivity to pacitaxel treatment in pacitaxel-sensitive

and -resistant prostate cancer cell lines. Therefore, when considered with our results for oesophageal cancer cells, it seems reasonable to conclude that miR-148a plays an important role in the cellular response to various chemotherapeutic agents including cisplatin, 5-FU and pacitaxel. In this context, the most reasonable explanation for the smaller effect of miR-148a transfection on 5-FU treatment in our study might lie in the differences in mechanism of action between cisplatin and 5-FU. Whilst cisplatin has cytostatic and cytotoxic effects, 5-FU presents mainly cytostatic properties. The relative cell survival after chemotherapy might therefore be affected earlier by cisplatin than by 5-FU treatment, and our assessment 72 h after induction of chemotherapy might underrepresent the impact of miR-148a expression on 5-FU therapy.

Other published studies reveal several interesting downstream targets for miR-148a, which might explain the observed improvement in sensitivity to anticancer treatment. First, the study of Fujita et al.[36] demonstrated that miR-148a directly targets mitogen- and stress-activated kinase 1 (MSK1). MSK1 knockdown was shown to reduce resistance to paclitaxel in their experiments, indicating that miR-148a acts, at least in part, via the regulation of MSK1 expression. Second, miR-148a-mediated modulation of response to chemotherapy might partly be explained by the regulation of de novo DNA methylation (miR-148a targets include DNA methyltransferase 3B (*DNMT3B*)[38,39] and DNA methyltransferase-1 (DNMT-1) or regulating the expression of methylation-dependent tumour suppressor genes.[40] Another interesting target for miR-148a is the pregnane X receptor (PXR). PXR is a nuclear receptor that belongs to the family of ligand-activated transcription factors and can be activated by a large number of compounds. This receptor upregulates several important drug-metabolizing enzymes or drug efflux transporters, including CYP3A4, MDR1 (P-gp) and MRP3, which consequently leads to enhanced biotransformation and/or clearance of drugs.[41,42] Therefore, PXR is believed to be "a novel master regulator of multidrug resistance in cancers,"[43] and elevated PXR expression is associated with resistance to anticancer drug treatment in several cancers, including prostate and colorectal cancer.[42,44] Takagi and colleagues[45] were the first to demonstrate that PXR is directly targeted by miR-148a and that the miR-148a-dependent decrease of PXR protein attenuated the induction CYP3A4 mRNA. One known substrate of CYP3A4 is cisplatin,[41] and overexpression of MDR1 with consequent elevated expression of its product P-gp has been shown to result in an increased efflux of, for example, 5-FU in malignant cells.[46]

There are limitations which should be considered when interpreting the results of our study. The most important limitation is the restriction to only two oesophageal cancer cell lines (OE19 and KYSE410, respectively). The reason behind this restriction was the hypothesis that the examination of sensitive and derivative, resistant, cells provides more crucial information about the effect of miRNA modification on response to anticancer treatment than the inclusion of multiple sensitive cell lines only. Only about 20–40% of patients with oesophageal cancer present a major response after neoadjuvant treatment (i.e. sensitive tumours), and only these patients benefit from treatment.[4,5] Hence, we chose to focus on a model representing those patients who do not achieve a complete response to neoadjuvant treatment (i.e. chemotherapy-resistant cells). Even though we evaluated only two oesophageal cells lines, the consistent miR-148a-mediated enhancement of chemotherapy response in both the original cell lines, and their chemotherapy-resistant derivatives, suggests that common mechanisms may be conserved in the two different tumour types. Whilst this requires further verification, both in other oesophageal cell lines and in vivo, it provides a foundation for understanding such mechanisms in oesophageal cancer. It also identifies the need for broader investigative studies that may identify other regulatory pathways which distinguish responses in squamous- and adenocarcinoma-derived cells.

Furthermore, our current study did not include a thorough validation of possible gene expression targets for miR-148a influencing resistance to chemotherapy in our oesophageal cancer cell lines. This was not one of the aims of our study as we were primarily interested in whether the reported effect of miR-148a on chemosensitivity in other tumour types was applicable in oesophageal cancer types, and our study has shown such an effect. However, elucidation of the mechanisms behind the effect of miR-148a, and whether the same mechanisms apply across all tumour types, is an important question for future studies.

There are two major options for establishing chemotherapy-resistant cell lines: pulsatile treatment with constant doses vs. continuous application of drugs with increasing doses. The mechanism of resistance development might differ between these two approaches. As pulsatile treatment might be a better approximation of the clinical situation, we chose this technique. However, pulsatile treatment is usually applied for very short periods (3–24 h) and uses high concentrations of drugs.[47] In our current study, we tried to imitate the clinical situation of cisplatin and 5-FU application in patients with oesophageal cancer more precisely by a 3- to 4-day exposure to the drugs. In order to prevent total cell death during this exposure time, we had to use 5-FU and cisplatin doses which corresponded to the lower limit of clinically relevant doses. Therefore, the resistance development under these conditions might slightly differ from the clinical situation. However, we were able to show that the cell lines generated do have resistance to these chemotherapy agents.

Unfortunately, we were unable to establish a 5-FU-resistant variant of the oesophageal adenocarcinoma cell line, OE19, due to technical problems. This missing cell line might inform further on the observed impact of miR-148a upregulation especially on 5-FU treatment. As the cisplatin-resistant variant of OE19 did not respond to miR-148a transfection with the expected increase in sensitivity to anticancer treatment, it would be very interesting to see if this also occurs in 5-FU-resistant cells. However, despite the limitations inherent in our study, it does provide the first good evidence that miRNAs provide a very promising target for new therapeutic strategies to support and improve existing anticancer treatments in oesophageal cancer patients.

In conclusion, we have shown for the first time that miR-148a upregulation sensitizes chemotherapy-resistant variants of both oesophageal adenocarcinoma and squamous cell carcinoma cell lines, to cisplatin and 5-FU in vitro, and further improves sensitivity in the corresponding chemotherapy-sensitive maternal cell lines. A review of the literature highlighted MSK1, de novo DNA methylation and PXR as potential mediators of these observations. These findings provide a basis for future studies to determine the altered chemotherapy response in other oesophageal lines following miR-148a administration. They also highlight a need to determine which pathways, affected by miR-148a in oesophageal adenocarcinoma and squamous cell carcinoma, modulate response to chemotherapy, and clinical studies using human tissue samples are required to confirm that this miRNA plays an important role in chemotherapy resistance in oesophageal cancer in vivo. Although therapeutic delivery of miRNAs is still a developing field, and there is much more work to be done before these molecules can be securely applied in clinical settings, miR-148a may one day have a therapeutic application in patients undergoing chemotherapy for oesophageal cancer.

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ORIGINAL ARTICLE

Nissen Fundoplication after Failure of Endoluminal Fundoplication: Short-Term Results

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Abstract
Background Endoluminal fundoplcation (ELF) with EsophyX™ is a new attractive investigational procedure for the control of gastroesophageal reflux disease (GERD). The aim of this work is to evaluate the short-term results of Nissen fundoplication (NF) after failure of ELF.
Method During the period April 2007–January 2010, nine patients previously treated with ELF for GERD were submitted to laparoscopic NF for persistent reflux.
Results All patients were symptomatic for GERD, had a pathological esophageal acid exposure at multichannel intraluminal impedance (MII pH/24 h), and all of them were on proton pump inhibitor. Mean duration of the NF was 85 min (range, 56–104). There were no intraoperative complications. One patient had a postoperative mild peritoneal bleeding treated conservatively. After a mean follow-up of 24.9 months (4–34), all patients are asymptomatic for reflux. Two patients have a mild or moderate dysphagia at follow-up. Five patients underwent MII pH/24 h 1 year after surgery. Mean total reflux time was 0.3%, and acid reflux percent time was 0.
Conclusions Patients with persistent symptomatic reflux after a failing ELF can still undergo NF with good results; the endoluminal procedure does not seem to modify the results of the laparoscopic procedure, although an increased incidence of dysphagia pos-NF may be observed.

Keywords Gastroesophageal reflux disease (GERD) · Endoluminal fundoplication (ELF) · Nissen fundoplication (NF)

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Introduction

Long-term proton pump inhibitor (PPI) antisecretive therapy and Nissen fundoplication (NF) are the standard treatment for gastroesophageal reflux disease (GERD).[1] In the last years, alternative methods for the endoscopic treatment of patients with persistent GERD have been developed.[2] EsophyX (EndoGastric Solutions, Redmond, WA, USA) is a novel instrument for antireflux endoluminal fundoplication (ELF): It constructs endoscopically a full-thickness valve at the gastroesophageal junction through tailored delivery of multiple fasteners during a single-device insertion.[3] The experimental results and the first clinical application of this device have shown that this

endoscopic valve seems to ameliorate GERD symptoms, but esophageal reflux exposure does not change significantly at 1 year follow-up.[4–6] Several patients may need to undergo surgical treatment of GERD after a failing ELF for persisting acid exposure.[5] In the current literature, there are no reports on the results of NF after a failed ELF with EsophyX: Several studies have reported the effects of a previous gastroplication with Endocinch on a subsequent NF,[7–9] but the two techniques are significantly different.

The aim of this work was to evaluate the short-term results of NF after failure of ELF with EsophyX in a group of patients treated for GERD.

Material and Methods

During the period April 2007–January 2010, nine patients (three women and six men) previously treated with ELF for GERD underwent laparoscopic NF for persistent symptomatic reflux at the Minimally Invasive and General Surgery Unit of Istituto Clinico Humanitas, Milan, Italy.

Seven of them belong to a group of 20 patients who underwent the endoscopic procedure at our department, within a clinical research protocol aimed at evaluating the results of ELF with EsophyX for the treatment of GERD.[5] This was a prospective independent study conducted at the Department of Gastroenterology of Istituto Clilnico Humanitas under a protocol, which was approved by the Ethics Committee and financially supported by the own Foundation for the Research. Informed consent was obtained before enrolling patients into the study. Patients with persistent or recurrent symptomatic GERD after ELF, with evidence of pathologic esophageal acid exposure at 24 h pH impedance recording, were offered a standard surgical fundoplication. The other two patients were referred to us after the endoscopic procedure had been performed elsewere; these patients were offered the same treatment as the first group and signed the same informed consent.

The data of these patients were prospectively collected into a database: Intraoperative data included duration of surgery and description, as made by the surgeon, of any intraoperative detail that would render the operation different from a classical LN. Early postoperative data included length of postoperative stay and postoperative complications.

The patients were scheduled a symptomatic assessment preoperatively and at 1–6–12 months: this assessement was made either by clinical follow up or by telephone interview. The GERD health-related quality of life (GERD-HRQL) questionnaire and a symptom severity scale were administered; PPI consumption, present symptoms, and the occurrence of adverse events (i.e., gas bloat, dysphagia, retrosternal pain, and nausea) were evaluated. The patients were asked to undergo an objective assessment within 12 months from surgery, with multichannel intraluminal impedance (MII) pH/24 h monitoring and endoscopy. The questionnaires were either given to the patients by an independent person who also acted as data manager before clinical assessment or were sent to the patients before telephone interview.

Statistical Analysis

The statistical analysis was carried out using Stat Software for Windows (StatSoft Inc., Tulsa, OK, USA). The Mann–Whitney U test was used to compare two independent groups of data. A $p<0.05$ was considered as statistically significant.

Table 1 Patients' characteristics before and after ELF

	Own series		Referred	
m/f	5/2		1/1	
Mean age (range)	46 (28-67)		32 – 63	
BMI (range)	25,4 (19,4-30,4)		22 – 27	
	Pre ELF	Post ELF	Pre ELF	Post ELF
Esophagitis: no / grade A [19]	4	6	2	2
Esophagitis: grade B / C [19]	3	1	0	0
GERD HRQL (range)	45 (22-77)	10 (0-16)	-	-
MII pH /24h Total reflux % time (range)	2.2% (1.4-13,5)	2.3% (1.2-8.1)	Pos	Pos
MII pH /24h Acid reflux % time (range)	2% (0.6-12.6)	1.9% (1-7.1)	Pos	Pos

Table 2 Clinical and instrumental follow-up after 24.9 months (range, 4.4–34.2)

Symptomatic follow-up (9/9)	
Median GERD-HRQL (range)	1 (0–29)
Off PPI (%)	8/9 (88.9)
Dysphagia (%)	No, 7 (77.8)
	Mild, 1 (11.1)
	Moderate, 1 (11.1)
Gas bloat (%)	Mild, 2 (22.2)
Instrumental follow-up (5/9)	
MII pH/24 h, total reflux % time (range)	0.3% (0.2–0.4)
MII pH/24 h, acid reflux % time	0
Esophagitis	0

Results

Table 1 reports the characteristics of the nine patients, before ELF, after the endoscopic procedure, and at inclusion in this study.

All nine patients were on PPI after the ELF procedure, and indication to surgery was, in all case, the presence of symptomatic pathologic esophageal acid exposure. Median time from ELF to NF was 13.3 months (range, 6.6–21.6). The mean duration of NF was 85 min (range, 56–104). At surgery in most patients, a fibroid reaction was found at the level of the left pillar of the diaphragm with some fasteners partially migrated from the serosa outside the esophagus and stomach. The plication was in most cases not consistent, resulting in a simple serosa adhesion, easy to be taken down. The fasteners were easily removed, and pre-EFL anatomy was restored before doing the Nissen. In one patient, there was a small mass protruding outside the esophagus mimicking a leiomyoma. The mass was isolated to be removed and proved to be a fastener encapsulated by esophageal tissue. Apart from these findings there were no intraoperative complications. In some cases, dissection was subjectively considered by the surgeon more tedious than usual, due to perihiatal fibrosis and extruded fasteners.

The postoperative course was complicated in one patient (11.1%) who presented postoperative acute anemia secondary to a mild peritoneal bleeding that stopped spontaneously; the patient was discharged on postoperative day 5, in good general conditions. Mean postoperative stay was 3.1 days (range, 2–5).

Table 2 reports the clinical results after a median follow-up of 24.9 months (range, 4.4–34.2 months). Eight patients (88.9%) were off PPI and asymptomatic for GERD at latest follow-up: One patient had resumed PPI, although she did not have an instrumental dignosis of pathologic acidic esophageal reflux. GERD-HRQL score decreased from a median of 10 to a median of 1 ($p<0.05$, Mann–Whitney U test).

Two patients (22.2%) had mild to moderate dysphagia: At instrumental evaluation, the plasty appeared to be correctly shaped and positioned in both patients. Two other patients (22.2%) had a mild gas bloat syndrome.

Instrumental follow-up was completed in five out of nine patients: One patient has not reached 1 year follow-up; the other three patients are feeling well and refuse instrumental follow-up. Endoscopy showed healing of esophagitis in all five patients. The MII pH/24 h report did not show pathologic esophageal acid exposure in any patient.

Discussion

Several endoscopic antireflux therapies have been proposed over the last years to reduce the need for chronic medical therapy or laparoscopic fundoplication for GERD.[10] Several trials evaluated these new procedures: The majority of these studies have not provided a sufficient clinical and instrumental evidence to determine the safety and efficacy of endoscopic procedures for GERD, particularly in the long term.[2] Therefore, some of the patients submitted to ineffective endoscopic procedures for treatment of GERD have been afterwards submitted to surgical treatment.[5,6]

Table 3 Results of NF after previous endoscopic plication with Endocinch and after previous endoluminal fundoplication with EsophyX (ELF endoluminal plication)

	Velanovich '02	Tierney '07	Furnée '10	Present series
N. patient	10	6	11	9
Months ELP to NF	-	11.5 (6-26)	23 (7-33)	13.3 (6.6-21.6)
NF op. time (min.)	-	160	119 (67-143)	85 (56-104)
Postop. stay (days)	-	2	4 (2-9)	3.1 (2-5)
Follow up (mos.)		20.4 (3-32)	32 (6-61)	24.9 (4.4-34.2)
Symptoms resol/improve.	8/10 (80%)	4/5 (80%)	9/11 (81,8%)	8/9 (88.9%)
Normal. acid exp. time	-	-	10/11 (90,9%)	5/5 (100%)
Dysphagia	2 (20%)	3 (50%)	3 (50%)	2 (22.2%)
	After Endocinch			After Esophyx

The results of anti-reflux surgery in these patients have been reported by several authors.[7–9]

The latest instrument for antireflux endoluminal fundoplication (ELF) is EsophyX (EndoGastric Solutions, Redmond, WA, USA), a novel transoral device, which was designed to endoscopically construct a partial fundoplication of 270° by attaching the fundus to the anterior and left lateral wall of the distal esophagus slightly below the esophagogastric junction through tailored delivery of multiple fasteners during a single-device insertion. The efficacy of ELF in the control of GERD is not clear: An improvement on symptoms and a relief from PPI use has been reported by others in almost 80% of patients at 6 month follow-up, but functional results are contradictory.[4]

We have recently published our first clinical experience with EsophyX for the treatment of GERD: In our experience, a mild symptom improvement was recorded in 55% and 46% of patients submitted to ELF at 6 and 12 month follow-up respectively.[5] Objective evaluation, however, showed that esophageal acid exposure did not change significantly after the endoscopic fundoplication, and several patients needed a revisional laparoscopic Nissen fundoplication for persistent or worsened symptoms. There are no data in the present literature reporting the results of NF after ELF procedure. Several papers addressed the results of NF after endoscopic full-thickness plication with Endocinch, a device for endoscopic suturing technique that creates a full thickness plication at the gastroesophageal junction and has some similarities to the EsophyX technique.[11,12] Table 3 summarizes the results of NF after previous endoscopic plication with Endocinch and after previous endoluminal fundoplication with EsophyX. These results demonstrate that a previous endoluminal plication does not seem to deteriorate the results of surgery in patients with failure of the endoscopic procedure: The NF does not seem to be technically more complicated than in the absence of the previous procedure. In our experience, at surgical exploration, a large number of fasteners were visible from the peritoneal side, close to the left diaphragmatic pillar, meaning an incomplete plication or a valve partial disruption. Their presence did not usually make the dissection more complicated, and in our experience, there were no intraoperative complications during NF. A different experience has been recently reported in abstract form by Furnée et al.:[13] Three out of 11 patients (27.3%) submitted to NF after ELF had perioperative complications; there were two intraoperative gastric perforations and one postoperative subphrenic abscess. This rate of perioperative complications is similar to that reported after redo Nissen fudoplication.[14,15] However, in our experience, the surgical field of the esophagogastric junction in patients submitted to previous ELF has few adhesions and is more similar to the one seen in untreated patients than in patients scheduled for a redo after a previous Nissen.

The efficacy of the NF in terms of control of heartburn and acid exposure does not seem to be influenced by the previous ELF. Symptom resolutions is reported between 80% and 90% of patients after NF, and normalization of esophageal acid exposure time is reported in 90–100% of patients, with results similar to those expected in untreated patients.[16,17]

However, most series report a rate of postoperative dysphagia in patients submitted to NF after ELF significantly higher than the rate reported after primary Nissen.[18] Although the number of patients in these series is small, all series report an incidence of dysphagia that seems to be higher than expected after a standard Nissen: The rate of postoperative dysphagia, in fact, is reported between 20% and 50% of patients, a rate that can significantly affect the quality of life of these patients after surgery. There are no clear explanations for this effect, although one may hypothesize that fibrosis caused by the previous ELF might affect the results of a conventional Nissen. These results may indicate the opportunity to choose a partial instead of a total wrap in patients undergoing surgical treatment of GERD after failing ELF.

Conclusion

In conclusion, patients with persistent reflux after a failing ELF can still undergo surgical fundoplication with good results on heartburn and control of pathologic esophageal acid exposure. The endoluminal procedure does not seem to modify the results of the laparoscopic procedure, although an increased incidence of dysphagia post-NF may be observed. This study shows that salvage of failures after ELF procedure are possible and devoid of major risks. Trials with the ELF2 technique, which might give better results in terms of GERD control than the ELF1, can be therefore conducted more safely.

Author disclosures Drs. Uberto Fumagalli, Roberta Barbera, Alessandro Repici, Matteo Porta, Alberto Malesci, and Riccardo Rosati have no conflicts of interest or financial ties to disclose.

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ORIGINAL ARTICLE

Neoadjuvant Therapy for Rectal Cancer: The Impact of Longer Interval Between Chemoradiation and Surgery

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Abstract
Purpose The aim of this study was to determine the effect of a longer interval between neoadjuvant chemoradiation and surgery on perioperative morbidity and oncologic outcomes.
Methods A colorectal cancer database was queried for clinical stage II and III rectal cancer patients undergoing neoadjuvant chemoradiation followed by proctectomy between 1997 and 2007. The neoadjuvant regimen consisted of long course external beam radiation and 5-fluorouracil chemotherapy. Patients with inflammatory bowel disease, hereditary cancer, extracolonic malignancy, urgent surgery, or non-validated treatment dates were excluded. Patients were divided into two groups according to the interval between chemoradiation and surgery (<8 and ≥8 weeks). Perioperative complications and oncologic outcomes were compared.
Results One hundred seventy-seven patients were included. Groups were comparable with respect to demographics, tumor, and treatment characteristics. Perioperative complications were not affected by the interval between chemoradiation and surgery. Patients undergoing surgery ≥8 weeks after chemoradiation experienced a significant improvement in pathologic complete response rate (30.8% vs. 16.5%, $p=0.03$) and had decreased 3-year local recurrence rate (1.2% vs. 10.5%, $p=0.04$). A Cox regression analysis was performed to assess the compounding effect of a complete pathologic response on oncologic outcome. A longer interval correlated with less local recurrence, although statistical significance was not reached ($p=0.07$).
Conclusion An interval between chemoradiation and surgery ≥8 weeks is safe and is associated with a higher rate of pathologic complete response and decreased local recurrence.

Keywords Rectal cancer · Neoadjuvant therapy · Pathologic complete response · Prognosis · Chemotherapy · Radiation · Radiotherapy

The work was presented at the podium session of the American Society of Colon and Rectal Surgeons annual meeting in Hollywood FL, May 4, 2009.

Synopsis This paper addresses the effect of a longer interval between chemoradiation and surgery on perioperative morbidity and oncologic outcomes in rectal cancer patients undergoing neoadjuvant chemoradiation followed by proctectomy.

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Introduction

For the past 20 years, neoadjuvant and adjuvant radiotherapy have been utilized to improve local control in patients with rectal cancer.[1–4] More recently, neoadjuvant chemoradiation has become the standard of care for patients with locally advanced rectal cancer (T3–T4 and or positive lymph nodes). This approach is associated reduced with local recurrence and increased treatment compliance compared to postoperative chemoradiation.[5]

In 1999, François et al.[6] (The Lyon Trial) advocated the adoption of an interval between chemoradiation and surgery of 6 to 8 weeks. This was based on a statistically non-

significant improvement in sphincter preservation rates without changes in perioperative complications, compared to an interval of 2–3 weeks. Based on these equivocal findings, an interval between chemoradiation and surgery of 6 to 8 weeks has become part of the standard protocol for the treatment of rectal cancer in the USA. The optimal interval, however, between neoadjuvant treatment and surgery remains debated.

Our group has recently reported on the clinical predictors of achieving complete pathologic response after neoadjuvant chemoradiation. The following variables were utilized to build a logistic regression model to predict pathologic complete response (pCR): gender, body mass, pretreatment tumor differentiation, tumor distance from the anal verge, clinical stage, serum carcinoembryonic antigen level, radiation dose, and time interval between completion of chemoradiation treatment and surgery. Of these variables, a time interval time between chemoradiation and surgery ≥8 weeks was the only independent predictor of pCR, which translated into improved oncologic outcomes.[7]

However, the effect of a longer interval on perioperative morbidity and oncologic outcomes remains unclear. Therefore, the present study was designed to determine whether an interval time between chemoradiation and surgery ≥8 weeks impacts perioperative morbidity and independently affects oncologic outcomes.

Methods

A single institution IRB-approved colorectal cancer database was queried to identify patients with clinical stage II and III rectal cancer undergoing neoadjuvant chemoradiation followed by proctectomy between 1997 and 2007. Patients who underwent to emergent surgical procedures were excluded from this study. Additionally, patients with inflammatory bowel disease, familial adenomatous polyposis syndrome, R1-2 resections, hereditary non-polyposis colorectal cancer syndrome, and other malignancy except for non-melanoma skin cancer and patients whose treatment dates or database information could not be validated were excluded.

The pretreatment oncologic assessment and clinical staging included rigid proctoscopy with tumor biopsy, colonoscopy, chest radiography, abdominal and pelvic computed tomography scan, and serum CEA. All patients underwent endorectal ultrasound and/or magnetic resonance image. Preoperative radiotherapy was delivered through a three- or four-field technique with a median dose 5,040 (inter-quartile range (IQR) 4,890–5,040 cGy).

The chemotherapy regimen was 5-fluorouracil (5-FU) based and delivered as continuous infusion, bolus, or oral preparation. The most common protocol for continuous infusion was 5-FU 225 mg/m^2/day over 6 weeks. Bolus chemotherapy was most commonly 5-FU 325 mg/m^2/day with leucovorin 20 mg/m^2/day given for two cycles of five consecutive days on weeks 1 (days 1–5) and 5 (days 29–33) of radiotherapy.

Patients underwent surgery at various intervals at the discretion of the surgeon and patient, with a flexible goal interval between 6 and 8 weeks. However, due to logistical, scheduling, and clinical factors, the actual interval varied. Surgery was performed by colorectal surgeons with adherence to the oncologic principals of total mesorectal excision and high vessel ligation as described in previous reports from our group.[8,9]

Pathologic complete response was defined as absence of viable adenocarcinoma cells in the surgical specimen, including primary tumor and lymph nodes.

The study population was divided into two cohorts based on interval between chemoradiation and surgery: interval<8 weeks and interval≥8 weeks. The IRB-approved database and patient medical records were reviewed to collect the following information: gender, age, American Society of Anesthesiology classification (ASA), the interval between chemoradiation and surgery, radiotherapy fields and dose, chemotherapy regimen, clinical T and N stage, pathologic stage, pretreatment distance from the anal verge, operative time, estimated blood loss, intraoperative complications, and postoperative morbidity and mortality. The oncologic outcomes evaluated were 3-year local recurrence, 3-year distant recurrence, 3-year disease-free survival, and 5-year overall survival.

For statistical analysis, categorical variables were expressed as absolute numbers and percentages and compared with Pearson χ^2 or Fisher's exact test. Parametric variables were summarized by means and standard deviation with the Student t test for comparison. Non-parametric data were described as medians and IQR and compared with the Wilcoxon rank sum test. Kaplan–Meier estimates, log-rank tests, and Cox regression analyses were used to assess the association of the interval between chemoradiation and surgery with 3-year local recurrence, 3-year distant recurrence, 3-year disease free survival, and 5-year overall survival.

Results

Demographics and Treatment Characteristics

A total of 177 patients were included. There were 129 (73%) males and 48 (27%) females. The median age was 57 (IQR, 48-64.5) years. All demographics, except body mass index (BMI), were similar between the groups as shown in Table 1. The median BMI was significantly higher in the longer interval group. Fifty-one patients (29%) underwent APR, and 125 (71%) patients underwent LAR. The interval between chemoradiation and surgery ranged from 4 to 14 weeks with a median of 8 weeks. There were

Table 1 Demographics and Clinical Characteristics

Variable	Interval<8weeks *N*=83	Interval≥8weeks *N*=94	*p*
ASA			0.57
1	1 (1.2%)	1 (1.1%)	
2	50 (60.2%)	44 (50.0%)	
3	32 (38.5%)	43 (48.8%)	
Gender			0.68
Female	20 (24.1%)	25 (26.6%)	
Male	63 (75.9%)	69 (73.4%)	
Age (years)	54.1 (45.8–64.5)	57 (49.5–66.9)	0.11
Body mass index (kg/m^2)	25.7 (23.7–30.4)	28.6 (24.9–30.5)	0.05
Pretreatment T			0.86
1	1 (1.2%)	0 (0%)	
2	3 (3.7%)	3 (3.4%)	
3	73 (90.1%)	83 (93.3%)	
4	4 (4.9%)	3 (3.4%)	
Pretreatment N			0.31
0	53 (65.4%)	50 (56.2%)	
1	26 (32.1%)	33 (37.1%)	
2	2 (2.5%)	6 (6.7%)	
Pretreatment cancer stage			0.24
2	53 (65.4%)	50 (56.2%)	
3	28 (34.6%)	39 (43.8%)	
Distance from anal verge (cm)	5.5 (4–7)	6 (3–7)	0.87

Continuous variables are expressed as median and inter-quartile range. A full dataset regarding chemoradiation regimen and/or pretreatment variables could not be obtained for a portion of the patients that had chemoradiation in other institutions

ASA American Society of Anesthesiologists Score

86 (49%) patients in the group with an interval <8 weeks and 91 (51%) in the interval ≥8 weeks.

One hundred two (57%) patients received neoadjuvant therapy at an institution other than the Cleveland Clinic. However, the chemoradiation regimen was not significantly different when compared to the patients treated at elsewhere. Table 2 outlines the chemoradiation regimen.

Surgical Characteristics and Perioperative Morbidity

The median estimated blood loss, median operative time, and rate of intraoperative complications were not influenced by the interval between chemoradiation and surgery. There was no significant difference in terms of rate and type of intraoperative complications between the two interval groups. Furthermore, the postoperative complications and need for reoperations were also similar in both interval groups. There was no 30-day postoperative mortality in either group (Table 3).

Pathologic Response

The median number of lymph nodes retrieved from the surgical specimen was 15 (IQR, 10–23). Forty-one (24%)

Table 2 Chemoradiation Regimen

Variable	Interval<8weeks *N*=83	Interval≥8weeks *N*=94	*p*
Radiotherapy fields			0.66
3	43 (75.4%)	49 (80.3%)	
4	14 (24.6%)	12 (19.7%)	
Radiotherapy dose (cGy)	5,040 (4,500–5,040)	5,040 (5,040–5,040)	0.25
Chemotherapy regimen			0.11
5-FU bolus	19 (27.5%)	18 (24.3%)	
5-FU continuous	48 (69.6%)	49 (66.2%)	
5-FU oral	2 (2.9%)	7 (9.5%)	
Neoadjuvant treatment institution			>0.99
Cleveland Clinic	36 (41.9%)	39 (43.3%)	
Referral Institutions	50 (58.1%)	51 (56.7%)	

Continuous variables are expressed as median and inter-quartile range. A full dataset regarding chemoradiation regimen and/or pretreatment variables could not be obtained for a portion of the patients that had chemoradiation in other institutions

Table 3 Surgical Characteristics

	Interval<8weeks *N*=83	Interval≥8weeks *N*=94	*p*
Surgical procedure			0.86
APR	23 (27.7%)	26 (27.7%)	
LAR	60 (72.3%)	68 (72.3%)	
Proximal diversion	56 (93.3%)	64 (94.1%)	1
Operative time (min)	229 (182–262)	238.5 (208–267)	0.47
EBL (mL)	450 (200–725)	448 (275–725)	0.79
Intraoperative complications			
Vaginal injury	2 (2.4%)	1 (1.1%)	0.61
Ureteral/urethral injury	2 (2.4%)	0 (0%)	0.23
Postoperative complications			
Cardiovascular	2 (2.4%)	2 (2.1%)	>0.99
Pneumonia	0 (0%)	2 (2.1%)	0.5
DVT/PE	0 (0%)	1 (1.1%)	1
Urinary retention	1 (1.2%)	5 (5.3%)	0.21
Ileus	10 (12.1%)	10 (10.6%)	0.89
Anastomotic leak	4 (6.7%)	4 (5.9%)	1
Abdominal abscess	2 (2.4%)	3 (3.2%)	1
Abdominal wound infection	2 (2.4%)	4 (4.3%)	0.68
Abdominal wound dehiscence	1 (1.2%)	1 (1.1%)	1
Perineal wound infection	1 (4.3%)	4 (14.8%)	0.35
Perineal wound dehiscence	1 (4.3%)	1 (3.8%)	1
Other complication	6 (7.2%)	3 (3.2%)	0.12
Reoperation	3 (3.6%)	6 (6.4%)	0.5
30-Day mortality	0 (0%)	0 (0%)	NA

Continuous variables are expressed as median and inter-quartile range

APR abdominoperineal resection, *LAR* low anterior resection, *EBL* estimated blood loss, *NA* not applicable, *DVT/PE* deep venous thrombosis and/or pulmonary embolism

patients achieved pCR, 54 (32%) experienced downstaging without pCR (i.e., pathologic stage<clinical stage), and 75 (44%) did not achieve downstaging (i.e., clinical stage≥ pathologic stage). Patients operated with an interval to surgery greater than or equal to 8 weeks had significantly higher rate of pCR and partial downstaging than those operated less than 8 weeks after chemoradiation (Table 4).

Oncologic Outcomes

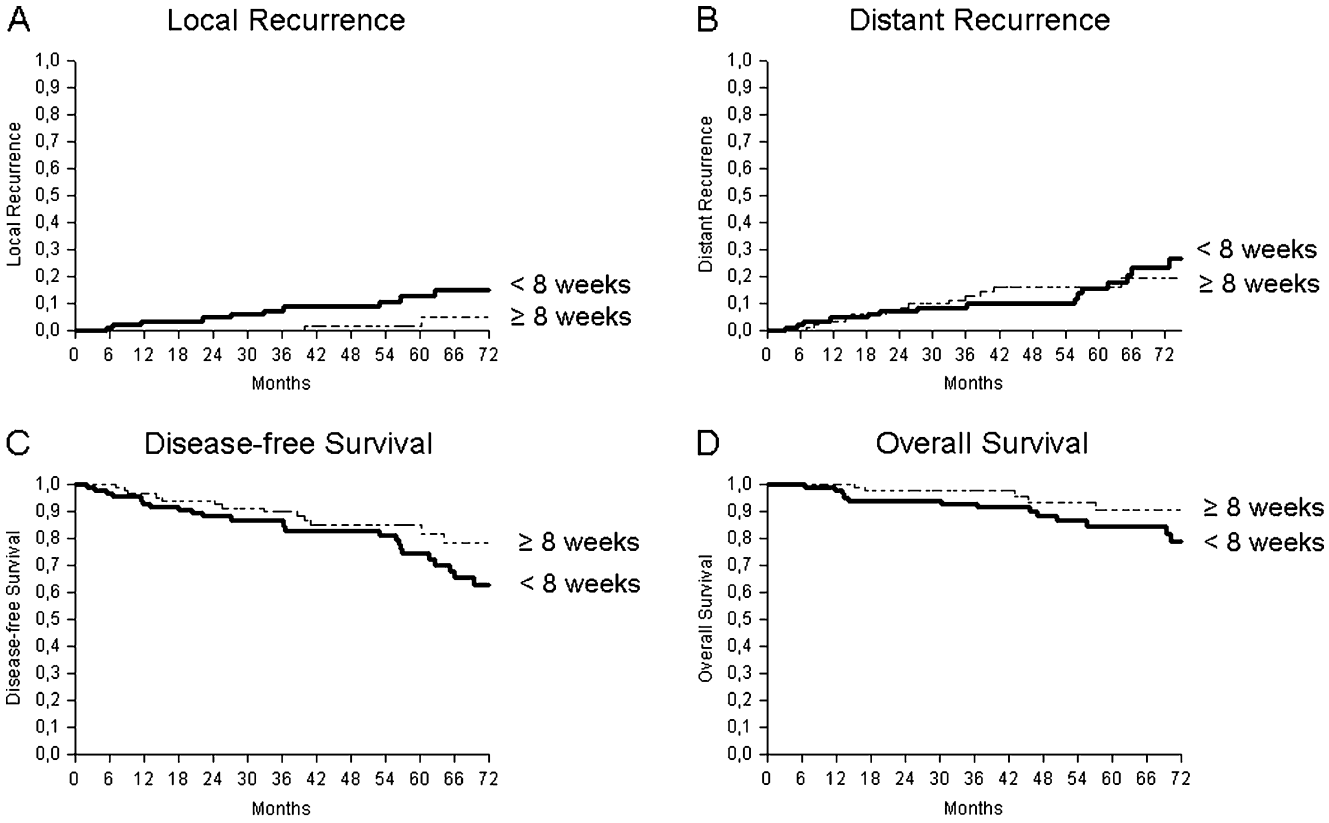
Thirty-six patients died during the study period, of which 26 of the deaths were secondary to rectal cancer. One hundred thirty-seven patients were alive at a median follow-up of 51 (IQR, 26.4–74.9) months. Of the alive patients, those in the shorter (66 patients) interval had longer median follow-up (59 months, IQR 40–80) than those in the longer interval group (44 months, range 25–66), $p=0.01$.

With respect to the oncologic outcomes, the longer interval group (≥8 weeks) was associated with significantly less local recurrence (1.2% vs. 10.5%, $p=0.04$). Sixty-three patients had either local or distant recurrence during the study period. In 53 patients (84%), the recurrence happened within 48 months after surgery, of which of 33 recurred within first 2 years. The distant recurrence, disease-free survival, and overall survival rates were not significantly different between the two groups (Fig. 1). The interval shorter than 8 weeks group had significantly longer follow-up time than the interval greater than or equal to 8 weeks' group. Thus, one might argue that the improvement in local control observed in the longer interval group is influenced

Table 4 Pathologic Response

Variable	Total	Interval<8weeks	Interval≥8weeks	*p*
No-downstaging	75 (44.1%)	43 (53.8%)	32 (35.6%)	0.027
Downstaging	54 (31.8%)	24 (30%)	30 (33.3%)	
Complete response	41 (24.1%)	13 (16.2%)	28 (31.1%)	

No-downstaging clinical stage≥pathologic stage, *Downstaging* clinical stage<pathologic stage, *Complete response* absence of viable adenocarcinoma cells in the surgical specimen, including primary tumor and lymph nodes



Kaplan – Meier Estimates for Oncologic Outcomes

3-year Oncologic outcomes	< 8 Weeks	≥ 8 Weeks	*p* - Value*
Local Recurrence %(SE)	10.5 (3.9)	1.2(1.2)	0.04
Distant Recurrence %(SE)	17.7(5.2)	14.1(4.6)	0.85
Disease-free Survival %(SE)	75.3(6.3)	84.7(4.9)	0.26
Overall Survival %(SE)	85.5(5.1)	88.2(5.4)	0.74

SE Standard Error

* Ajusted for age at surgery with Cox regression analysis

Figure 1 Oncologic outcomes—Kaplan–Meier estimates.

by the shorter follow-up. However, patients operated with at least 8 weeks after chemoradiation still had a median follow-up time of nearly 4 years, during which time most recurrences occurred.

In order to assess whether the interval is a prognostic factor regardless of the tumor response, Cox regression was utilized to adjust the survival analysis for pCR. Accounting for the effect of pCR on oncologic outcomes, a longer interval between chemoradiation and surgery suggested the effect of decreased local recurrence, although statistical significance was not reached (hazard ratio 0.53, 95% confidence interval 0.21–1.05, $p=0.07$).

Discussion

Neoadjuvant chemoradiation is the gold standard for the initial treatment of stage II and III rectal cancers. It decreases local recurrence, improves disease-free survival, and is associated with reduced long- and short-term toxicity when compared to adjuvant chemoradiation.[5,10–14]

Despite attempts to improve tumor response by varying chemotherapy and radiation regimens, no significant impact on oncologic outcomes has been made.[2,15–18] Previous work by our group has demonstrated that a prolonged interval between chemoradiation and surgery was an independent predictor of achieving a pCR.[7] This study shows that a longer interval is safe for patients as it does not increase peri- or postoperative morbidity. Furthermore, an interval of greater than or equal to 8 weeks resulted in a decreased rate of local recurrence.

It has been argued that an extended interval between neoadjuvant chemoradiation completion and surgery may result in increased surgical morbidity due to increased adhesions and tissue friability.[19] Moore et al.[20] observed more frequent anastomotic leaks and pelvic abscesses among 73 patients undergoing surgery more than 44 days after chemoradiation. Our data refute this argument, as there was no difference in anastomotic leak, pelvic abscess, or other complications between the two study groups. Moreover, our results are supported by a study conducted by Stein et al. in which 14 patients operated between 10 and 14 weeks after chemoradiation had no difference in operative morbidity and mortality compared to 19 patients operated within an interval time of 4 to 8 weeks.[21]

The main goal of this study was to prove that waiting longer to perform surgery would not affect patient morbidity with the expectation that waiting would not affect oncologic outcomes other than through the influence of improved rates of pCR. However, using Cox regression analysis to adjust for whether patients achieved pCR, a longer interval was associated with a reduced risk of local recurrence, albeit not significant at a 5% level. The inability to demonstrate statistical significance could be related to the relatively small sample size. The benefits of delayed surgery on oncologic prognosis have been proposed. In a small study with shorter follow-up, Tulchinsky et al.[22] demonstrated that an interval greater than 7 weeks was associated with higher pCR rate and improved disease-free survival. However, the unexpected suggestion that waiting longer than 8 weeks independently yields improved local control is a novel report. This enhanced effect of a prolonged time interval on tumor response may be supported by the fact that radiation induced necrosis is a time-dependent phenomena.[23] Therefore, the persistent effects of neoadjuvant treatment would continue to cause cell death over time, and consequently, waiting longer before surgery could yield less viable carcinoma at the time of surgery.

Despite improved local recurrence, overall survival was not affected by a prolonged interval. Overall survival is a consequence of several different issues, such as age, comorbidities, distant recurrence, and even life style. Secondly, the biology of metastatic lesions may be different than that of the primary tumor, and the local control may not affect distant disease.[24] Lastly, improved overall survival may indeed occur, but the numbers and follow-up in this study are not powered to show a difference.

Due to the retrospective nature of this study, it is subject to potential bias and certain limitations. Although the two groups were not prospectively matched, the demographics, pretreatment TNM stage, and chemoradiation regimen were not significantly different between the two groups. Because a percentage of patients in this study received neoadjuvant chemoradiation at institutions other than Cleveland Clinic, the variability in treatment centers yielded some heterogeneity in chemoradiation regimens, although all patients received long-course radiation and 5-FU.

Since the study was not prospectively designed, the time interval between chemoradiation and surgery was decided according to the individual surgeon preference. Traditionally, our general approach has been to wait 6 to 8 weeks after completion of chemoradiation before surgery, but often, factors such as patient morbidity and logistical scheduling issues also influenced the interval.

Unfortunately, our study lacks specific data on postoperative chemotherapy, which might well affect distant control.[25] A 5-FU-based postoperative regimen is currently preferred in our institution for all cases of locally advanced rectal cancer, regardless of their response to preoperative treatment.[25,26] It is therefore unlikely that in our specific study postoperative chemotherapy made an impact limited to a specific interval to surgery group or dependent on whether complete response occurred or not.

Although this is not a randomized controlled trial, there are several strengths to the data presented in this study. This

is one of the largest reports of rectal cancer patients treated with neoadjuvant therapy. All cases were managed by strict adherence to oncologic principles with emphasis on total mesorectal excision by high-volume surgical teams, thus limiting technical factors associated with negative outcome and placing more emphasis on the treatment paradigm.

Conclusion

In summary, this study demonstrated that an interval between chemoradiation and surgery greater than or equal to 8 weeks was not associated with adverse effects in peri- and postoperative morbidity while yielding higher tumor response and improved local control. Although a prospective trial is warranted to better define the optimal interval between chemoradiation and surgery, we recommend an interval of least 8 weeks.

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ORIGINAL ARTICLE

Suture-Free Anastomosis of the Colon Experimental Comparison of Two Cyanoacrylate Adhesives

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Abstract
Background We explored the potential of two cyanoacrylate tissue adhesives for constructing colonic anastomoses.
Method The study involved 12 female domestic pigs. The animals were divided into two equal groups. In both groups, the sigmoid colon was transected. An intestinal anastomosis was constructed with a modified circular stapler (all staples were withdrawn) and cyanoacrylate tissue adhesives. Glubran 2® was used in group A and Dermabond® was applied in group B. Fourteen days after the first operation, a follow-up surgery was performed in both groups. The glued section of the colon was resected, processed with the standard paraffin technique and stained with haematoxylin–eosin. The finished specimens were examined under light microscopy. Assessments were made for the presence of fibroblasts, neutrophils, giant polynuclear cells, neovascularisation and collagen deposits. Adhesions, anastomotic dehiscence, peri-anastomotic inflammation and intestinal healing were assessed peri-operatively.
Results All anastomoses in group A healed with no signs of pathology. In group B, fibrotic adhesions and stenoses tended to occur in areas surrounding the anastomoses. Histological examinations confirmed increased fibrosis.
Conclusion The tissue adhesive Glubran 2 appears to be (under experimental conditions) a promising synthetic adhesive for colonic anastomosis construction; conversely, the tissue adhesive Dermabond was unsuitable for suture-free anastomosis construction.

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Keywords Colon · Anastomosis · Suture-free · Cyanoacrylate · Adhesive

Introduction

Correct technical execution is the fundamental prerequisite for success in any surgical procedure. Anastomotic dehiscence is one of the most serious post-operative complications in colorectal surgery. Anastomotic dehiscence occurs in 2–7% of patients after planned colon resection operations[1–4] and in 7–15% of patients after planned rectal surgeries.[5–7] This complication contributes substantially to morbidity and mortality rates associated with colorectal surgery.[1,3,7] The occurrence of dehiscence depends on a range of factors that have long been the subject of research and analyses. Surgical technique, tension in the area of anastomosis, type of sewing material, previous therapy, patient's overall health and nutritional status and the

erudition of the surgeon have been explored as potential impacting factors.[4,5,8–10]

Parallel to the research on factors that might negatively impact anastomosis healing, researchers are also exploring new materials and techniques that could prevent or minimize the risk of anastomosis dehiscence. The basic and seemingly simple aim of sutured or stapled anastomosis construction is to secure an appropriate edge-to-edge apposition for healing. It is necessary to achieve optimal distance, freedom from tension and suitable suture or staple tightness to ensure appropriate blood perfusion to the connected parts of the intestine.[11]

Cyanoacrylate tissue adhesives provide another option and alternative approach to traditional suture techniques. Considering their mechanical, physical and biological properties, tissue glues should facilitate an optimal bond between anastomosed sections of the intestine with negligible negative effects on intestinal wall perfusion.[11,12]

The aim of this experimental study was to explore the technical and biological potential of two types of cyanoacrylate tissue glues used for large intestine anastomosis construction (sigmoid colon). We compared their properties and ascertained their reliability when used as a single supportive element in intestinal anastomosis.

Materials and Methods

Experimental Animals

The study involved 12 experimental animals: female domestic pigs of medium weight (average 32.7 kg). The animals were divided into group A (six animals) and group B (six animals).

The experiments were conducted in accordance with the Protection of Animals against Cruelty Act No. 246/92 Coll. as amended. The experiments were approved by the joint Departmental Committee of the Faculty of Military Health Sciences and Faculty of Medicine, Charles University in Hradec Kralove, Czech Republic.

Anaesthesia

The animals were fasted 1 day prior to surgery but were allowed fluid intake. Fluid intake was stopped on the day of operation. Animals were pre-medicated with ketamine, 15 mg/kg of body weight delivered intramuscularly (IM; Narkamon, Zentiva, Prague, Czech Republic); azaperone, 1.0 mg/kg IM (Stresnil, Janssen, Beerse, Belgium) and atropine, 0.02 mg/kg IM (Atropin, Hoechst-Biotika, Martin, Slovakia). Next, an orotracheal intubation was performed; subsequently, the animal was artificially ventilated with managed volume ventilation (Cirrus-Trans, Datex-Ohmeda, GE Company, Fairfield, CT, USA).

General anaesthesia was maintained by titration with midazolam, 0.05 to 0.1 mg/kg delivered intravenously (Dormicum, Roche, Prague, Czech Republic), combined with propofol, 2 to 4 mg/kg/h (Diprivan, Astra Zeneca, Cheshire, UK) and metamizol, 5 mg/kg/h (Novalgin, Aventis Pharma, Frankfurt on Main, Germany). Muscle relaxation during the surgery was maintained with pipecuronium, 40 mg/kg, delivered intravenously (Arduan, Budapest, Gedeon Richter, Hungary). The blood pressure was monitored invasively via an axillary artery cannulation.

A single intramuscular bolus dose (‘one shot’) of Betamox LA at 15 mg/kg (amoxicillin, Norbrook Laboratories, Newry, UK) was administered as an antibiotic prophylaxis after the induction of anaesthesia. Continual volume maintenance therapy consisted of a combination of crystalloids (Infusio Hartmanni, Medicamenta, Vysoke Myto, Czech Republic) and colloids (Hemohes 6%, Braun, Melsungen, Germany). The electrocardiogram, O_2 saturation and end-tidal CO2 were monitored throughout surgery.

Surgical Procedure

A midline laparotomy was conducted after standard aseptic preparation of the surgical field. A urine catheter was introduced into the urinary bladder through a small incision at the bladder apex and fixed with a circular suture to ensure derivation of urine and management of diuresis throughout surgery.

The sigmoid colon was transected. Circular monofilament sutures (Prolene 3/0, Johnson & Johnson, division of Ethicon, Somerville, NJ, USA) were placed on the ends of the disconnected intestinal tubes. An intestinal anastomosis was constructed with a modified circular 25 mm stapler (Circular Stapler CDH25, Johnson & Johnson, division of Ethicon, Somerville, NJ, USA). All staples were withdrawn from the stapler, and only the circular blade and mechanical part of the stapler was used. A stapler anvil was placed into the oral section of the intestine and secured in place with a circular suture. Following sphincter divulsion, the main part of the stapler was inserted transrectally into the sigmoid colon. The metal shaft of the stapler was ejected and the aboral part of the intestine was fixed to the stapler with a purse-string suture (Fig. 1). Then, cyanoacrylate tissue glue was sparingly applied to both ends of the connected intestine (Fig. 2). One millilitre of Glubran 2 (*N*-butyl-2-cyanoacrylate + methacryloxysulpholane, GEM s.r.l., Viareggio, Italy) was used in group A and 1 ml of Dermabond (2-octyl-cyanoacrylate, Johnson & Johnson, division of Ethicon, Somerville, NJ, USA) was applied in group B. The subsequent procedures were identical in both groups.

The circular stapler was closed in order to achieve tight apposition of the glued surfaces. Any remaining glue was cleared away from the glued surfaces with a gauze swab.

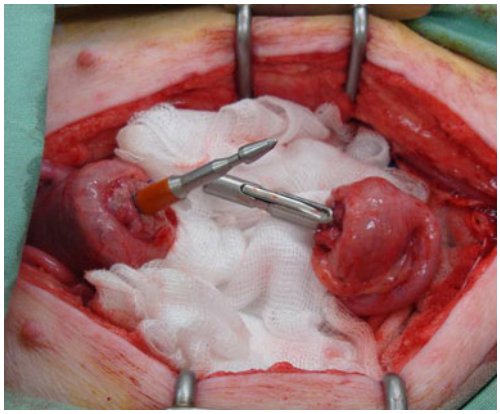

Fig. 1 Intestinal sections fixed on the stapler with circular sutures

After 90 s (time to glue polymerisation), the stapler was fired, opened with two turns and withdrawn from the intestine. Anastomosis was visually checked over the entire diameter and the tightness was verified by 'water test'. The abdominal cavity was lavaged with 10% Betadine solution (Povidonum iodinatum, Egis Pharmaceutical Ltd., Budapest, Hungary) and then dried. The abdominal cavity was closed in one layer, similar to a 'mass closure', with an absorbable monofilament suture PDS-loop (polydioxanon, Johnson & Johnson, division of Ethicon, Somerville, NJ, USA). The abdominal skin was closed by stapling (Stapler PMR 35, Johnson & Johnson, division of Ethicon, Somerville, NJ, USA). Following surgery, the experimental animal was extubated and placed into a warm end-of-anaesthesia booth.

Post-surgery Period

The animals were started with liquid feed the second day after surgery. Standard granulated feed was started on the third day after surgery. The skin staples were left in place until the follow-up operation.

Fourteen days after the first operation, a follow-up surgery was performed in both groups to evaluate the integrity of the anastomosis (Fig. 3) and uncover any potential strictures or leaks. We also reviewed the presence of abscesses or other forms of inflammation in the area of anastomosis, other pathological processes in the abdominal cavity and the incidence and the extent of adhesions. A scale modified by Houston and Rotstein[13] was used to assess adhesions: 0 = no adhesions; 1 = minimal adhesions, mainly between the small part of omentum and intra-abdominal organs or abdominal wall or freely separable adhesions between organs; 2 = small adhesions, i.e. between omentum and anastomotic site or between anastomosis and small bowel, oviducts, urinary bladder or other organs and 3 = extensive adhesions with partial obliteration of abdominal cavity.

The glued section of the intestine was resected to include at least 5 cm of the intestine on both sides of the anastomosis. Resected segments were cut lengthwise and macroscopic evaluation was performed on the mucosal side of the anastomosis. The animals were euthanized by intravenous administration of T61 (Hoechst, Frankfurt on Main, Germany) at the end of the operation.

Histopathological Examination

The resected parts of the intestine were fixed in a 10% formalin solution, processed with the standard paraffin technique and stained with haematoxylin–eosin. The finished specimens were examined under light microscopy. Assessments were made for the presence of fibroblasts, neutrophils, giant polynuclear cells, neovascularisation and collagen deposits. Collagen deposit area was evaluated with Masson's trichrome stain. Semi-quantitative histopathological evaluation of the presence of cell elements in the glued area of the anastomosis was performed according to the Ehrlich–Hunt numeric scale.[14]

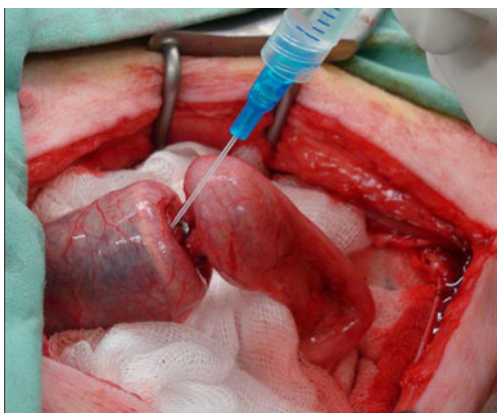

Fig. 2 Application of the cyanoacrylate adhesive on the intestinal sections

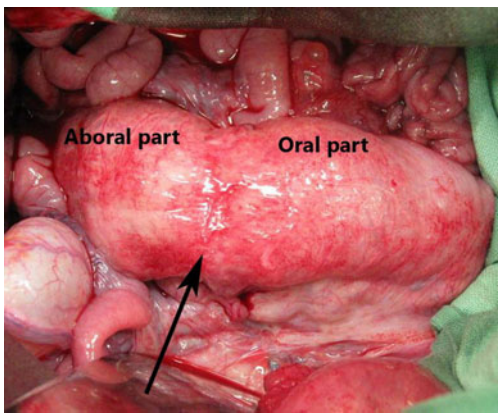

Fig. 3 Healed anastomosis 2 weeks after it was glued with Glubran 2. The *arrow* shows the anastomosis

For fibroblasts and neutrophils, one to 40 cells in ten high-power fields was graded as 1+, 41 to 80 as 2+ and 81 to more as 3+. One to 3 giant polynuclear cells or vessels in neovascularisation or collagen deposits areas in ten high-power fields was graded as 1+, four to six as 2+ and seven to more as 3+. The histopathologist who assessed the specimens was blinded to the type of glue used on the evaluated specimens.

Variables were expressed as medians (25th percentile, 75th percentile), and Wilcoxon two-sample test was used to compare them. Probability values were two-tailed and were considered significant if <0.05 (Table 2).

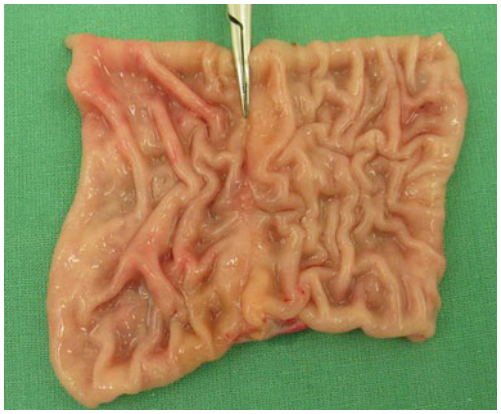

Fig. 4 Healed mucosal layer of anastomosis 2 weeks after it was glued with Glubran 2

Results

Macroscopic Picture—Group A (Glubran 2)

All anastomoses created with Glubran 2 healed, and no dehiscence or leaks were observed. Adhesions were minimal or small in all cases. The adhesions mostly involved adhesions of the omentum to the abdominal wall, but in one animal, the small intestinal loop had adhered to the abdominal wall. It is likely that these adhesions were related more to the laparotomy than to the method of constructing the anastomosis. Peri-anastomotic adhesions mostly involved freely separable adhesions of the oviducts. The presence of these adhesions was attributable to the anatomic arrangement of the female pig pelvis; the bicornuate uterus and the adjacent oviducts pressed ventrally and laterally upon the sigmoid colon. In one animal, the wall of the urinary bladder was drawn up against the anastomosis (Table 1).

No abscesses or other inflammatory changes to the abdominal cavity were identified, but one subcutaneous abscess was observed in one animal. Macroscopic assessments of the resected anastomoses revealed nearly complete healing of the mucosal layers in all specimens (Fig. 4). No stenosis was found.

Macroscopic Picture—Group B (Dermabond)

Complete healing of the anastomosis occurred in four animals in the Dermabond group. One animal had to be euthanized the third day after surgery due to signs of acute peritonitis; an autopsy revealed dehiscence of 1/3 of the perimeter of the anastomosis. During the planned follow-up surgery performed 2 weeks later, one animal displayed a small peri-anastomotic abscess and a small covered dehiscence of the posterior anastomotic wall. The incidence and extent of adhesions within the abdominal cavity were more extensive (Table 1). Peri-anastomotic adhesions were, compared to group A, more fibrotic. The most frequent adhesions were observed in the oviducts, and in two animals, the wall of the urinary bladder was tightly fixed to the anastomosis. Apart from the one case of a local abscess at the anastomosis, no signs of peritonitis were observed in any of the surviving animals.

Resections of the anastomoses revealed fibrotic restructuring of the mucosal layer (Fig. 5). The anastomoses were characterized by a partially stenotic ring and intestinal dilatation above the anastomosis, exceeding the diameter of the intestine below the anastomosis by 1/3 to 1/2 in all the cases. The narrowing was consequent to protuberance of the fibrotic tissue into the lumen of the intestine within the area of glued anastomosis.

Microscopic Picture—Group A (Glubran 2)

The histological findings in the area of the glued anastomosis were virtually uniform in all animals in group A. The adhesive was spread evenly over the entire perimeter of the anastomosis and was surrounded by a wide layer of granulated tissue with mixed inflammatory infiltrate rich

Table 1 The incidence and the extent of adhesions

Extent of adhesions		Group A Glubran 2 (N=6)	Group B Dermabond (N=5)
0	No adhesions	0	0
1	Minimal adhesions	4	0
2	Small adhesions	2	5
3	Extensive adhesions	0	0

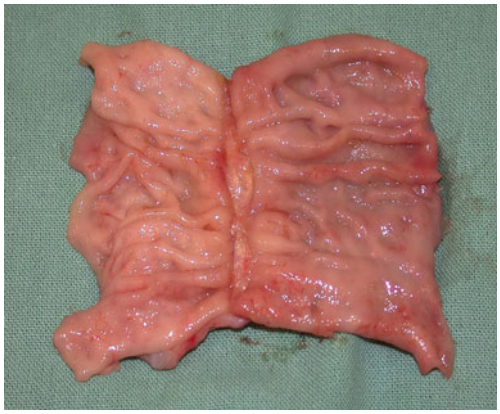

Fig. 5 Healed mucosal layer of anastomosis 2 weeks after it was glued with Dermabond fibrous restructuring of mucosal lining and narrowing of the lumen at anastomosis is evident macroscopically

in neutrophils. A fresh thin layer of cellular fibrous tissue was evident at the periphery. Giant polynuclear cells were either absent or infrequent (Fig. 6). Part of the mucosal surface was replaced with granulated tissue of mixed inflammatory infiltrate, and the other part showed a tendency towards complete healing. The presence of cellular elements, vascularisation and collagen deposits was assessed semi-quantitatively (Table 2).

Microscopic Picture—Group B (Dermabond)

An even, thin layer of the adhesive was found within the entire perimeter of the anastomoses in group B specimens. This was surrounded by a very thin border of granulated tissue with acute or mixed inflammatory infiltrate rich in neutrophils and encircled with a wide layer of cellular fibrous tissue containing numerous polynuclear cells that are common in

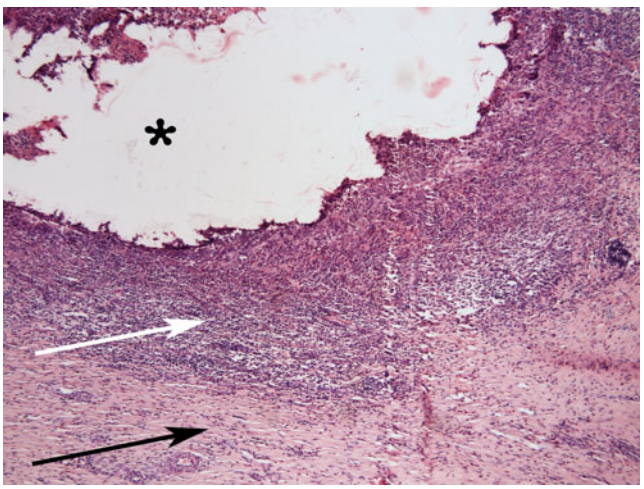

Fig. 6 Glubran 2. The amorphous mass of tissue adhesive (*asterisk*) encircled with granulation tissue layer with mixed inflammatory infiltrate rich in neutrophils (*white arrow*). Fresh thin layer of cellular fibrous tissue is present on the periphery (*black arrow*; enlarged ×10)

response to a foreign body (Fig 7). The mucosal tissue above the anastomosis was ulcerated and replaced with granulated tissue with inflammatory infiltrate. Also, fibrous hypertrophy was evident in the serous layer. The presence of cellular elements, vascularisation and collagen deposits was assessed semi-quantitatively (Table 2).

Discussion

Tissue glues are biological, semi-synthetic or synthetic substances. The basic feature of tissue glue is a strong adherence to living tissue surfaces. Tissue glues are classified as those with haemocoagulation factors (biological glues), including fibrin and thrombin glues, and those without haemocoagulation factors, i.e. cyanoacrylates, polyethyleneglycols, albumin with glutaraldehyde, cellulose, gelatine and collagen.[15,16]

Initially, abdominal surgery was predominantly associated with fibrin glues. These adhesives mimic the last step in the haemocoagulation cascade, i.e., conversion of fibrinogen into fibrin. Concentrated fibrinogen forms the primary component of these glues, and minor components include fibronectin, factor VIII and plasminogen.[16]

At present, research regarding tissue adhesives focuses mostly on cyanoacrylate-based agents. With this type of tissue glue, a firm bond between tissues occurs as a result of the transformation of monomer cyanoacrylate components (clear, colourless liquids) into polymer chains. The polymerization process is induced by anions (I−, CH3COO−, OH−), weak organic bases and amino acids.[17] Polymerization induced by the amino acids in the proteins of living tissues results in the formation of a thin polymer film firmly fixed to the tissue surface. Consequently, when polymerization takes place between two apposed tissue sections, they become firmly attached.[18] The polymerized film is then biodegraded by the gradual hydrolysis of alkyl-group bonds by esterases contained in cellular lysosomes. The by-products of degradation (polycyanoacrylate acids) are water-soluble and are excreted renally.[17,18]

Methyl-cyanoacrylate was the first glue used for medical purposes in 1964; it was used to close a 3-cm-long cystostomy in a dog. However, this derivative was not widely used in practice due to its rapid biodegradation and tissue toxicity.[18]

It was not until the 1990s, when derivatives with long polymer chains had become available, that wider utilisation of cyanoacrylates was reported in experimental and clinical practices. Currently, the most widely used cyanoacrylate is 2-octyl-cyanoacrylate (Dermabond®, Nexaband®, Liqui-Band®, SurgiSeal®). This derivative is now routinely used to form suture-free closers in skin injuries, predominantly in paediatric and plastic surgery practices.

Table 2 Semi-quantitative histopathological score of the presence of cellular elements, neovascularisation, and collagen deposits; examined under light microscopy

	Glubran 2 (N=6)					Dermabond (N=5)					p value
Grading	0	1+	2+	3+	–[c]	0	1+	2+	3+	–[c]	
Fibroblasts [a]	–	4	2	–	1 (1, 2)	–	–	3	2	2 (2, 3)	<0.05
Neutrophils [a]	–	–	2	4	3 (2,3)	–	3	2	–	1 (1, 2)	<0.05
Giant polynuclear cells[b]	4	2	–	–	0 (0, 1)	–	–	2	3	3 (2, 3)	<0.05
Neovascularisation[b]	–	4	2	–	1 (1, 2)	–	3	2	–	1 (1, 2)	NS
Collagen deposits[b]	4	2	–	–	0 (0, 1)	–	3	2	–	1 (1, 2)	<0.05

[a] For fibroblasts and neutrophils, one to 40 cells per ten high-power fields (HPF) was graded as 1+, 41 to 80 as 2+ and 81 to more as 3+

[b] One to three giant polynuclear cells, neovascularisation or large collagen deposits per ten HPF was graded as 1+, four to six as 2+ and seven to more as 3+

[c] Variables are expressed as median (25th percentile, 75th percentile). Wilcoxon two-sample test was used to compare them. Probability values were two-tailed and were considered significant if <0.05

2-Octyl-cyanoacrylate (Dermabond®) was successfully used in in vivo experiments with pigs to create closures of urinary bladder incisions. Two comparative studies demonstrated healing of 7.5-cm-long incisions made through the entire thickness of the bladder wall.[19,20]

Only two experimental studies focusing on creating suture-free colonic anastomosis with cyanoacrylate glues have been conducted so far. Both studies involved laboratory rats. Similar experiments on a large laboratory animal (domestic pig) have not been performed so far.

The first study compared colonic closures created with a monofilament fibre (polypropylene) and cyanoacrylate tissue glue *n*-butyl-2-cyanoacrylate (Histoacryl Blue®).[21] The comparisons between the cyanoacrylate glue and the suture groups were made with respect to outcome measures including anastomotic leakage, anastomotic stricture, peritonitis and wound infection. Also, histological appearance of tissue samples from anastomotic site was evaluated, and anastomotic bursting pressure was measured. The measurement was made on the third and seventh post-operative day.

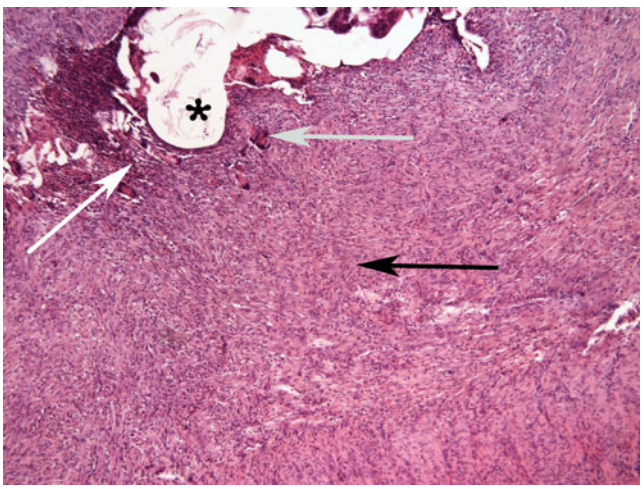

Fig. 7 Dermabond. The glue (*asterisk*) is surrounded by a thin layer of granulation tissue rich in neutrophils (*white arrow*) with evidence of giant polynuclear cells (*grey arrow*). This layer is surrounded by a wide layer of cellular fibrous tissue (*black arrow*; enlarged ×10)

The authors concluded that the use of Histoacryl Blue® in rat colonic anastomosis does not improve the healing process due to significantly higher incidence of anastomotic stricture, adhesion formation and higher bursting pressure in the suture group. There were no significant differences in histological scores.

The second study investigated the effects on healing in high-risk experimental intestinal anastomosis rats. The colonic closures created with a monofilament fibre (polypropylene) and 2-octyl-cyanoacrylate (Dermabond®) were compared under standard and high-risk conditions, where the intestinal wall was intentionally bruised with a Pean clamp.[22] The investigated end-points were mechanical strength, gross adhesion formation, hydroxyproline concentration and histological healing parameters. The study demonstrated a comparable degree of intestinal healing on the third and seventh post-surgery day. No differences between the groups regarding gross peri-anastomotic changes and hydroxyproline concentration were identified on the seventh post-operative day, representing a late phase of healing in a rat. Compared to the third post-operative day, there were fewer general inflammatory changes; granulocyte infiltration level, representing the acute inflammatory reaction, was still increased in the high-risk and octyl-cyanoacrylate groups compared to the normal anastomosis group. Also in these groups, the presence of necrosis, exudate and peritonitis was more evident. Regarding to mechanical strength, there were no differences between the groups on the third post-operative day, but on the seventh day, the sewn anastomoses resisted higher pressure during the bursting pressure test.

The authors concluded that the tested adhesive was not suitable for construction of colonic anastomoses, due to the lower resistance to pressure and the higher (but not significant) incidence of inflammatory changes in the area

of anastomosis compared to sewn anastomoses.[22] Both studies used cyanoacrylates intended primarily for skin closures, not for organ adhesions; in our opinion, this is the likely explanation for the unsatisfactory results with the cyanoacrylate tissue glues.

Currently, the only commercially manufactured cyanoacrylate that is intended primarily for surgical bonding of organs is Glubran 2 (GEM S.r.l., Viareggio, Italy), which is a combination of *N*-butyl-2-cyanoacrylate and methacryloxysulpholane. The product conforms to the European Directive on Medical Devices 93/42/CEE for internal and external surgical use.[23] *N*-Butyl-2-cyanoacrylate alone is available under various brand names (Indermil®, Histoacryl®, Xoin®, GluStitch®) as a tissue glue intended for suture-free skin closures or sclerotisations of esophageal varices.[24–26] The second component of the adhesive, methacryloxysulpholane monomer provides the adhesive with important properties; it reduces the temperature needed for exothermic polymerization (approximately 45°C), increases the elasticity of the glue after polymerization, reduces tissue toxicity and prevents microbial invasion. These properties may play a crucial role in the healing process of the glued tissue. The proportions of the two substances in the glue and its particular biological, physical and mechanical properties are subject to the manufacturer's trade secret. In the present study, the combination of *N*-butyl-2-cyanoacrylate and methacryloxysulpholane contained in Glubran 2 provided significantly better healing of anastomoses, as assessed macro- and microscopically. Our microscopic evaluations corresponded to physiological healing of the colon in the second week post-surgery.[27,28]

In contrast to Glubran 2, 2-octyl-cyanoacrylate (Dermabond) was associated with pronounced fibrosis of the anastomosis and a relatively high incidence of peri-anastomotic adhesions. These results are in line with the results of previous studies and provide evidence for some degree of organ toxicity. However, these complications did not occur when the glue was used for its original purpose, i.e. for skin closures.[29]

Wider use of cyanoacrylate adhesives in gastrointestinal tract surgery has been hindered by reports of relatively unreliable outcomes and, as already mentioned, various extents of histological toxicity. Our study found statistically significant difference in the occurrence of fibroblasts, neutrophils, foreign-body giant polynuclear cells and collagen deposits; this likely results from the different responses of the organism to the type of the applied glue. Dermabond, in comparison to Glubran 2, causes greater fibrosis. This leads to formation of scar-like fibrous stenosis in the area of anastomosis, representing an important negative adverse effect of the glue. The presence of foreign-body polynuclear cells is expected in areas where a foreign material is present. Glue is a foreign material that gradually disintegrates; this disintegration is proportional to the pace of glue fragmentation, i.e. reduction into smaller sections that cause increase in the number of polynuclear cells. Another mechanism contributing to the polynuclear cell elevation includes direct tissue toxicity that causes destruction of the nearby cells—their necrosis. Therefore, it can be assumed that Dermabond is associated with faster fragmentation and possibly higher biological toxicity. This is most probably the reason for greater presence of giant cells associated with Dermabond use compared to Glubran 2.

Previous experimental studies on the use of cyanoacrylate glues for intestinal anastomoses frequently focused, apart from macroscopic and microscopic assessment of anastomotic healing, on measuring the maximal intraluminal pressure ('bursting pressure') that could be resisted by the glued anastomosis. These experiments on laboratory rats demonstrated that glued anastomoses did not resist the same pressures as sewn anastomoses.[21,22] The focus of the present study was to determine whether the use of an adhesive facilitated primary healing of an intestinal anastomosis. We chose not to measure bursting pressures on incompletely healed anastomoses because high pressures would negatively impact the integrity of the anastomosis. Consequently, the damaged anastomosis would require reapplication of the adhesive, and this could bias the results of the experiment. This might cause significant damage to the pigs and result in the devaluation of this challenging research work involving large experimental animals. Bursting pressure measurement was not performed during the follow-up surgery as it is very likely that, after 2 weeks, a healed anastomosis is so strong that it is not important what technique or material was used to make it. Nonetheless, intra-luminal pressure resistance values and comparisons represent valuable information. Therefore, in future phases of this research project and in a wider time frame, it is the authors' intention to systematically measure resistance of glued anastomoses to intra-luminar pressure at different stages of anastomosis healing.

Hand-sewn and stapled anastomoses are well-established and reliable techniques but may also be associated with certain risks. In order for the intestinal tissue to be joined, its structure is partly disturbed with a needle or a staple. This results in tissue microtraumas and might cause mild bleedings and haematomas in the area of anastomosis. Intestinal wall disruption with a suture or a staple might theoretically facilitate bacterial contamination. Hand-sewn anastomosis also depends on an individual surgeon's technique. Tight and dense sutures may result in local ischemia, necrosis and dehiscence. When a stapler with unsuitable staple length is used, the intestinal wall connection may be too tight and lead to ischemia or may be too loose. These, together with other factors, may contribute to development of a leak and dehiscence.

It is advantageous that tissue glue is active only on the intestinal wall (generally any glued organ) surface and that the entire glued area is being joined; its activity is thus not limited to restricted junctures as with the classical anastomoses. The tight interconnection with a cyanoacrylate polymer chain does not in any way affect internal structure or integrity of the connected tissue. This is the main reason why the authors are researching the use of tissue glue in colorectal surgery.

The present study aims not only to compare two glues, i.e. Dermabond, a skin glue that had been previously applied in a similar study,[22] and Glubran 2 intended for gluing organs and not previously investigated in a similar study, but it also seeks to answer whether it is of value to continue researching glued anastomoses. According to our results and in case of Glubran 2, it seems to be justifiable to continue. Obviously, it will be necessary to perform further experiments focusing on comprehensive comparisons of glues with classical suture materials. It is a view of the authors that rational use of glues in colorectal and gastrointestinal surgery, respectively, could at first be achieved through a combination of classical, sparsely sutured (i.e. less traumatic for the tissue) stitched anastomosis and tissue glue or a combination of a stapled anastomosis (possibly with a lower number of staples) and tissue glue.

Conclusion

The combination of *N*-butyl-2-cyanoacrylate and methacryloxysulpholane in Glubran 2® appears to be (under experimental conditions) a promising synthetic adhesive for colonic anastomosis construction. It demonstrated a high level of reliability, with minimal impact on the surrounding organs or the entire abdominal cavity throughout the course of anastomotic healing. It did not facilitate adhesion formation. Further studies are needed to determine the long-term effects of the adhesive on intestinal wall tissue.

Our results also showed that 2-octyl-cyanoacrylate (Dermabond®), used to glue organs in recent experimental studies, was unsuitable for suture-free anastomosis construction. We found that its organ toxicity led to intensive inflammatory reactions and intestinal wall fibrosis.

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ORIGINAL ARTICLE

Clinical Characteristics of Rectal Cancer Involving the Anal Canal

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Abstract
Background This study evaluates the clinical characteristics of rectal cancer involving the anal canal.
Methods A total of 346 consecutive patients with primary low rectal cancer located below the peritoneal reflection were reviewed in this study. Patients were divided into two groups according to whether the lower edge of the tumor came in contact with the anal canal (P group, $n=78$) or not (Rb group, $n=268$). Clinical and pathological parameters, recurrence rates, and survival rates were compared between the two groups.
Results The occurrence of uncommon histological types of tumor was significantly higher in the P group than in the Rb group. P group patients also had a significantly higher lateral pelvic node metastasis rate ($p<0.001$), lower 5-year overall survival rate ($p=0.0491$), and higher 5-year local recurrence rate ($p=0.0171$) than Rb group patients. Multivariate analysis revealed that tumor location was a significant risk factor for local recurrence. In the P group, multivariate analysis showed that uncommon histological tumor types were a significant prognostic factor.
Conclusion Rectal cancer involving the anal canal should be treated with special care, considering the particularly high lateral pelvic lymph node metastasis rate and high local recurrence rate.

Keywords Rectal cancer · Local recurrence · Lateral pelvic node · Anal canal

Introduction

The high incidence of local recurrence after curative operation leading to poor prognosis is the biggest problem when treating rectal cancer. Many studies have been conducted to reveal the risk factors of local recurrence in rectal cancer, such as positive circumferential resection margin, nodal positivity, and advanced T stage.[1–3]

Total mesorectal excision has played a major role in reducing the rates of local recurrence and improving survival in rectal cancer.[4,5] One reason for this is the higher frequency of complete resection of the tumor together with its lymphatic and venous drainage that is achieved by complete removal of the mesorectum.[6] This procedure also increased the rate of sphincter-preserving surgery for low rectal cancer[7]; moreover, the recently developed surgical technique of intersphincteric resection has been proposed to offer sphincter preservation in patients with very low rectal carcinomas such as those involving the rectal canal.[8]

However, there still remains the question whether very low rectal cancer which involves the anal canal has the same clinical and pathological characteristics as cancer situated higher in the rectum. Whereas several studies have revealed that the distance from the anal verge was one of the risk factors for local recurrence, none of these reports addressed the clinical differences between very low rectal cancer involving the rectal canal and low rectal cancer which does not. In this study, we discuss the clinical characteristics of rectal cancer involving the anal canal in comparison to those with other types of low rectal cancer,

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Table 1 Clinical characteristics

Characteristic		P group (n=78)	Rb group (n=268)	p value
Gender	Male	52	185	
	Female	26	83	N.S.
Age (range)		63 (39–85)	61 (28–87)	N.S.
CEA (ng/ml)		7.1	9	N.S.
Tumor size (mm)		49	44	N.S.
Tumor differentiation	Well/Mod	62	257	
	Others	16	11	<0.001
Lateral pelvic node metastasis	Negative	60	249	
	Positive	18	19	0.015
TNM T	T1	6	59	
	T2	18	77	
	T3	44	123	
	T4	10	9	N.S.
TNM N	N0	45	156	
	N1	18	81	
	N2	15	31	N.S.
TNM stage	Stage I	20	100	
	Stage IIA	23	52	
	Stage IIB	2	4	
	Stage IIIA	3	23	
	Stage IIIB	15	58	
	Stage IIIC	15	31	N.S.
Surgical procedure	Sphincter-preserving surgery	5	173	
	Others	73	95	<0.001

N.S. not significant

our goal being to define the optimum treatment strategy for this particular kind of rectal cancer.

Materials and Methods

Patients

A total of 346 consecutive patients with primary low rectal cancer located below the peritoneal reflection and who underwent curative resection at the Yokohama City University Hospital, Japan, between 1993 and 2003 were reviewed in this study. Tumor location was determined before surgery by digital rectal examination, endoscopy, barium enema, computed axial tomography (CAT scan), and magnetic resonance imaging. All rectal cancers were adenocarcinomas. Those carcinomas originating from squamous or transitional epithelium were excluded from study. The patients were seen at the outpatient clinic at 3-month intervals for 5 years and at 12-month intervals thereafter. Tumor markers were examined at every patient visit. CAT scan of the liver and lung or abdominal ultrasonography

Table 2 Recurrence pattern

	P group (n=78)	Rb group (n=268)	p value
Liver	5	15	N.S.
Lung	6	16	N.S.
Local	9	11	<0.001
Inguinal lymph node	7	1	<0.001
miscellaneous	2	4	N.S.

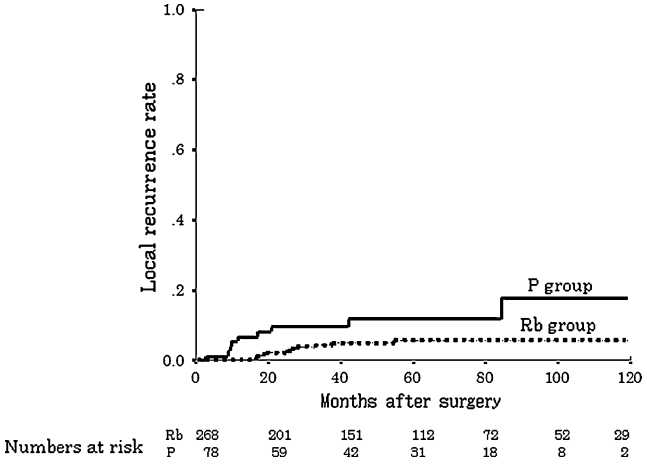

Fig. 1 Five-year local recurrence rate was 5.9% in the Rb group and 11.9% in the P group (p=0.0171)

with chest X-rays was performed at least every 6 months. Colonoscopy was performed every 12 months. Recurrences were clinically determined by colonoscopy or radiological images. Pathological stage III patients were given adjuvant chemotherapy with oral fluorinated pyrimidine.

Surgical Treatment

Total mesorectal excision (TME) was performed in all cases. In patients with T4 tumors, we performed a combined resection of those tissues and/or organs invaded by the cancer. At our institution, the diagnosis of stage II or III cancer is an indication of the need for lateral pelvic node dissection, which was performed on 231 patients in this study. In lateral pelvic node dissection, the fatty and connective tissues outside the pelvic plexus, around the internal iliac and common iliac vessels, and in the obturator cavity were removed, resulting in the iliac vessels becoming completely exposed, with or without pelvic autonomic nerve preservation. The surgical margin including radial margin was negative in all cases, as confirmed by histological examination. No patients underwent pre- and/or post-radiation therapy.

Clinical and Pathological Analysis

Patients were divided into two groups according to whether the lower edge of the tumor reached the anal canal (P group, n=78) or not (Rb group, n=268). To determine the location of the lower edge of the tumor, anoscopy and digital examination were performed in all cases, and when the distance between the lower edge of the tumor and the anal verge was within 3 cm, we defined that the tumor involved the anal canal. In this study, 73 out of 78 in the P group underwent abdominoperineal resection or total pelvic exenteration. In all those cases, it was histologically confirmed that the lower edge of the tumor exceed the anorectal ring.

Standard oncological analysis was performed on all the patients and specimens in accordance with the TNM classification. Clinical and pathological parameters, recurrence rates, and survival rates were then compared between the two groups of patients.

Statistical Analysis

Local recurrence rates and survival rates were calculated by the Kaplan–Meier method and differences were compared statistically by the log-rank test. Cox's proportional hazards model was used for multivariate analysis. Data differences between groups were considered statistically significant at $p<0.05$.

Results

Clinical characteristics of the two groups are shown in Table 1. There were no differences in gender, age, serum CEA level, TNM T, TNM N, or TNM stage between the two groups. Average tumor size was 5 cm larger in the P group than in the Rb group; however, there was no significant difference. In the P group, there were three poorly differentiated adenocarcinomas, nine mucinous carcinoma, two endocrine cell carcinomas, and one anaplastic carcinoma, whereas in the Rb group, there were one poorly differentiated adenocarcinoma and ten mucinous carcinomas. The incidence of mucinous carcinoma and poorly differentiated carcinoma were 11.5% and 3.8% in the P group and 3.7% and 0.4% in the Rb group, respectively. The rate of occurrence of unusual histological tumor types was significantly higher in the P group than the Rb group ($p<0.001$). P group patients also suffered

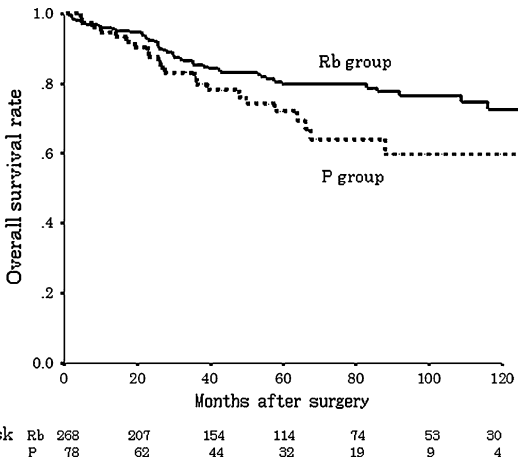

Fig. 2 Overall 5-year survival rate was 80% in the Rb group and 72.2% in the P group (p=0.0491)

Table 3 Uni- and multivariate analysis of local recurrence risk factors

	Univariate *p* value	Multivariate *p* value	Odds ratio	95% CI
Age (>60 vs. ≤60)	N.S.			
Sex (male vs. female)	N.S.			
CEA (>5.0 vs. ≤5.0)	N.S.			
Histology (well/mod vs. others)	N.S.			
Tumor size (>45 mm vs. ≤45 mm)	N.S.			
Lateral pelvic node metastasis				
(Negative vs. Positive)	0.033	N.S.		
TNM T (T1/T2 vs. T3/T4)	N.S.			
TNM N (N0 vs. N1/N2)	0.027	N.S.		
Group (Rb vs. P)	0.017	0.028	2.793	1.156–6.757

CI confidence interval

significantly more lateral pelvic node metastasis than Rb group patients (23.0% vs. 7.1%, $p<0.001$).

The recurrence pattern of the two groups is shown in Table 2. The rate of liver and lung metastases did not differ significantly between the two groups; however, local recurrence was significantly higher in P group compared with Rb group patients. Moreover, most inguinal lymph node metastases were observed in P group patients, with the exception of one Rb group case.

The 5-year local recurrence rate was significantly higher in P group compared with Rb group patients (11.9% vs. 5.9%, $p=0.0171$; Fig. 1), while the 5-year overall survival rate was significantly higher in Rb group compared with P group patients (80% vs. 72.2%, $p=0.0491$; Fig. 2).

Uni- and multivariate analyses of the risk factor for local recurrence were conducted to examine clinical factors. The presence of lateral pelvic node metastasis, TNM N, and tumor location were shown to be statistically significant risk factors for local recurrence by univariate analysis, while multivariate analysis found tumor location to be the only significant risk factor for local recurrence (Table 3).

The risk factor for local recurrence and prognostic factor in the P group were examined. No significant risk factor for local recurrence was detected in this study, while the histological types of the tumor (well/mod vs. others, $p=0.237$, odds ratio=2.330) seemed to most affect the outcome.

Of these factors, univariate analysis found that histology, lateral pelvic node metastasis, and TNM N were significant prognostic factors in the P group, while multivariate analysis revealed histology to be significant (Table 4).

Discussion

This study found several characteristics typical of rectal cancer involving the anal canal compared to other low rectal cancer. First, a significantly higher occurrence of different kinds of histological tumor types occurred; in particular, mucinous carcinomas and poorly differentiated adenocarcinomas were observed in 15.4% of P group patients compared with 4.1% of Rb group patients ($p<0.001$ for all unusual tumor types). This supports the hypothesis that mucinous carcinomas arising in the ano-rectal region are associated with anal glands or fistula in anus.[9] Moreover, poorly differentiated adenocarcinoma has a potentially high invasive tendency that leads to involvement of the anal canal.

Second, the rate of lateral pelvic lymph node metastasis was higher in rectal cancers involving the anal canal, which agrees with the finding of Ueno et al.[10] that the lower the tumor location, the higher the risk of lateral nodal involvement. The rate of lateral nodal metastasis in T3/T4 low rectal tumors below 8 cm was 17%, but this varied according to location from the anal verge: 42% at 0–2.0 cm and 10.5% at 6.1–8.0 cm. Division of the rectum into two zones was proposed in 1895 by Gerota and supported in 1904 by Poirier and colleagues.[11,12] They described lateral

Table 4 Multivariate analysis of prognostic factor of the P group

	p value	Exp(*B*)	CI
Histology (well/mod vs. others)	0.014	3.09	1.264–8.048
Lateral pelvic node metastasis (negative vs. positive)	0.218	1.916	0.680–5.396
TNM N (N0 vs. N1/N2)	0.671	1.282	0.407–4.049

lymphatic channels consisting of three pedicles: anterior, running along the prostate and bladder to end at nodes near the external and internal iliac vessels; lateral, along the middle rectal vessels; and posterior, along the middle and lateral sacral vessels. The result of this study suggested the possibility that the main lymphatic drainage channel to the lateral region may be located more closely to the sphincter muscle or that the existence of the channel along the inferior rectal artery pass through Alcock's canal to the lateral region.

In the present study, the local recurrence rate was significantly higher in P group patients, while multivariate analysis showed that anal involvement was the factor that most affected the likelihood of local recurrence. In this series, the surgical margin was negative in all cases, which was confirmed by histological examination.[13] Moreover, TNM N and presence of lateral pelvic lymph node metastasis were not significant factors by multivariate analysis, though these were significant by univariate analysis. Several studies showed that clinical N stage, gender, CRM, and distance from anal verge were independent risk factors for local recurrence.[14,15] Our study suggests that lateral pelvic node metastasis is another reason for the observed high frequency of local recurrence. In this way, our observations indirectly support the findings of other investigators; for example, Sugihara et al.[16] reported that positive lateral lymph node was the strongest predictor in both patient survival and local recurrence. Although radiation therapy is regarded as an essential option for advanced low rectal cancer in the western world,[17,18] we do not perform pre- and/or post-radiation therapy, whereas lateral pelvic node dissection is performed in stage II and stage III cancers, as is the common practice in many Japanese institutions.[19,20] As discussed above, lateral pelvic node metastasis is a risk factor for local recurrence, so lateral pelvic node dissection would be expected to reduce this risk. We are currently undertaking a clinical trial to compare TME with TME and lateral pelvic lymph node dissection (JCOG0212; ClinicalTrials.gov Identifier: NCT00190541).

In this study, however, we observed a 5-year cumulative local recurrence rate of 11.9% in patients with rectal cancer involving the anal canal. Though there are no previous reports which specifically mention the local recurrence rate of anal canal involving rectal cancer, our results cannot be expected to be better in comparison to other investigations of TME plus radiation therapy. For instance, Rullier et al.[21] found that after a median follow-up time of 40 months, the rate of local recurrence was 2%. This previous study concluded that preoperative radiochemotherapy allowed sphincter-saving resections to be performed, resulting in good local control and functional results in patients with T3 low rectal cancers that would have otherwise required abdominoperineal resections. This suggests that when it comes to cases of anal canal-involving rectal cancer, treatment with TME plus lateral pelvic node dissection is insufficient, so we should consider performing preoperative chemoradiation therapy.

In this series, we could not find the significant risk factor of local recurrence in the P group mainly because of the relatively small sample size. However, according to the clinical characteristics of the P group, uncommon histological types of tumor and high frequency of lateral pelvic node metastasis may be the relevant risk factors for local recurrence.

Finally, we used multivariate analysis to find independent prognostic factors, which were revealed as uncommon histological tumor types. The rate of uncommon histological tumor type was about 21%. These histological types are associated with less response to chemoradiation and poorer prognosis.[22] Therefore, the development of a new preoperative chemoradiation regimen which is effective for poorly differentiated adenocarcinoma is necessary. We believe that this will improve the prognosis of an otherwise difficult care of anal canal-involving rectal cancer.

Conclusion

Rectal cancer involving the anal canal should be treated with special care, considering the particularly high lateral pelvic lymph node metastasis rate and high local recurrence rate. Development of an effective chemoradiation regimen for uncommon histological tumor types is necessary for a better prognosis.

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ORIGINAL ARTICLE

Lateral Internal Sphincterotomy for Chronic Idiopathic Anal Fissure: An Alternative Approach

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Abstract
Background An alternative approach to lateral internal sphincterotomy in the management of chronic anal fissure is presented and its potential advantages are described.
Methods Using the conventional diathermy, the internal sphincter along with its overlying anoderm is cut to the caudal border of the dentate line.
Results This prospective study included 350 patients. Twenty-six patients (7.4%) reported spotting of blood with defecation and 266 patients (76%) reported minimal perianal discharge. The cessation of the discharge and spotting of blood correlated with the complete healing of the sphincterotomy wound. Urine retention requiring temporary catheterization was encountered in 19 patients (5.4%). Neither abscesses nor fistulae were encountered. Cure was achieved in all patients. Neither recurrences nor permanent fecal incontinence were encountered throughout the study period.
Conclusion The alternative approach is efficient and safe and may be added to the surgeon's armamentarium when attempting lateral internal sphincterotomy for chronic anal fissure.

Keywords Anal fissure · Internal sphincterotomy · Anal incontinence

Introduction

Lateral internal sphincterotomy (LIS) is the surgical treatment of choice for refractory anal fissure.[1] Classic LIS entails cutting the internal anal sphincter till the level of the dentate line. Techniques available for the performance of LIS include both the closed and the open technique.[2,3] In both techniques, precise adjustment of the length of sphincterotomy to the level of the dentate line may be technically difficult. In the closed technique, LIS is performed blindly without visualization of either the internal sphincter or the dentate line.[2,3] In the open technique, although LIS is performed under direct vision; however, the internal anal sphincter is either mobilized, divided, and allowed to return to its place or dissected, divided in situ with the dentate line pushed away along with the overlying anoderm.[2,3] In the present study, an alternative approach for performing LIS under direct vision while simultaneously visualizing both the internal anal sphincter and the dentate line is presented and its potential advantages described.

Patients and Methods

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Alexandria. An informed consent was obtained from all patients included in the study. From June 2005 through December 2009, 350 patients with chronic idiopathic anal fissure were operated upon. Chronicity was defined as “history of pain lasting more than 4 weeks or with pain of less duration but similar

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episodes in the past and was Physically evidenced by the presence of a sentinel pile at the distal margin of the fissure, heaped up edges of the fissure and visible sphincter fibers at the base of the fissure."[4] All patients were suffering from recurrent symptoms following at least three previous painful episodes that were treated medically. Medical therapy consisted of frequent warm sitz baths, Diclofenac Potassium tablets 50 mg three times daily (Cataflam®, Novartis Pharma, Cairo, Egypt), Lactulose® (Egyptian Int. Pharmaceutical Industries CO., Tenth of Ramadan City, Egypt) 20 ml twice daily, and glyceryl trinitrate cream 0.2% (GTN®, Leader CO, Cairo, Egypt) topically three times daily. Botulinum toxin is expensive to a degree that would prohibit its use in the management of chronic idiopathic anal fissures and topical Diltiazem is not available in the Egyptian drug market. In the present study, failure of medical treatment did not mean failure to relieve pain and end the painful episode (a situation not encountered in the present study) but rather failure to prevent the recurrence of painful episodes and failure to provide a definitive treatment for the disease. Exclusion criteria included prior anal surgery, any degree of fecal incontinence, and concomitant anal conditions requiring surgical treatment at the time of sphincterotomy; e.g., hemorrhoids or fistula. Patients' demographics were obtained. Preoperative fecal continence was scored using a validated incontinence scoring system.[5] No preoperative bowel preparation was required.

All operations were performed under a standardized spinal anesthesia technique with the patient in the prone Jackknife position and the buttocks strapped apart. Spinal anesthesia produces complete relaxation of both the internal and the external anal sphincters with loss of tone in both muscles. This is considered an important step in identifying both structures separately using the current approach. The procedure starts by the sequential introduction of two fingers into the anus to allow a Parks retractor to be admitted into the anal canal. The retractor is opened slowly till the anal canal becomes in taut. At this stage, the lowest border of the anal canal is formed only by the caudal border of the internal anal sphincter which can thus be easily identified. Under spinal anesthesia, the external anal sphincter relaxes and its lower end moves laterally under the perianal skin outside the field of the operation. Inspection of the anal canal allows easy identification of the dentate line. Lateral internal sphincterotomy is next performed as follows. The index finger is placed immediately lateral to caudal border of the internal anal sphincter to delineate the sphincter and mark the radial extent of cutting at this level (Fig. 1). Using the conventional diathermy, in the coagulation mode with the power level set at level 5, starting from the caudal border of the internal sphincter and proceeding cephalad, the internal sphincter

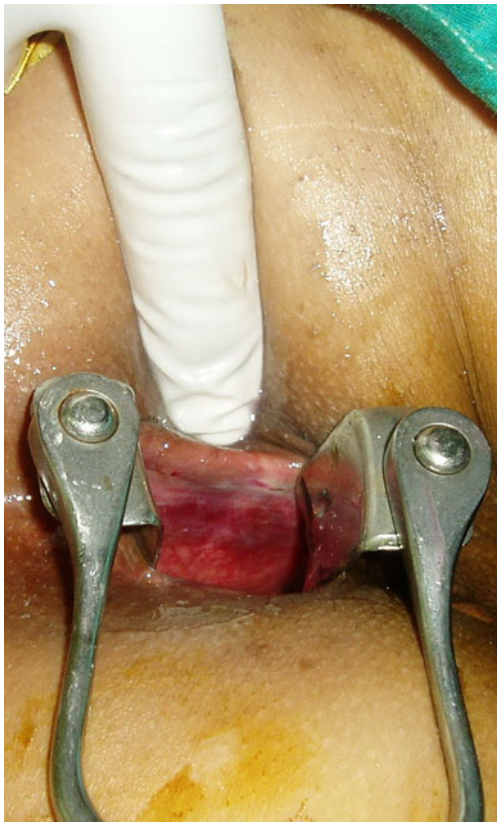

Fig. 1 The Parks retractor is opened in the anal canal to put it in taut and the index finger is placed lateral to caudal border of the internal anal sphincter for delineation

along with its overlying anoderm are cut. The cephalic end of this cut usually lies at the caudal end of the dentate line and is never at or cephalad to it. Care is taken to ensure cutting the full thickness of the internal sphincter along the full length of the sphincterotomy. This is ascertained when the diathermy touches the inner aspect of the external sphincter thus producing visible contraction of this sphincter. The right lateral position is usually chosen for this internal sphincterotomy to avoid the hemorrhoidal plexus at the left lateral position. However, in absence of hemorrhoidal disease, either side could be chosen. The wound was left open. The completed LIS is demonstrated in Fig. 2. Neither the fissure nor the sentinel piles are excised, but hypertrophied anal papillae were excised when encountered. No anal packing is used. Patients were discharged 6 h later after ensuring that there was no urine retention. Diclofenac Potassium 75 mg IM injections (Cataflam®, Novartis Pharma, Cairo, Egypt) were given before discharge on patient demand. Diclofenac Potassium tablets 50 mg were prescribed for home use when needed (Cataflam®, Novartis Pharma, Cairo, Egypt).

Follow-up was performed by inspection of the anal canal for healing of wounds in the outpatient clinic at the end of the first and second postoperative weeks. At the end of the

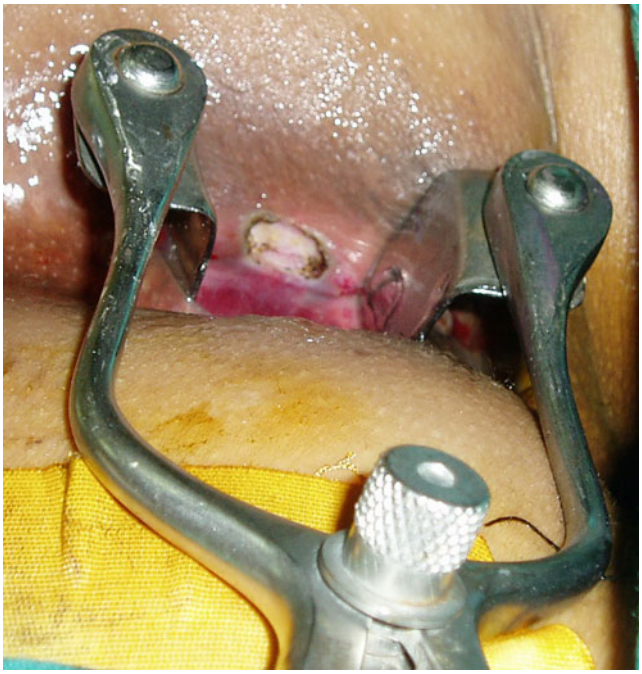

Fig. 2 The completed lateral internal sphincterotomy

sixth postoperative week, the anal canal was inspected for healing of the fissure and fecal continence was rescored. Follow-up was then performed by phone calls to the patients on the sixth postoperative month. Afterwards, patients had accessibility to the surgeon's phone number and were asked to report any problems encountered. Office visits were welcomed on patients demand.

Results

The present study included 206 males (58.9%) and 144 females (41.1%). Their age ranged from 24 to 62 years with a mean of 38.4±8.2 years. In 291 patients (83.1%), the fissure was located posteriorly; in 32 patients (9.1%), the fissure was located anteriorly and the remaining 27 patients (7.7%) had both anterior and posterior fissures. Forty-six females (13.1%) gave history of prior vaginal delivery. The duration of illness ranged from 12 to 240 months with a median of 18 months. The operation was bloodless, and the operative time was usually 1 min.

At discharge, only 89 patients (25.4%) demanded one analgesic ampoule. Oral analgesics after defecation were required by 86 patients (24.6%) for 1–2 days postoperatively with the remaining 264 patients (75.4%) describing their first postoperative defecation as painless. Urine retention requiring temporary catheterization was encountered in 19 patients (5.4%). At the end of the second postoperative week, complete healing of the sphincterotomy wound was achieved in all patients. Twenty-six patients (7.4%) reported one to two episodes of spotting of blood with defecation, and 266 patients (76%) reported minimal perianal discharge that gave a sense of perianal wetness. The cessation of the discharge and spotting of blood by the end of the second postoperative week correlated with the complete healing of the sphincterotomy wound. At the end of the sixth postoperative week, all fissures were completely healed and patients were symptom-free. Neither abscesses nor fistulae were encountered.

Preoperatively, all patients scored 0 on the incontinence scoring system denoting perfect continence in all patients. At 6 weeks postoperatively, again all patients scored 0 on the incontinence scoring system denoting perfect continence. In the intervening period, five patients (1.4%) had temporary incontinence to flatus that resolved completely by the end of the sixth postoperative week. Throughout the first six postoperative months and the study period, none of the patients developed recurrent symptoms.

Discussion

Over the past decade, there has been great enthusiasm for the use of pharmacologic treatments for chronic anal fissure. Various agents have been extensively studied, e.g., topical nitric oxide donors and calcium channel blockers. Despite their initial encouraging results, such agents were found to be only marginally better than placebo in healing chronic anal fissures.[4,6,7] There were problems with compliance owing to side effects (namely headache), a lower rate of healing and a dramatically higher recurrence rate than surgical treatment.[6] Injection of Botulinum toxin into the internal sphincter has yielded better results allowing healing at a rate higher than placebo.[7] However, there is no consensus on dose, site, or number of injections.[8] These results have led the standards practice task force of the American society of colon and rectal surgeons to conclude that surgery (i.e., LIS) may be appropriately offered without a trial pharmacologic treatment and have thus kept LIS as the gold standard treatment for the refractory chronic anal fissure.[6]

Between 1.2% and 30% of patients undergoing LIS experience variable degrees of incontinence, most having temporary incontinence to flatus that usually resolves over time.[9,10] Although still controversial, a number of factors have been suggested as possible contributors including surgical technique (open vs. closed), type of anesthesia (local vs. general), additional procedures performed, obstetric history, and coexisting occult sphincter defects among others.[11–14] Incontinence after LIS has also been found to be directly related to the length of sphincterotomy.[15–17] Garcia-Aguilar et al. compared the functional and anatomic characteristics of 13 incontinent patients to those of 13 continent patients after LIS.[15] The length of sphincterotomy reported by the surgeon

as the percentage of sphincter divided was statistically significantly longer in the incontinent group than in controls (75% vs. 57%, respectively).[15] Sultan et al. prospectively evaluated the extent of internal sphincter division following open LIS in 15 patients by anal endosonography.[16] In nine of the ten females included, there was full length division of the internal sphincter. They concluded that division of the internal sphincter in most females tends to be more extensive than intended.[16] Lindsey et al. reported on 17 incontinent patients following LIS.[17] Of the 15 patients (88%) demonstrating an overextensive internal sphincterotomy, in 11 patients (64%), the sphincterotomy extended two thirds of the length of the internal sphincter and in four patients (24%), it went the complete length. One half of the patients with an overextensive sphincterotomy were males. They concluded that LIS in both men and women is difficult to standardize and that incontinence after this procedure is not confined to high risk patients.[17] On the other hand, incomplete sphincterotomy has been shown to be associated with treatment failure.[18] The main point of difference between the present technique and other classic techniques is that the anoderm overlying the sphincterotomy is incised in the present technique and is preserved in the other classic techniques.[2,3] The main reason for incising the anoderm along with the internal sphincter was to allow both the dentate line and the internal sphincter to be under the surgeon's direct vision throughout the procedure. Having both structures under direct vision allowed accurate and precise adjustment of the length of sphincterotomy in relation to the dentate line. In the classic open technique, although LIS is performed under direct vision, such visual correlation between both structures is absent. In the present study, temporary incontinence to flatus was encountered in five patients (1.4%) and resolved completely by the end of the sixth postoperative week. None of the patient included developed permanent incontinence. The present technique may prove beneficial in avoiding some technical pitfalls of LIS, e.g., the overextensive, the full length, and the incomplete sphincterotomies. Although a future anal endosonographic study is required to provide evidence to this suggestion, the results of the present technique in terms of recurrence and fecal continence seem to support it.

Although unintentionally, incising the anoderm overlying the sphincterotomy offered two additional advantages. First, there was no dissection, i.e., the anoderm was not lifted from the underlying internal sphincter and an intersphincteric plane was not developed, and consequently, there was no dissection-induced bleeding. This, coupled with the use of diathermy, has rendered the present technique bloodless. This bloodlessness was extremely helpful in achieving fine adjustment of the length of sphincterotomy in relation to the dentate line and has, in part, contributed to the 1 min operative time achieved with the present technique. Second, because the anodermal incision overlaid the full length of the sphincterotomy and because the procedure was bloodless, no complications requiring surgical intervention were encountered, i.e., abscesses or fistulae.

Excision of the fissure was not practiced in the present study since there is no evidence that fissurectomy promotes healing.[2,3,19] In a prospective study, Khubchandani and Reed concluded that excision of the fissure was not necessary as time to healing and success in healing following sphincterotomy were not influenced by excision of the fissure.[19] Sentinel piles were not excised after explaining to the patient that this was not the fissure itself (a common belief of the lay people in our country). Patients were advised against the excision of sentinel piles on the basis that such excision will create a wound that will cause postoperative pain and will require a relatively long time to heal without influencing fissure healing positively. Avoiding excision of the fissure and the sentinel piles has, in part, contributed to the short operative time achieved with the present technique although this was not the aim of such policy.

More than two thirds of the patients did not require analgesia at the time of discharge. Furthermore, more than 75% of the patients described their first postoperative defecation as painless and did not therefore require oral analgesics after defecation. Such findings suggest that the anodermal incision was not associated with neither significant nor prolonged postoperative pain. On the other hand, as a consequence to incising the anoderm, twenty-six patients (7.4%) reported one to two episodes of spotting of blood with defecation and 266 patients (76%) reported minimal wound discharge that gave a sense of perianal wetness. The cessation of the discharge and spotting of blood within the second postoperative week correlated with the complete healing of this anodermal incision.

Follow-up was performed by physical examination at the end of the first, second, and sixth postoperative weeks. At the sixth postoperative month, it was difficult to convince patients who became symptom-free for a long time that physical examination was necessary for the follow-up of such a minor procedure and they usually ignored the office visit. For this reason, phone calls were used for follow-up beyond the sixth postoperative week.

The present alternative approach, owing to its bloodlessness and very short operative time, seems suitable for application as an office procedure under local anesthesia. However, this is difficult to ascertain in our country since the use of local anesthesia for anal procedures is not accepted by patients.

The present alternative approach has achieved cure in all patients included. Furthermore, neither recurrences nor permanent fecal incontinence were encountered throughout the study period. Although longer follow-up durations are still required to draw more definite conclusions, however,

the preliminary results seem encouraging. Whether these potential advantages would translate into an advantage for this approach over the currently applied techniques awaits the results of future prospective randomized studies comparing the present technique to other techniques.

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ORIGINAL ARTICLE

Variation in Lymph Node Assessment After Colon Cancer Resection: Patient, Surgeon, Pathologist, or Hospital?

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Abstract
Background Evaluation of ≥12 lymph nodes after colon cancer resection has been adopted as a hospital quality measure, but compliance varies considerably. We sought to quantify relative proportions of the variation in lymph node assessment after colon cancer resection occurring at the patient, surgeon, pathologist, and hospital levels.
Methods The 1998–2005 Surveillance, Epidemiology, and End Results—Medicare database was used to identify 27,101 patients aged 65 years and older with Medicare parts A and B coverage undergoing colon cancer resection. Multilevel logistic regression was used to model lymph node evaluation as a binary variable (≥12 versus <12) while explicitly accounting for clustering of outcomes.
Results Patients were treated by 4,180 distinct surgeons and 2,656 distinct pathologists at 1,113 distinct hospitals. The overall rate of 12-lymph node (12-LN) evaluation was 48%, with a median of 11 nodes examined per patient, and 33% demonstrated lymph node metastasis on pathological examination. Demographic and tumor-related characteristics such as age, gender, tumor grade, and location each demonstrated significant effects on rate of 12-LN assessment (all $P<0.05$). The majority of the variation in 12-LN assessment was related to non-modifiable patient-specific factors (79%). After accounting for all explanatory variables in the full model, 8.2% of the residual provider-level variation was attributable to the surgeon, 19% to the pathologist, and 73% to the hospital.
Conclusion Compliance with the 12-LN standard is poor. Variation between hospitals is larger than that between pathologists or surgeons. However, patient-to-patient variation is the largest determinant of 12-LN evaluation.

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Introduction

Colon cancer causes over 50,000 deaths per year in the USA, and over 100,000 new cases are diagnosed annually.[1] Lymph node metastasis is a critical predictor of survival, and adequate ascertainment of lymph node status determines the use of adjuvant chemotherapy, which has been proven to improve survival.[2] Adequate harvest and evaluation of lymph nodes is therefore essential to accurately identify those patients most likely to benefit from chemotherapy. In fact, increased lymph node harvest has been reported to be independently associated

with improved long-term survival after colectomy for colon cancer.[3–6] The National Quality Forum (NQF), in collaboration with the American College of Surgeons, American Society for Clinical Oncology, and National Comprehensive Cancer Network, has endorsed the evaluation of at least 12 lymph nodes after colon cancer resection as a hospital quality surveillance measure.[7]

Compliance with this 12-lymph node (12-LN) metric varies considerably, with only 44% of patients who underwent a colectomy in 2001 having 12 or more lymph nodes assessed.[8] The reason for the wide variation in compliance is poorly understood. Factors that influence lymph node assessment may relate to the patient, surgeon, pathologist, or hospital. At the patient level, variations in lymph node yield may be related to age, gender, or tumor characteristics such as stage, grade, or site of resection.[8–11] Patient-related determinants of lymph node examination are important to understand in order to fairly apply and standardize any quality measures related to lymph node assessment. Although not well studied, factors related to the surgeon, pathologist, or hospital are of special interest because, unlike most patient-related factors, they may be modifiable.[12] Such data may also allow better targeting of hospital quality improvement efforts. The lack of provider-level data to explain the wide variability in compliance with the 12-LN standard has led some investigators to call its utility as a quality care measure into question.[13]

One problem in understanding the variation in lymph node assessment is that detailed provider-specific operative or pathological data are typically not available in population-based data sets. All retrospective studies are constrained by the variables available for analysis; some variation in outcome will inevitably remain unexplained by the finite number of variables included in explanatory models. A potentially useful approach, in addition to identifying individual variables that predict adequate lymph node assessment, is to quantify the relative proportions of the variation in lymph node assessment occurring at the patient, surgeon, pathologist, and hospital levels. Such an approach would allow targeting of quality improvement efforts at the appropriate level even when the relevant variables affecting care are not explicitly known. To our knowledge, no previous study has explicitly assessed variability at all of these levels. We sought to conduct such an analysis using the most recent available data from the Surveillance, Epidemiology, and End Results—Medicare (SEER-Medicare) database.

Methods

Data Source and Study Population

Using data from the 1998–2005 SEER-Medicare database,[14,15] patients aged 65 years and older with Medicare parts A and B coverage undergoing curative-intent surgery for adenocarcinoma of the colon were identified. Patients with any previous cancer diagnosis, appendiceal tumors, and those with stage IV disease were excluded in accordance with NQF guidelines,[7] as were those receiving preoperative radiation therapy. The number of lymph nodes examined for each patient is reported by SEER, and patients reported as having either no lymph nodes (n=654) or an unknown number examined (n=505) were excluded. Treating surgeons, pathologists, and hospitals were identified using encoded Unique Physician Identification Numbers from Medicare claims. Mean annual colon cancer volumes were calculated for each provider using the same cohort used for analysis, and for descriptive purposes, providers were grouped into terciles such that an approximately even number of patients were in each group. Previous studies have demonstrated that use of SEER-Medicare-derived volumes is an acceptable approach that has little impact on volume–outcome relationships for colon cancer, although some misclassification due to non-Medicare volume and SEER geographic boundaries does occur.[16–18]

Statistical Analysis

All variables for analysis were chosen based on clinical plausibility and were force-entered into the final model. Multilevel logistic regression was used to model lymph node evaluation as a binary variable (≥12 versus <12) with a nesting structure of patients nested within surgeons, nested within pathologists, and nested within hospitals (with each level of correlation representing a "cluster"). Random intercepts at each level were included in the model, allowing for additional variation at each level that was not explained by variables included in the model. Cluster-level variances at each level were obtained from both null (i.e., no explanatory variables) and full (i.e., all explanatory variables) models, with the patient-level variance constrained to $\pi^2/3$ (by definition for the logistic distribution).[19] These cluster-level variances were used to calculate the relative proportion of variance in lymph node assessment attributable to each level. To facilitate comparison with other explanatory variables, cluster-level variances were also expressed on the odds ratio (OR) scale using the median odds ratio, which quantifies the variation between clusters as the median value of odds ratios obtained by comparing sets of two patients from two randomly chosen, different clusters (e.g., two hospitals).[20] All tests of statistical significance were two-sided, and statistical significance was established at α=0.05. Statistical analyses were performed using the GLLAMM package[19] for Stata/MP 10.1 for Windows (StataCorp, College Station, TX, USA). This study was deemed exempt from review by the Johns Hopkins University School of Medicine Institutional Review Boards.

Results

Study criteria identified 27,101 eligible patients. Patient characteristics are presented in Table 1, and tumor and operative characteristics are presented in Table 2. Of note, the majority of colon resections were right colectomies (n=15,701, 58%), and only 7% of all colectomies were performed laparoscopically (n=1,886). The majority of tumors were T3 lesions (n=16,183, 60%), and 33% demonstrated lymph node metastasis on pathological examination. Provider characteristics are presented in Table 3. The 27,101 patients in this study were treated by 4,180 distinct surgeons and 2,656 distinct pathologists at 1,113 distinct hospitals. The majority of colectomies were performed by general surgeons (n=23,349, 86%).

The overall compliance with 12-LN evaluation was 48% (n=13,003), with a median of 11 nodes examined per patient (Fig. 1a). Based on empirical Bayes estimates, the median percent compliance with 12-LN evaluation at the 1,113 hospitals was 43% (Fig. 1b). Rates of 12-LN assessment stratified by patient, tumor and operative characteristics, and provider characteristics are presented in Tables 1, 2, and 3, respectively. The effects of these characteristics were assessed using a multivariable multilevel logistic regression model that explicitly accounted for correlations in 12-LN assessment among patients treated by the same surgeon, pathologist, or hospital (Table 4). Demographic characteristics such as age, gender, and race demonstrated significant effects. Patients admitted to the hospital emergently rather than electively (OR 0.87, P< 0.001) and those who died in the hospital (OR 0.67, P< 0.001) had lower rates of 12-LN assessment. The type of colectomy had a pronounced effect (transverse versus right colectomy, OR 0.34, P<0.001), but there was no difference between laparoscopic and open approaches. The presence of any lymph node metastasis was associated with increased odds of 12-LN evaluation (OR 1.17, P<0.001).

Surgeon, pathologist, and hospital characteristics were also evaluated in the multivariable model (Table 4). Colorectal surgeons had higher rates of 12-LN assessment as compared to general surgeons (OR 1.21, P=0.001). There was a modest effect of surgeon volume (high volume

Table 1 Patient characteristics

Variable	Number	Percent	Percent with 12-LN assessment	P value
Number of patients	27,101		48	
Age (years)				
65–69	4,362	16	50	<0.001
70–74	5,575	21	49	
75–79	6,535	24	49	
80–84	5,716	21	48	
>85	4,913	18	45	
Gender				
Female	15,630	58	49	<0.001
Male	11,471	42	46	
Race				
White	23,289	86	48	<0.001
Black	2,021	7	49	
Asian	851	3	44	
Hispanic	359	1	36	
Other/unknown	581	2	51	
Admission type				
Elective	16,324	60	50	<0.001
Urgent	5,483	20	47	
Emergent	5,294	20	44	
In-hospital death	976	4	38	<0.001
Year of diagnosis				
1998	1,769	7	43	<0.001
1999	1,816	7	42	
2000	3,600	13	42	
2001	3,785	14	44	
2002	4,072	15	48	
2003	4,144	15	49	
2004	4,011	15	53	
2005	3,904	14	56	

Table 2 Tumor and operative characteristics

Variable	Number	Percent	Percent with 12-LN assessment	P value
Type of resection				
Right colectomy	15,701	58	56	<0.001
Transverse colectomy	1,492	6	34	
Left colectomy	3,492	13	40	
Sigmoid colectomy	6,178	23	35	
Total colectomy	238	<1	65	
Operative approach				
Open	25,215	93	47	<0.001
Laparoscopic	1,886	7	56	
Tumor T classification				
T1	3,252	12	33	<0.001
T2	4,703	17	44	
T3	16,183	60	52	
T4	2,963	11	51	
Lymph node metastasis				
Yes	8,900	33	53	<0.001
No	18,201	67	46	
Tumor differentiation				
Well	2,261	8	42	<0.001
Moderate	18,751	69	48	
Poor	5,255	19	54	
Undifferentiated	217	<1	56	
Unknown	617	2	34	

Table 3 Provider characteristics

Variable	Number[a]	Percent	Percent with 12-LN assessment	*P* value
Surgeon specialty				
General surgery	23,349	86	46	<0.001
Colorectal surgery	3,521	13	61	
Surgical oncology	231	<1	53	
Surgeon volume				
Low volume (1–2)	9,923	37	46	<0.001
Mid-volume (2–3)	8,197	30	47	
High volume (3–16)	8,981	33	52	
Pathologist volume				
Low volume (1–3)	9,369	35	48	0.052
Mid-volume (3–5)	9,145	34	49	
High volume (5–61)	8,587	32	47	
Hospital volume				
Low volume (1–7)	9,068	33	42	<0.001
Mid-volume (7–14)	9,084	34	48	
High volume (14–51)	8,949	33	55	
Hospital control				
Non-profit	21,873	81	50	<0.001
For-profit	2,098	8	42	
Government	3,130	12	39	
Teaching hospital				
Yes	14,509	54	53	<0.001
No	12,592	46	42	
ACOSOG member				
Yes	5,339	20	57	<0.001
No	21,762	80	46	
NCI designation				
None	26,408	97	48	<0.001
Clinical center	104	<1	56	
Comprehensive center	589	2	67	
Rural hospital				
Yes	2,629	10	38	<0.001
No	24,472	90	49	

ACOSOG American College of Surgeons Oncology Group, *NCI* National Cancer Institute

[a] Refers to number of patients

versus low volume, OR 1.10, $P=0.039$) and a more pronounced effect of hospital volume (high volume versus low volume, OR 1.31, $P=0.027$), but no effect of pathologist volume, on 12-LN assessment. Among other hospital characteristics, treatment at a National Cancer Institute (NCI) comprehensive cancer center was associated with the largest difference in 12-LN assessment (OR 2.57, $P<0.001$). Teaching hospitals (OR 1.24, $P=0.001$) and American College of Surgeons Oncology Group member hospitals (OR 1.17, $P=0.025$) also achieved higher rates of 12-LN assessment.

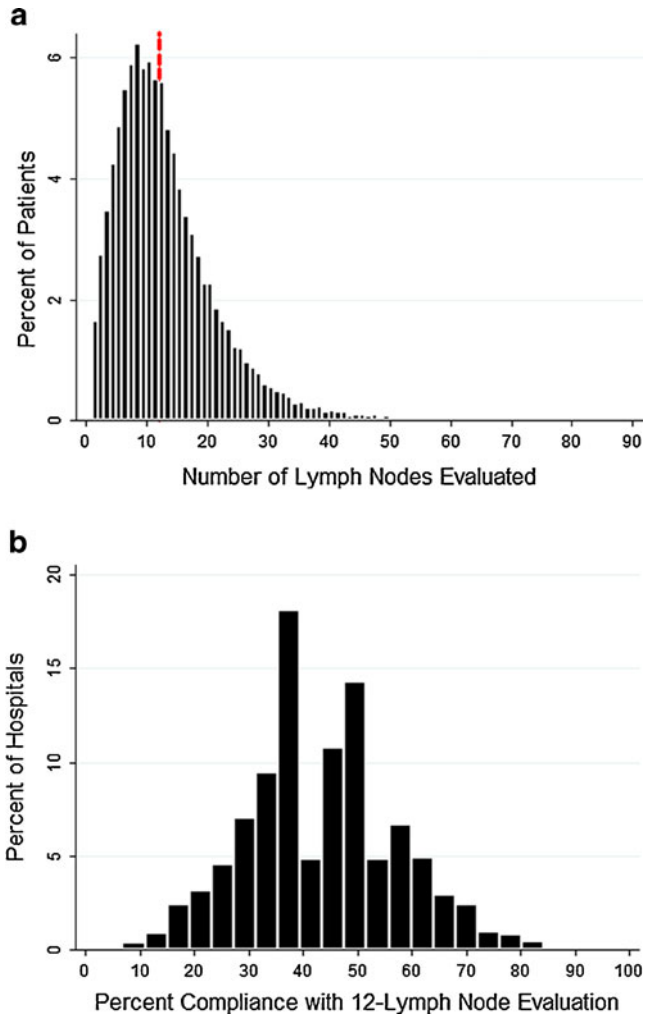

Fig. 1 The overall compliance with 12-LN evaluation was 48% ($n=$ 13,003), with a median of 11 nodes examined per patient (**a**; *dotted line* denotes 12-LN threshold). Based on empirical Bayes estimates, the median percent compliance with 12-LN evaluation at the 1,113 hospitals was 43% (**b**)

In aggregate, the majority of the variation in the 12-LN assessment was related to non-modifiable patient-specific factors (Fig. 2a). The modifiable provider-related variation in 12-LN assessment was then assessed in further detail (Table 5). In the null model that included no explanatory variables, 8.3% of provider-level variation occurred at the surgeon level, 18% at the pathologist level, and 74% at the hospital level (Fig. 2b). Similarly, after accounting for all explanatory variables in the full model, 8.2% of the residual provider-level variation was attributable to the surgeon, 19% to the pathologist, and 73% to the hospital. These

Table 4 Multivariable multilevel logistic regression analysis of 12-lymph node assessment

Variable	OR	95% CI	*P* value
Age (years)			
65–69	1.00	–	Ref.
70–74	0.93	0.85–1.02	0.124
75–79	0.91	0.83–1.00	0.047
80–84	0.84	0.76–0.92	<0.001
>85	0.72	0.65–0.80	<0.001
Female	1.14	1.07–1.21	<0.001
Race			
White	1.00	–	Ref.
Black	0.99	0.88–1.12	0.883
Asian	0.81	0.67–0.98	0.030
Hispanic	0.62	0.48–0.80	<0.001
Other/unknown	1.09	0.88–1.34	0.437
Admission type			
Elective	1.00	–	Ref.
Urgent	0.96	0.89–1.04	0.365
Emergent	0.87	0.81–0.94	<0.001
In-hospital death	0.67	0.58–0.79	<0.001
Year of diagnosis			
1998	1.00	–	Ref.
1999	0.93	0.79–1.09	0.352
2000	1.03	0.89–1.18	0.722
2001	1.18	1.02–1.35	0.022
2002	1.40	1.22–1.60	<0.001
2003	1.43	1.24–1.64	<0.001
2004	1.78	1.55–2.05	<0.001
2005	2.15	1.86–2.48	<0.001
Type of resection			
Right colectomy	1.00	–	Ref.
Transverse colectomy	0.34	0.30–0.38	<0.001
Left colectomy	0.44	0.41–0.48	<0.001
Sigmoid colectomy	0.36	0.34–0.39	<0.001
Total colectomy	1.46	1.07–2.00	0.016
Laparoscopic resection	0.94	0.83–1.06	0.325
Tumor T classification			
T1	1.00	–	Ref.
T2	1.81	1.62–2.02	<0.001
T3	2.81	2.54–3.10	<0.001
T4	2.59	2.28–2.94	<0.001
Lymph node metastasis	1.17	1.10–1.25	<0.001
Tumor differentiation			
Well	1.00	–	Ref.
Moderate	1.12	1.00–1.25	0.049
Poor	1.16	1.03–1.32	0.018
Undifferentiated	1.40	0.99–1.96	0.054
Unknown	0.82	0.65–1.02	0.079
Surgeon specialty			
General surgery	1.00	–	Ref.
Colorectal surgery	1.21	1.08–1.35	0.001
Surgical oncology	1.01	0.72–1.41	0.968
Surgeon volume			
Low volume	1.00	–	Ref.
Mid-volume	1.06	0.98–1.14	0.151
High volume	1.10	1.00–1.19	0.039
Pathologist volume			
Low volume	1.00	–	Ref.
Mid-volume	1.06	0.96–1.17	0.239
High volume	1.00	0.89–1.12	0.979
Hospital volume			
Low volume	1.00	–	Ref.
Mid-volume	1.15	0.96–1.37	0.125
High volume	1.31	1.03–1.66	0.027
Hospital ownership			
Non-profit	1.00	–	Ref.
For-profit	0.89	0.73–1.09	0.274
Government	0.81	0.68–0.97	0.021
Teaching hospital	1.24	1.09–1.41	0.001
ACOSOG member	1.17	1.02–1.33	0.025
NCI designation			
None	1.00	–	Ref.
Clinical center	1.88	0.79–4.46	0.152
Comprehensive center	2.57	1.59–4.14	<0.001
Rural hospital	0.99	0.84–1.15	0.852

OR odds ratio, *CI* confidence interval, *Ref.* referent, *ACOSOG* American College of Surgeons Oncology Group, *NCI* National Cancer Institute

quantities were transformed to the odds ratio scale to allow more intuitive interpretation of their significance alongside the explanatory factors in the multivariable model (Table 4). When viewed as median odds ratios, the surgeon-related

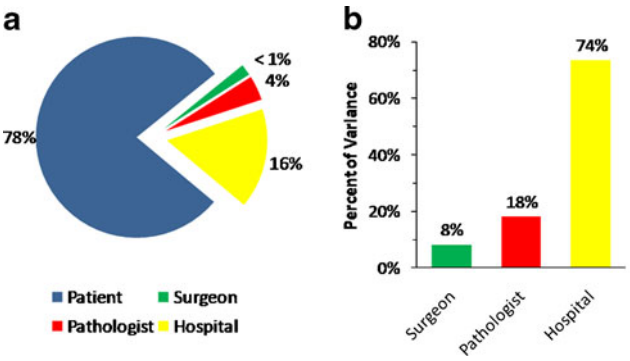

Fig. 2 The majority of the variation in 12-LN evaluation was related to non-modifiable patient-specific factors (**a**). When the modifiable provider-related variation in 12-LN evaluation was assessed in further detail, 8% of provider-level variation occurred at the surgeon level, 18% at the pathologist level, and 74% at the hospital level (**b**)

Table 5 Provider-level variation

Level	Null model	Full model[a]
Surgeon		
Share of total variation (%)	1.8	1.8
Share of provider-related variation (%)	8.3	8.2
Median odds ratio	1.30	1.30
Pathologist		
Share of total variation (%)	4.0	4.1
Share of provider-related variation (%)	18	19
Median odds ratio	1.48	1.49
Hospital		
Share of total variation (%)	16	16
Share of provider-related variation (%)	74	73
Median odds ratio	2.20	2.18

All $P<0.05$

[a] Residual variation after accounting for all variables in Table 4

effect was analogous to an odds ratio of 1.30, the pathologist-related effect 1.49, and the hospital-related effect 2.18. In all cases, cluster-level variances were significantly different from zero ($P<0.05$), indicating that the surgeon, pathologist, and hospital exerted statistically significant effects on 12-LN assessment rates in addition to the effects accounted for by variables in our model.

Discussion

Colorectal cancer lymph node examination rates have been a topic of considerable study and, recently, much debate.[4,8,13,21–23] Based largely on retrospective observational studies[3–6] that noted an independent association between increasing lymph node count and long-term survival, the assessment of 12 lymph nodes after colon cancer resection has been endorsed as a hospital quality surveillance standard.[7] The use of lymph node count as a quality indicator has been criticized, however.[13,21,22] Because variation in lymph node count is multifactorial, targeting quality improvement strategies based on evaluation of lymph node count is problematic.[22] Variations in lymph node count have been noted at the patient,[8,11,24,25] surgeon,[26,27] pathologist,[27–30] and hospital[6,21,31] levels, but no previous study has quantified variation at all of these levels simultaneously. Understanding the factors that contribute to the number of lymph nodes evaluated is critical to targeting improvements in lymph node evaluation and, by extension, patient outcome. However, the relative contribution of each factor and exactly what or who (i.e., hospital versus surgeon versus pathologist) is being evaluated when interpreting lymph node count remains ill-defined. Additionally, the use of a multilevel framework allows variation at different levels of care to be quantified even if the specific variables acting at these levels are not explicitly defined in the available data. The present study quantitatively assesses these relative contributions and demonstrates that differences in lymph node count are largely related to patient-level variation. When potentially modifiable provider-related variability is specifically examined, hospital-level variation is much larger than pathologist- or surgeon-level variation. These results suggest that while significant determinants of adequate LN assessment remain out of the control of providers, provider-related variation in 12-LN evaluation is largely a hospital-level phenomenon.

A wide spectrum of provider-related factors may influence the quality of patient care and help explain variations in quality of care. The Donabedian framework organizes such factors into those related to structure or process and relates them to outcome.[32] While conceptually useful, such a model belies the complex relationship between various structural factors such as case volume or hospital characteristics and process measures such as 12-LN assessment. Furthermore, no analysis of compliance with such process measures can exhaustively and explicitly identify all determinants of compliance. The use of population-based data, while allowing greater generalizability, further limits the variables available for analysis to those in the data set. For example, ascertaining whether a specific hospital had formally adopted an institution-wide quality-control program for 12-LN assessment was not feasible using SEER-Medicare data. In the present study, we addressed this issue by using a multilevel regression model that allowed us to characterize the variation at each level (patient, surgeon, pathologist, or hospital) without explicitly accounting for all the variables at that level. This approach allowed us to explore aspects of the variation in 12-LN assessment that have been inadequately studied by prior studies.

The relative contributions of surgeon versus pathologist in attaining the 12-LN count are one such ill-defined issue[23,26] addressed by our analysis. Previous studies have suggested that differences in surgical technique or pathological examination may explain some of the variation in lymph node assessment.[30,31,33–36] Rieger et al.[26] compared the lymph node yield after colon cancer surgery of a single high-volume surgeon who operated at two hospitals with separate pathology departments. In this study, pathology provider A had a median LN count of 10 compared with 19 for pathology provider B. Of note, following an intervention in the pathology protocol at hospital A, the median lymph node yield increased to 12. In the present study, 8.2% of the residual provider-level variation was attributable to the surgeon, versus 19% to the pathologist. Collectively, these data suggest that the pathologist can have a significant influence on the number of lymph nodes reported. It is important to note, however, that most of the

provider-related variation in lymph node count was not at the surgeon or pathologist level but rather at the hospital level (78%). In fact, the effect of hospital-level variation had a larger impact on 12-LN count than many other effects we studied, including surgeon and pathologist characteristics. The dominance of hospital-level variation does not imply that surgeons and pathologists have a less important role to play; we note that if a significant majority of surgeons or pathologists at a given hospital act cohesively as a group to improve lymph node evaluation, this would manifest as a hospital-level improvement. Some such hospital-level factors (e.g., teaching status, NCI designation) were explicitly accounted for in our analysis. Other such hospital-level factors were not identified in the SEER-Medicare data but were implicitly accounted for in the hospital-level variation. We speculated that these might include pathology department-wide policies regarding adequacy of lymph node evaluation or insistence by surgeons at a hospital that final pathology reports include adequate lymph node review.

Our analysis revealed that most of the modifiable (i.e., non-patient) variation in 12-LN assessment occurs on the hospital level as a functional unit. While others have noted that hospital characteristics such as NCI center designation may be associated with lymph node assessment,[37] these characteristics are likely proxies for hospital-level quality-control measures. Such data suggest that certain hospitals may have a higher baseline level of quality that may be generalized across other areas of care throughout that institution. This concept is based on the belief that shared elements of structures and processes of care across certain individual hospitals may relate to overall performance. With this in mind, future quality improvement measures aimed at lymph node assessment may be better directed at the hospital level rather than individual physicians.

Previous studies have largely focused exclusively on either hospital-level[13,21] or patient-level ("biology"-related) factors.[9,11] Past studies, however, have not addressed the relative importance of hospital versus patient factors associated with lymph node count variation. In the present study, we quantified provider-related variation using median odds ratios, allowing intuitive comparison between explanatory variables and residual provider-related variation on the odds ratio scale. For example, the median effect on 12-LN assessment of residual hospital-to-hospital variation was analogous to the effect of a left versus right colectomy. As such, these data call into question the usefulness of the 12-LN standard as a hospital quality surveillance measure. The current formulation of this measure does not, for example, account adequately for variations in patient and tumor characteristics (such as tumor location). We noted significant variation in 12-LN evaluation related to patient-specific factors such as patient age, tumor location, tumor stage, and tumor grade, as have previous studies.[8,9,11,22] However, unlike any previous study, we included specific patient- and provider-level data in our explanatory model. In turn, we were able to demonstrate that individual patient-level variables exert larger effects on 12-LN assessment compared with provider-level factors, as the vast majority (78%) of residual variation was still attributable to patient-level factors even after accounting for all other variables in the model. As Baxter has noted,[22] achieving the goal of adequate and consistent staging using a benchmark assumes that lymph node count does not vary substantially between individual patients. We would suggest that if a benchmark is to be used in the presence of such patient-to-patient variation, the reporting standards should at least be sufficiently standardized as to insulate the measure from such case mix variation as much as possible. If the impact of patient-level variation on the number of lymph nodes evaluated is indeed as great or even greater than that of provider-level variation, as our analysis demonstrates, then quality measures such as the 12-LN benchmark need to be standardized with respect to the relevant patient and tumor characteristics. Our data, therefore, clearly call into question the use of 12-LN quality measure as currently formulated. Rather, our data strongly suggest that if the 12-LN benchmark is to be retained as a hospital quality measure, the guideline needs to extensively account for differences in patient case mix.

We acknowledge several limitations to our analysis. First, we focused on patients aged 65 years and older because of our reliance on Medicare data for this study. While we would expect compliance rates with the 12-LN rates to be higher in younger patients, we would not expect a priori that our conclusions regarding variation in compliance to be significantly different—however, other studies would need to verify this hypothesis. Second, in assessing provider-level variables, we were limited to those available in the SEER-Medicare data. However, this was a major reason that we used a modeling approach that allowed quantification of variability without needing to explicitly identify all important variables at each level. We also did not specifically examine the impact of laparoscopic versus open colectomy on 12-LN rates. Previously published randomized data,[38] however, have shown that the median number of harvested lymph nodes in laparoscopic versus open colectomy was similar. As such, these data suggest that the impact of increasing utilization of laparoscopic colectomy was unlikely to impact our findings. Finally, SEER data are based on a geographic sampling and as such do not guarantee representative sampling of hospitals and physicians with respect to the provider-level characteristics we evaluated. Again, other sources of data will need to be used to corroborate our findings.

In conclusion, overall compliance with the 12-LN evaluation standard after colon cancer resection is poor, although it has improved over time. While variation in

lymph node count can in part be attributed to differences among pathologists and surgeons, the largest share of modifiable variation was found to be at the hospital level. Importantly, however, patient-to patient variation was much greater than that attributable to health care providers, both with respect to individual patient-specific factors and the residual variation after accounting for such variables. The 12-LN threshold may, in part, be a "quality" measure. However, our data strongly suggest that while some determinants of lymph node assessment are provider-related, much of the variation in LN assessment is due to hospital and patient-level factors. As such, our findings suggest that the 12-LN hospital quality measure should be cautiously used and that its reporting should be more rigorously standardized.

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ORIGINAL ARTICLE

Surgery for Hilar Cholangiocarcinoma: A Multi-institutional Update on Practice and Outcome by the AFC-HC Study Group

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Abstract
Introduction Surgical resection is the only option for long-term survival in patients with hilar cholangiocarcinoma (HC), but it is associated with high morbidity and mortality. The aim of the present study was to prospectively assess the perioperative management and short-term outcomes of surgical treatment of HC in a recent, multi-institutional study with a short inclusion period.
Methods Between January and December 2008, a register prospectively collected data on patients operated on for HC (exploratory or curative surgery) in eight tertiary centers. The register focused on perioperative management, resectability, surgical procedures employed, morbidity, and mortality. The study cohort consisted of 56 patients (40 men and 16 women) with a median age of 63 years (range, 33–83 years).
Results Among the 56 patients, 47 (84%) were jaundiced and 42 (75%) tumors were classified as Bismuth–Corlette type III–IV. Nine patients (16%) underwent staging laparoscopy and four (7%) received neoadjuvant chemotherapy. Preoperative biliary drainage (endoscopy, 42%) was performed in 38 (81%) jaundiced patients and portal vein embolization (right side, 83%) was performed prior to surgery in 18 patients (32%). Among these 56 patients, curative resection was achieved in 39 (70%). All underwent major liver resection (>3 segments), bile duct resection, and lymphadenectomy. Thirteen patients (36%) underwent portal vein resection, one of whom also required pancreaticoduodenectomy. Eighty-two percent of resected patients (n=32) had no proof of malignancy prior to hepatectomy. Clear surgical margins were obtained in 77% (n=30). The postoperative mortality was 8% and complications occurred in 72% of the resected patients. Seven (25%) patients required reoperation, and 15 (54%) patients required percutaneous drainage. In a univariate analysis, the risk factors for morbidity were intraoperative blood transfusion (p=0.009) and vascular clamping (p=0.006). The median length of hospitalization was 20±13 days.

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Conclusion Curative resection for HC is associated with a high rate of R0 resection. However, surgery is associated with high levels of morbidity and mortality, despite intensive perioperative management.

Keywords Hilar cholangiocarcinoma · Preoperative management · Morbidity · Mortality

Abbreviations

HC	Hilar cholangiocarcinoma
PVE	Portal vein embolization
SD	Standard deviation

Introduction

Hilar cholangiocarcinoma (HC), also known as Klatskin tumor, is challenging in terms of staging and surgical treatment. It has long been accepted that surgical resection with complete removal of all cancerous tissue is the only way to provide patients with a chance of cure or long-term survival.[1,2] This radical treatment requires extended hepatectomy (with combined caudate lobectomy and bile duct resection) and lymphadenectomy.[3] The management of HC patients has changed over recent decades. In the more recently developed Neuhaus concept, the portal vein bifurcation is always resected, in order to provide sufficiently clear lateral margins and avoid tumor entry.[3–5] Most of these complicated procedures are performed in patients with cholestatic livers and are associated with a high risk of postoperative mortality and morbidity. To reduce the risk of postoperative liver failure in jaundiced patients, preoperative biliary drainage and portal vein embolization (PVE) have been recommended.[3,6,7] Lastly, HC treatment is hampered by the low accuracy of preoperative tumor extension staging; up to 29% of resected patients turn out to have an R1 or R2 resection,[8] whereas the resected specimen is not cancerous in 5% of patients.[9]

Due to the rarity of HC, most large-scale surgical series have featured a long inclusion period.[10–12] As a result, perioperative management techniques tend to change over the course of a study and thus introduce bias. An exception is constituted by the two randomized, prospective studies of HC resection[13,14] performed at the University of Nagoya (Japan). The studies featured a short inclusion period and looked at the pre- and postoperative outcomes of surgical resection. However, it is unclear whether these results can be extrapolated to other countries and surgical centers.

Hence, with a view to establishing a prospective registry of operated HC patients, the AFC-HC study group was created under the auspices of the *Association Française de Chirurgie* (the French Society of Surgery, AFC).

The aim of this prospective, cross-sectional study was to describe perioperative management procedures and short-term outcomes for HC surgery in France in 2008.

Methods

Patient Selection

All members of the AFC-HC-2009 study group were encouraged to participate in a prospective multicenter study of the outcomes of HC surgery from January 1 to December 31, 2008. The present study, focusing on perioperative management and short-term outcomes, included the patients who underwent surgery (laparotomy or staging laparoscopy) for resectable HC. The total number of patients referred for HC in 2008 in these different centers was unknown. A standardized, structured questionnaire collected 289 data items per patient. For each patient, a copy of the histology report on the tumor specimen was requested.

A diagnosis of HC was adopted when the histology report explicitly confirmed the presence of an adenocarcinoma of biliary origin (i.e., a cytokeratin $CK7^+$ $CK20^-$ phenotype) arising from the biliary confluence or either of the main ducts. No patients underwent resection for benign disease in this series due to inclusion criteria. Patients with intrahepatic or gallbladder tumors with secondary involvement of the biliary confluence or the first-order biliary branches were excluded from the analysis.

Over the course of the 12-month inclusion period, a total of 56 patients (40 men and 16 women, with a median age of 63 years (range, 33–11 years) underwent surgery (exploratory or with curative-intent) in eight tertiary centers scattered throughout France. The management was not standardized: staging and use of preoperative biliary drainage, portal vein embolization, or laparoscopic assessment was variable according to the center. Jaundice was the most common symptom (n=47; 84%). The lesions were classified according to Bismuth–Corlette's classification.

Criteria Studied

Demographic data, risk factors, comorbidities, presenting symptoms, and time to histologic diagnosis are presented in Table 1. The preoperative parameters included tumor staging and preoperative management data: biliary drainage, PVE, future remnant liver volume (as a percentage), histologic evidence of cancer, neoadjuvant therapy, and laparoscopic assessment.

Other parameters included perioperative findings, the surgical techniques used (hepatobiliary resection, vascular resection, and/or resection of adjacent organs), total blood loss, perioperative blood transfusions (i.e., intraoperative volumes and those delivered in the first 48 h following surgery), and tumor characteristics (dimensions, surgical

Table 1 Characteristics of the study population

	n (%)
Number of patients	56
Gender (M/F)	40/16
Age (mean±SD)	63±11
Body mass index (mean±SD, kg/m^2)	24±3
Risk factors	
Primary sclerosing cholangitis	0
Chronic inflammatory bowel disease	0
Papillomatosis	0
Caroli disease	0
Variation of biliopancreatic junction	0
Gallstones	5 (9%)
Cirrhosis	0
Comorbidities	
Diabetes mellitus type 1	1 (2%)
Diabetes mellitus type 2	6 (11%)
Hypertension	21 (39%)
Ischemic heart disease	4 (7%)
Chronic obstructive pulmonary disease	1 (2%)
Chronic renal failure	1 (2%)
Dyslipidemia	9 (16%)
Alcohol	10 (19%)
Tobacco	15 (29%)
Cancer	7 (13%)
Initial symptoms	
Symptoms present	48 (91%)
Jaundice	47 (84%)
Weight loss	30 (54%)
Pruritus	18 (32%)
Asthenia	13 (23%)
Right upper quadrant abdominal pain	12 (21%)
Cholangitis	4 (7%)
Non-specific abdominal pain	4 (7%)
Time between first symptoms and surgery (median)	4 months [0–62]

margins, and lymph node metastasis). A clear resection margin (R0) was defined as negative resection margins. R1 resection was defined as the microscopic presence of tumor tissue at the resection margin. R2 resections included the presence of peritoneal deposits, liver metastases, or para-aortic lymph node metastases. The dissection margins that correspond to the surfaces toward the vascular structures and liver parenchyma were also analyzed.

Postoperative complications, reoperations, percutaneous drainage, length of hospitalization, and in-hospital mortality were also recorded. Complications were graded according to the Dindo–Clavien classification.[15] Postoperative mortality was defined as admission or 90-day mortality. Postoperative ascites was defined as effusion ≥400 ml via the drain after postoperative day 4. Postoperative liver failure was defined by a rise in serum total bilirubin levels of over 50 μmol/L and a prothrombin time below 50% at postoperative day 5.

Statistical Analysis of Risk Factors for Morbidity and Mortality

Due to the study's prospective design, only 3% of the data were missing. The univariate analysis used a Chi-square test or Fisher's exact test (when $n<5$) for qualitative variables and Student's *t* test for quantitative variables. A Mann–Whitney *U* test was used for non-parametric variables. A *p* value of 0.05 or less was considered to be statistically significant. Multivariate analysis was deliberately not performed (given the sample size). Although data on recurrence and long-term survival were not analyzed in this prospective study, at least 6 months of follow-up data were available for all patients.

Results

Preoperative Staging and Management (n = 56)

Preoperative morphological staging included a computed tomographic (CT) scan in all patients, magnetic resonance imaging, or MR cholangiography in 37 patients (63%), a PET-CT scan in eight patients (14%), preoperative endoscopic ultrasound assessment in 12 patients (21%), and angiography in one patient (2%). The median size of the lesion was 16 mm (8–30) and nine patients (16%) had lesions larger than 25 mm. There was a suspicion of portal vein involvement in 12 (20%) patients. Eight (14%) patients had marked unilateral liver atrophy and 2 (4%) patients had suspected lymph node metastasis. Tumors were classified as Bismuth–Corlette type III–IV in 42 (75%) patients.

Nine (16%) patients underwent staging laparoscopy and uncovered a contraindication to resection (peritoneal carcinomatosis) in one of the nine patients (11%).

Preoperative biliary drainage was performed in 38 of the 47 jaundiced patients (81%), and details concerning this procedure are presented in Table 2. The percutaneous route was used slightly more frequently than the endoscopic route (58% vs. 42%). When preoperative endoscopic drainage was employed; this drains the liver that was intended to be preserved in all patients. Drainage-related morbidity was 34% (n=13), and the most frequent problems were cholangitis (69%; n=9) and hemorrhage (15%; n=2). One patient developed duodenal perforation during prosthesis placement and required duodenal suture via laparotomy. Complications were neither related to the type of prosthesis nor the transpapillary nature of the drainage. Only 57% of drained patients had a serum total bilirubin level <50 μmol/L at the time of the operation, and 10% had a serum total bilirubin level >200 μmol/L.

Eighteen patients (32%) underwent preoperative PVE and all of them underwent surgery 4 weeks after the procedure. Data on this procedure are summarized in Table 3. PVE was performed after biliary drainage in 14 patients (78%). A single patient underwent PVE of the left portal vein, but six patients had occlusion of segment 4 branches, in addition to occlusion of the right portal vein. A single complication (11%) resulted in limited portal branch thrombosis, with no lasting impact on resectability. Of the patients in whom extended right hepatectomy was planned, the median future remnant liver volumes before and after PVE were 18% and 31%, respectively. The median gain was 33%, and the post-PVE future remnant liver accounted for more than 40% of the total liver volume in 22% of patients.

Table 2 Modality, safety, and efficiency of preoperative biliary drainage

Jaundiced patients	n=47
Preoperative biliary drainage	38/47 (81%)
Endoscopic	15 (42%)
Percutaneous	23 (58%)
Transpapillary	4
Indications for biliary drainage	
Jaundice	32 (84%)
Cholangitis	6 (16%)
Type of biliary stent	
Metallic stent	5
Plastic stent	33
Number of stents (mean)	1.2 [1–5]
Number of procedures (mean)	1.3 [1–5]
Drainage-related complications	13 (34%)
Cholangitis	9 (69%)
Severe	3
Hemorrhage	2 (15%)
Other	2
Death	0
Total serum bilirubin (μmol/L) before biliary drainage (mean)	188 [136–760]
Total serum bilirubin (μmol/L) before surgery (mean)	68 [85–360]
Total serum bilirubin before surgery <50 μmol/L n (%)	22 (57%)
Total serum bilirubin before surgery >50 μmol/L n (%)	16 (43%)
Total serum bilirubin before surgery >100 μmol/L n (%)	10 (26%)
Total serum bilirubin before surgery >200 μmol/L n (%)	4 (10%)

Table 3 Portal vein embolization (PVE) techniques, morbidity, and outcomes

No. patients	*n*=18 (32%)
Segments occluded	
Right liver	9
Right lobe	6
Segments 6–7	1
Left liver	1
Unknown	1
Material	
Glue	12
Coils	6
PVE-related complications	
Portal vein thrombosis	2 (11%)
Remnant liver volume[a]	
All operations *n*=18	
Before PVE	18%
After PVE	31%
After PVE >30%	33%
After PVE >40%	22%
Gain (median)	33% [17–150%]
Gain (quartile 25)	21%
Gain (quartile 75)	43%
Scheduled, extended right hepatectomy *n*=18	
Before PVE	19%
After PVE	28%
Gain (median)	41% [25–150%]

[a] Remnant liver volume/total liver volume among the 18 operated patients who underwent surgery

One patient received neoadjuvant chemotherapy, another received neoadjuvant radiation therapy, and two patients received both treatment modalities. Chemotherapy was always based on gemcitabine. According to a CT evaluation, the tumor was stable in all patients. Ten (18%) patients received antibiotic prophylaxis for the week prior to liver resection, and five patients (8%) received preoperative synbiotic treatment.

Surgical Procedures

No resection was performed in 17 patients (30%), since laparotomy (*n*=16) or laparoscopy (*n*=1) had revealed a contraindication. These included distant lymph node metastasis (celiac trunk or para-aortic sites; *n*=6), vascular involvement (*n*=4), peritoneal carcinomatosis (*n*=3), malignant infiltration of the hepatoduodenal ligament (*n*=3), and liver metastasis (*n*=1). Of the contraindicated patients, 38% were Bismuth–Corlette type III–IV, one had received neoadjuvant therapy, two had undergone PVE, and four had undergone preoperative laparoscopic assessment [a positive celiac trunk lymph node (*n*=2) and malignant infiltration of the hepatoduodenal ligament (*n*=2)]. Among the 39 resected patients, 32 (82%) had no proof of malignancy prior to surgery. Histologic proof of malignancy had been obtained endoscopically in 61% of the other patients.

All resections performed are described in Table 4. All the resected patients underwent major liver resection (>3 segments), bile duct resection, and lymphadenectomy. Segment 1 was resected in 77% of patients. Lymphadenectomy variously concerned the hepatoduodenal ligament (100% of patients), the hepatic artery (91%), the celiac trunk (82%), and para-aortic nodes (3%). Vascular clamping was necessary in 27 patients (69%). Frozen-section examination of the bile duct margins was performed for 23 (59%) patients. Positive frozen sections were significantly associated with definitive R1 resection ($p < 0.0001$). Portal vein resection was performed in 13 patients (33%), including three patients in whom the policy of Neuhaus was followed ([4]). Four patients (10%) underwent associated resection of adjacent organs, including one pancreaticoduodenectomy prompted by a positive frozen section on the lower part of the common bile duct. Seventeen patients (44%) required blood transfusions.

Short-Term Outcomes in Resected Patients (*n* = 39)

Mortality

The overall postoperative mortality rate was 7.6% (*n*=3). The causes of death were (1) acute liver failure (*n*=1), after extended right hepatectomy and combined portal vein resection despite PVE, (2) sepsis with multi-organ failure after extended left hepatectomy and portal vein resection (*n*=1), and (3) pancreatic fistula in the patient with an extended right hepatectomy and pancreaticoduodenectomy. Two of the three patients were over 70 years. The deaths occurred in three different high-volume centers.

Morbidity

Seventy-two percent of the patients (*n*=28) experienced complications after surgery (grade I complications in 21% of patients (*n*=6), grade II in 11% (*n*=3), grade IIIa in 32% (*n*=9), grade IIIb in 18% (*n*=5), grade IV in 7% (*n*=2), and grade V in 11% (*n*=3)). Biliary leakage (*n*=14) and sepsis (*n*=14) accounted for half of all complications. The most severe complication was liver failure (*n*=7, 25%). These complications prompted an invasive procedure in 57% of patients, including reoperation in seven patients, percutaneous drainage in 15 patients [non-infected perihepatic collection (*n*=10), biloma (*n*=3), and hematoma (*n*=2)] and interventional endoscopy in one case. The mean length of hospitalization was 20±13 days. In a univariate analysis, vascular clamping

Table 4 Surgical procedures performed in the 39 resected patients

Liver parenchyma	*n*=39 (100%)
Major hepatectomy (≥3 segments)	39 (100%)
Extended right hepatectomy	22
Extended right hepatectomy + 1	15
Left hepatectomy	16
Anterior hepatectomy	1
Resection of segment 1	30
Bile duct	39 (100%)
Bile duct resection only	*n*=0
Number of biliary anastomoses	1.15 [1 - 4]
Intubation of biliary anastomoses	5 (13%)
Vascular clamping	27 (69%)
Transfusion	16 (41%)
Mean number of units of packed blood cells	4.1 [2 - 13]
Operating time (min)	342 [125 - 798]

($p=0.006$), intraoperative transfusion ($p=0.009$), and the duration of surgery ($p=0.006$) were found to be risk factors for postoperative complications. Jaundice, preoperative synbiotic treatment, PVE (tested in candidates for extended right hepatectomy), and portal vein resection were not identified as factors influencing morbidity and mortality. Likewise, preoperative biliary drainage did not influence morbidity and mortality.

Histopathological Data—Predictive Criteria for Resectability

Resection margins were R0 in 30 patients (77%), R1 in eight patients and R2 in one patient. None of the available preoperative criteria (age, gender, jaundice, serum bilirubin level, liver atrophy, suspicion of portal vein involvement, adjacent organ involvement, and the Bismuth–Corlette classification) was significantly correlated with R0 resection status. The incidence of R0 resection did not correlate with the presence or absence of portal vein resection (86% vs. 67%, respectively, $p=0.27$). Twenty-eight percent ($n=11$) had lymph node involvement.

Analysis of the histology reports showed a lack of information concerning surgical margins: the nature of dissection margins was specified in 18% ($n=7$) of patients and the length of margins was mentioned in 21% of the reports ($n=8$). Bismuth–Corlette resectability is specified in Table 5.

Discussion

The present series provided a short inclusion, prospective, multicenter, up-to-date overview of the management of HC patients. As such, it contrasts strongly with recent single-group reports and generated crucial data on preoperative management in "real life", in terms of preoperative histologic confirmation of malignancy (82% of resected patients had no proof of malignancy prior to hepatectomy), frequency of biliary drainage (81%), and the drainage route (endoscopic, 42%) in jaundice patients, PVE (right side, 83%) prior to surgery (in 32% of patients), resectability (70%), R0 resection margin (77%), surgical procedure used (including liver resection in all patients) morbidity, and mortality (72% and 8%, respectively), and postoperative interventional procedures (25% patients required reoperation and 54% had percutaneous drainage).

Eighty-four percent of patients were jaundiced at the time of diagnosis; of these, 81% underwent preoperative biliary drainage (endoscopic drainage in 42% of patients). Drainage was associated with 30% of morbidity, and only 57% of drained patients had a low serum bilirubin level (<50 μmol/l) at the time of surgery. Overall, preoperative management for decreasing the serum bilirubin level is interventional and has its own morbidity. Surgeons fail to take account of the failure of these procedures and thus maintain the scheduled surgery in approximately 40% of patients.

Table 5 Resectability of HC, according to the Bismuth–Corlette classification

Bismuth–Corlette staging	*n* (%)	Resectability
Bismuth–Corlette I	6 (11%)	17%
Bismuth–Corlette II	8 (14%)	62%
Bismuth–Corlette III		
Bismuth–Corlette IIIa	19 (34%)	79%
Bismuth–Corlette IIIb	18 (32)	89%
Bismuth–Corlette IV	5 (9%)	60%

We believe that some points are of major importance for future practice. Firstly, we should select HC patients in whom we truly need to perform preoperative biliary drainage—it should not be implemented systematically. For example, Kennedy et al. have recently showed that preoperative biliary drainage of the liver remnant improved the outcome of extended liver resection in HC patients only when the preoperative remnant volume was below 30% (i.e., mortality was associated with a lack of preoperative biliary drainage in patients whose preoperative remnant volume was below 30%).[16] Likewise, in a specific analysis of the impact of preoperative biliary drainage before resection in the 595 resected HC patients in the whole AFC-HC register (unpublished data), Farges et al. showed that there was a linear correlation between preoperative bilirubin levels and mortality after right liver resection; for patients with equivalent bilirubin levels prior to right-side resection, mortality was always lower in those who had undergone biliary drainage. In contrast, there were no correlation between preoperative bilirubin levels and mortality after left liver resection and (for equivalent bilirubin levels) mortality was greater in patients who had undergone biliary drainage.[17] In the literature, preoperative biliary drainage is performed in nearly 40% of patients presenting type IIIA (left-side) HC. A similar debate can be added for PVE, given that only 22% of the patients with embolism had a future remnant liver volume >40% at the time of resection. Secondly, we have to find alternatives for patients in whom preoperative interventional procedures have failed. This study highlighted the fact that failure of preoperative interventional management (biliary drainage and PVE) is observed in 30% of the resected patients. In cases of persistently high preoperative bilirubin levels, the presence of chronic cholangitis or fibrosis must be ruled out and adjunct procedures for greater bilirubin clearance (such as extracorporeal albumin dialysis, used in the treatment of refractory cholestatic pruritus) can be envisaged in protocols.[18–20] The indications for preoperative biliary drainage and PVE must be refined, and the outcomes of these interventional procedures must be analyzed more accurately.

The present series showed that, in 2008, 82% of resected patients (with postoperative mortality of 8%) had no proof of malignancy prior to hepatectomy. Around two thirds of the biopsies were performed endoscopically. In fact, differential diagnoses of malignant hilar lesions are rare (less than 5%), and a few predictive factors for malignancy have been reported: the involvement of second-order bile ducts, vascular invasion, and lobar atrophy are more likely in patients with malignant hilar lesions. The combination of vascular invasion and lobar atrophy significantly increases the likelihood of diagnosis for malignant hilar lesions. The absence of these signs should prompt the physician to consider an alternative diagnosis and attach greater importance to performing a PET scan, laparoscopic examination, and biopsy of the hilar region.[9] The treatment of differential diagnoses of malignant hilar strictures is usually surgical and endoscopic management of benign lesions has given disappointing long-term results.[21,22] Furthermore, laparoscopic assessment was not routinely performed in France in 2008 (even for advanced HC). However, the accuracy of laparoscopic assessment (when performed in selected patients) appeared to be low in the present series, with only one patient contraindicated for peritoneal carcinomatosis.

Hilar cholangiocarcinoma remains a challenge for surgeons because of its propensity for local invasion and its proximity to the portal vein, hepatic arteries, and liver parenchyma. Until recently, locally advanced disease at diagnosis and surgical inaccessibility resulted in low resectability, few R0 resections, and poor survival. However, since the 1990s, the surgical treatment of HC patients has evolved. Hepatic resection almost always includes segment I [3] and Bismuth–Corlette type III and type IV lesions no longer represent contraindications.[23] Moreover, an aggressive approach for HC with systematic portal vein resection is now recognized as offering a better chance of long-term survival [4] and has been adopted by most leading liver teams. These new procedures are associated with acceptable morbidity and mortality,[24] a greater proportion of R0 resections and increased 5-year survival (between the survival times for resected pancreatic cancer and colorectal cancer liver metastases). The present series confirms that resection was achieved in 70% of patients, with major hepatectomy and combined bile duct resection and lymphadenectomy in all patients. None of the patients underwent bile duct resection without hepatectomy. Moreover, most liver resections included caudate lobectomy. Systematic portal vein resection (the Neuhaus concept) was achieved in only three patients, and therefore, we are not able to make any recommendation about this policy. Most portal vein resections were decided on preoperatively because of suspected portal vein involvement. In the present series, most of the patients (77%) had Bismuth–Corlette III or IV lesions: of these, 76% underwent resection, including three Bismuth–Corlette IV patients. However, the overall R0 resection rate was high (77%).

This prospective study is one of four having provided prospective data on morbidity and mortality after HC surgery.[13,14,25] Fifteen of our 42 patients with biliary drainage and/or PVE (36%) experienced complications (three of which were severe). Nevertheless, none were contraindicated as a result of these complications. Despite better perioperative management of patients, morbidity still remains significant—even in high-volume centers—and ranges from 14% to 62%.[1,10–12,26–28] In the present series, 72% of the complications were benign (i.e., Dindo–Clavien grade ≤IIIa); however, 25% of the patients with complica-

tions required reoperation, and more than half required percutaneous drainage of an abdominal collection. Morbidity was dominated by septic complications and bile leakage (the most frequent complications) and postoperative liver failure (associated with the highest mortality). Invasive procedures were required in 57% of patients with postoperative complications. This high rate underlines the need for a large, multidisciplinary care team which includes physicians highly skilled in interventional radiology and/or endoscopy. In the present study, vascular clamping and intraoperative transfusion were found to be risk factors for morbidity, corroborating the results published by Hirano et al.[10] In the present series, post-resection mortality was 7.6%. It is difficult to compare this rate with literature values,[1,10–12] in view of our multicenter design and our focus on the management of HC (rather than the results from a single, high-volume center). Hence, 84% of centers reported 0% mortality and the three deaths occurred in three different high-volume centers. We did not find a treatment–center interaction in this study. Furthermore, our statistical analysis should be interpreted with a degree of caution, in view of the heterogeneity of management procedures and the small number of patients included in this series. This is especially true for the potential role of preoperative biliary decompression or PVE in the prevention of postoperative mortality in patients undergoing major hepatectomy. With a view to decreasing morbidity and mortality after major resection (all resections were major in the present series), the recent, disturbing publication by Chen et al. must be considered. The authors evaluated the extent of liver resection for HC in a prospective series: minor hepatectomy (minimal perihilar liver resection, in order to obtain an R0 margin) was performed in 93 patients with Bismuth–Corlette type I, II or III HC (in the absence of hepatic arterial or portal venous invasion) and major hepatectomy was performed in 45 patients with type III HC with hepatic arterial or portal venous invasion or type IV HC. The overall mortality and morbidity rates were 0% and 29.7%, respectively, and the bile leak rate was 1.4 %. Long-term survival was similar in the minor and major resection groups.[25]

In conclusion, the present study provides an overview of the treatment of HC patients in 2008. Curative resection for HC is associated with a high rate of R0 resection. However, surgery is associated with high levels of morbidity and mortality, despite intensive perioperative management.

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For *Drafting of manuscript:* JM. Regimbeau, D. Fuks;

For *Critical revision of manuscript:* JM. Regimbeau, O. Farges, D. Fuks;

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All authors agree with the submission and have given their final approval.

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ORIGINAL ARTICLE

Radical Surgery in the Presence of Biliary Metallic Stents: Revising the Palliative Scenario

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Abstract
Background The application of endobiliary self-expandable metallic stents (SEMS) is considered the palliative treatment of choice in patients with biliary obstruction in the setting of inoperable malignancies. In the presence of SEMS, however, radical surgery is the only curative option when the resectability status is revised in case of malignancies or for overcoming complications arising from their application in benign conditions that masquerade as inoperable tumours. The aim of our study was to report our surgical experience with patients who underwent an operation due to revision of the initial palliative approach, whilst they had already been treated with biliary SEMS exceeding the hilar bifurcation.
Methods Three patients with hilar cholangiocarcinoma that was considered inoperable and one patient with IgG4 autoimmune cholangio-pancreatopathy mimicking pancreatic cancer underwent radical resections in the presence of biliary SEMS.
Results After a detailed preoperative workup, two right trisectionectomies, one left extended hepatectomy and a radical extrahepatic biliary resection were performed. All cases demanded resection and reconstruction of the portal vein. R0 resection was achieved in all the malignant cases. Two patients required multiple biliodigestive anastomoses entailing three and seven bile ducts respectively. There was one perioperative death due to postoperative portal vein and hepatic artery thrombosis, whilst two patients developed grade III complications. At follow-up, one patient died at 13 months due to disease recurrence, whilst the remaining two are free of disease or symptoms at 21 and 12 months, respectively.
Conclusions Revising the initial palliative approach and operating in the setting of biliary metallic stents is extremely demanding and carries significant mortality and morbidity. Radical resection is the only option for offering cure in such complex cases, and this should only be attempted in advanced hepatopancreaticobiliary centres with active involvement in liver transplantation.

Keywords Self-expanding metallic stents · Cholangiocarcinoma · Bile duct · Hilar tumours · Hepatic confluence

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Introduction

The application of self-expanding metallic stents (SEMS) as palliative treatment of inoperable biliary and pancreatic malignancies is currently a widely established and accepted practice.[1] SEMS offer improved patency and significantly lower rates of obstruction compared with the plastic stents, and they are considered as the palliative method of choice for patients with a life expectancy of more than 6 months.[2,3] However, the disadvantage of occlusion due to in-growth or overgrowth phenomena does exist, whilst their rapid incorporation into the bile duct wall due to the foreign body reaction makes them almost irremovable.[4] So far, the only indication for

embarking in a surgical attempt in the setting of metallic stents' presence is either the revision of a "non-resectable" diagnosis for malignant entities, or the necessity for relieving the complications of the SEMS when they xare placed in benign cases mimicking inoperable neoplasms.[3–5] Scarce reports exist in the literature regarding the surgical experience and the challenges arising in the presence of biliary metallic stents. We report herein our experience in four patients within the last 2 years who were initially treated palliatively with uncovered SEMS as inoperable cases.

Materials and Methods

Between September 2007 and September 2009, 81patients with a diagnosis of inoperable cholangiocarcinoma (CCA) were assessed in the setting of our multidisciplinary hepatopancreaticobiliary (HPB) meetings for biliary malignancies. Seventeen patients were already treated by SEMS insertion elsewhere as inoperable hilar CCA, and they were referred for palliative treatment or potential enrolment in clinical trials. Diagnosis of inoperability was revised for three of these 17 patients following evaluation by our multidisciplinary team. The initial assessment precluding surgical resection was either extension of the tumour in second-order biliary radicles or portal vein involvement. Additionally, one more patient was referred due to multiple episodes of cholangitis in the presence of a SEMS inserted for palliation of metastatic pancreatic cancer with nodal involvement. However, final histology revealed IgG4 autoimmune cholangio-pancreatopathy (AICP).

Description of the Stents

In all cases, the proximal end of the SEMS was located above the biliary bifurcation. In two patients with cholangiocarcinoma, bilateral metallic stents were placed during the initial management. The third patient with CCA had a well-functioning solitary metallic stent draining the main right hepatic duct. The stent was placed after an exploratory laparotomy where the patient was judged as inoperable due to positive biopsies of the portal nodes. The fourth patient with autoimmune cholangio-pancreatopathy had a stent just passing the hepatic confluence proximally, but entering the duodenum distally (Table 1).

Description of Stents' Side Effects

The duration from the time of stenting until the time of operation was 5±8.5 months. Only one among these patients remained free of episodes of cholangitis after stent insertion, reaching the time of operation without any additional drainage intervention. The other three patients had all dysfunctioning stents which required endoscopic retrograde cholangiopancreatography (ERCP) for the removal of debris, ERCP twice plus percutaneous transhepatic drainage (PTD) and ERCP twice with placement of plastic stents through the metal ones plus PTD, respectively. The duration of primary stent patency was 45 and 92 days for the two patients with CCA who experienced cholangitis, whilst the first episode of stent obstruction in the patient with AICP developed at 213 days. Preoperative values of bilirubin were 67, 98, 12 and 16 μmol/L for the cases, respectively.

Preoperative Workup

All patients underwent a thorough preoperative evaluation with multi-detector contrast-enhanced computed tomography according to standardised liver and pancreatic protocols and/or magnetic resonance cholangiopancreatography. Repeat ERCP was performed in all cases for the assessment of pathology, definition of the proximal and distal stent level or as attempts to relieve jaundice prior to surgery. In order to achieve preoperative levels of bilirubin <50 μmol/L and treat segmental cholangitis as well, two patients required percutaneous transhepatic drainage.

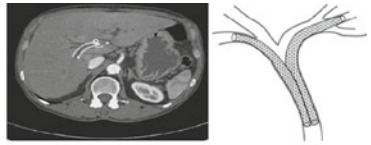
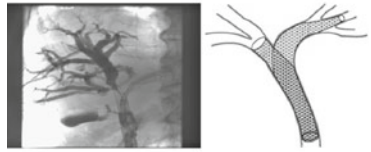
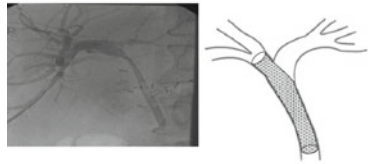
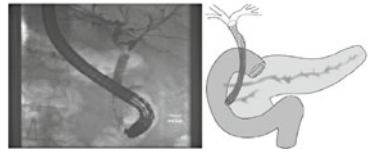
Additionally, positron emission tomography was performed in two CCA patients for the exclusion of any distant metastatic deposits. Based on CT volumetry, one patient underwent portal vein embolization prior to the planned extended right hepatectomy for inducing hypertrophy of the future left liver remnant.

Laparoscopic assessment of the abdominal cavity for disease dissemination was performed in two patients with CCA and also in the patient with the presumed pancreatic cancer.

Results

All patients with diagnosis of hilar cholangiocarcinoma underwent extended liver resections. According to the Brisbane classification, there were two right trisectionectomies and one left extended hepatectomy (SgI+middle hepatic vein).[6] The patient with AICP required extrahepatic bile duct resection and a high hilar hepaticojejunostomy entailing both the major ducts. All cases required portal vein resection and reconstruction either for securing negative resection margins, or due to technical demands arising from the dense fibrosis caused by the inflammatory response of the existing stent. However, no vein graft was used. Additionally, one patient (case 3) required resection and reconstruction of the right hepatic artery in combination with portal vein resection.

Table 1 Patient characteristics

Patients	Imaging and SEMS location	Type of resection and complications
Case 1: 58-year-old male CCA type IIIb, stents in situ 2,5 months, cholangitis free, Bil 67 μmol/L		R trisectionectomy (SgI+IV, V–VIII) 1HJ to 1 bile duct PV resection T3N0Mx, R0 Grade III complication (drainage of abdominal collection–wound infection)
Case 2: 73-year-old male CCA type IV, stents in situ 5 months, 1st cholangitis at 40 days, Bil 98 μmol/L		R trisectionectomy (SgI+IV, V–VIII) 1 HJ to 3 ducts PV+HA resection T3N1Mx, R0 Grade IV complication (PV+HA thrombosis, relaparotomy, MOF)
Case 3: 65-year-old male CCA type IV, stent in situ 5 months, 1st cholangitis at 3 months, Bil 12 μmol/L		Left extended hepatectomy(S1+MHV) 3HJs to 7 bile ducts PV resection T2N1Mx, R0 No complications
Case 4: 49-year-old male AICP, stent in situ 21 months, 1st cholangitis at 7 months, Bil 16 μmol/L		Extrahepatic bile duct resection Supra-pancreatic stent transection Hilar HJ to Rt+Lt bile ducts PV resection Grade III complication (PV thrombosis)

The stents were removed en bloc with the surgical specimen in two cases and in the remaining two, they had to be removed wire by wire due to extensive incorporation into the tissues. In the case of the patient with AICP, the distal part of the stent was cut at the level of the supra-pancreatic margin of the common bile duct (CBD) and remained in situ in order to avoid injury of the ampulla and the option of a pancreatoduodenectomy. The stump of the distal CBD was closed in a running fashion with Prolene 3/0. The number of the anastomosed bile ducts was one, three, seven and two, respectively. In all the cases of liver resections where multiple anastomoses were needed, every effort was made to approximate the exposed neighbouring subsegmental ducts into common channels for reducing the number of biliodigestive anastomoses. A fine-bore feeding catheter (4F) was used as stent of the anastomosis in two cases for minimising the risk of bile leak from high-risk reconstruction and this was externalised through the stump of the intestinal Roux-en-Y loop. The catheter was left in place for 35 and 42 days, respectively. Mean operative time was 8.58 h (range, 6.1–12 h), whilst the average operative blood loss was 2.7 U (range, 0–6 U). The number of frozen-section biopsies for the three malignant cases was three, five and nine, respectively. All the vascular resection margins were proven free of tumour invasion, and an R0 resection was achieved in all the malignant cases. Mean duration of hospital stay was 19 days (range, 9–27 days).

In histology, evidence of cholestasis was present in one case, whilst large duct obstruction and ascending cholangitis with abscess formation was prominent in the background liver of another patient (Fig. 1).

One patient with CCA died within the immediate postoperative period. He developed portal vein and hepatic artery thrombosis following a right trisectionectomy combined with portal vein and hepatic artery resection and reconstruction. Despite relaparotomy and embolectomy, he died on the ninth postoperative day due to multiple organ failure. There was only one patient with postoperative bile leak after liver resection, in combination with severe wound infection. He was treated with drainage of the collection under ultrasound guidance and long-term application of a vacuum-assisted closure pump. Additionally, the patient with AICP developed portal vein thrombosis and required long-term treatment with anticoagulants. In accordance with the classification for surgical complications of Dindo et al.,[7] there were 25% grade IV, 50% grade III and 25% grade II complications, respectively.

In follow-up, one patient with CCA died at 13 months due to disease recurrence, whilst the third one remained free of disease at 21 months after the operation. The patient with AICP remains free of cholangitis episodes in 12 months of follow-up, with evidence of cavernous transformation of the porta hepatis.

Discussion

Endobiliary self-expanding metallic stents are considered the management of choice for palliation of inoperable biliary and pancreatic malignancies.[1–9] However, in the setting of high volumes referral centres, the possibility of revising the initial diagnosis of irresectability or facing the complications of metallic stents inserted for benign conditions is indeed a reality and a challenging situation as well.

According to a systematic appraisal of the role of SEMS in the treatment of benign bile duct stricture, Siriwardana et al.[3] reported the need for operative removal of occluded metallic

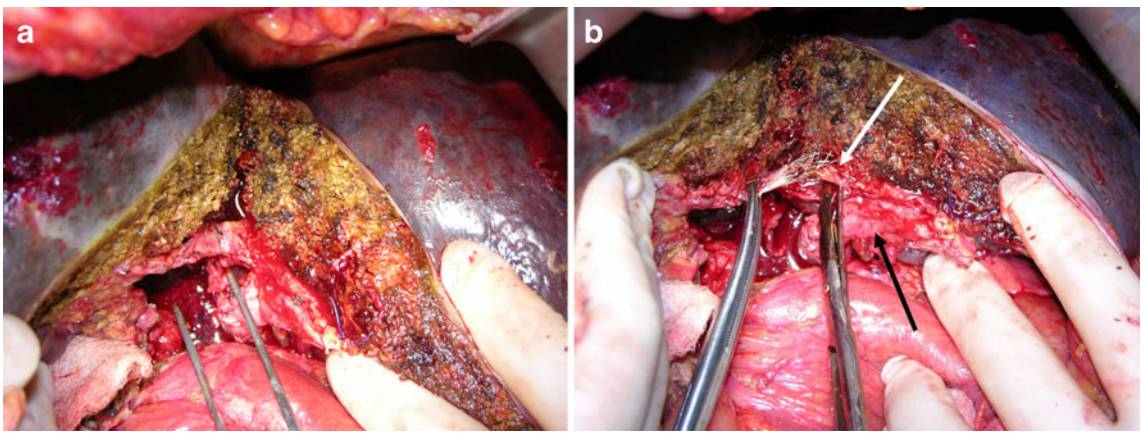


Fig. 1 **a**, **b** Right trisectionectomy (case 1). **a** "Parenchyma first" approach and complete exposure of the left Glissonian pedicle at the level of SgII–SgIII bile duct bifurcation. Metal stent (*white arrow*) within the left hepatic duct. **b** Transection of the left hepatic duct above the proximal end of the metal stent. SgII–III bile duct (*white arrow*), umbilical branch of left portal vein (*black arrow*)

stents in 9% among 400 cases due to complications not amenable to conservative endoscopic management. Additionally, Vibert et al.[8] has reported in five cases with benign disease where SEMS were inserted in the setting of a diagnosis which was later revised at a tertiary HPB institute.

A detailed multimodal imaging evaluation is the first and most important great importance, step in the initial assessment of these patients. We consider the most useful combination to be of CT/MRI compounded with biliary imaging and particularly percutaneous transhepatic cholangiography. However, overcoming the difficulties in imaging interpretation due to artefacts caused by the presence of SEMS is mandatory in order to attempt a surgical intervention in this setting.[10] Evaluating the proximal and distal extent of both the disease and the SEMS, as well as the potential vascular involvement in the radial axis as, is extremely challenging in the background of the inflammatory response caused by the SEMS. Lack of advanced imaging and interventional modalities, as well as HPB-focused radiologists and endoscopists in the referring institutes, might explain, in part, the primary palliative management of these cases. Referral biases may also influence the number of cases reaching a tertiary centre whilst still operable or before the development of complications due to the long-term presence of SEMS.

All our patients with a diagnosis of CCA were managed with extended liver resections in an effort to achieve both the mandatory clear margins from oncological perspective and to overcome the proximal level of the inserted stent as well. This necessity resulted in performing multiple biliodigestive anastomoses in two out of the three cases, entailing three and seven segmental ducts, respectively.

In all our cases, vascular resection and reconstruction was proven necessary since the dense fibrotic reaction at the porta hepatis and the hepatoduodenal ligament demanded meticulous sharp dissection of the stented biliary tree and radical resections of the adjacent portal vein. The combination of the desmoplastic reaction of the cholangiocarcinoma tumours and the inflammatory tissue response caused by SEMS, must be taken into account either for oncological reasons or due to the operative difficulties during the dissection of the common bile duct from major vascular structures. The rate of vascular resections in our series is significantly higher compared with that reported by Vibert et al. where only one among five cases demanded portal vein resection. The most likely explanation is the malignant nature of the pathology in most of our cases, in contrast with the benign entities faced by the previous group of authors. It could be speculated that, in our series, this fact resulted in higher vascular resection and reconstruction rates. In support of this, high rates of vascular resections up to 38%, have been reported by Mullen et al.[11] in pancreatoduodenectomies for pancreatic malignancies that were stented with metallic stents prior to the operation. Although the duration of SEMS' presence and the progressive incorporation of the stents into the tissues could be another predisposing factor to this, it was, however, substantially shorter (5 months in our series) compared with the one reported in the study by Vibert et al.

We did not find necessary the use of interposition grafts for portal vein reconstruction in our cases. Indeed, when performing a right trisectionectomy, the mobilisation of SgIV portal branch and the extensive mobilisation of the portal vein (PV) trunk, in addition to the mobilisation of the umbilical portion of the left PV at the groove of Rex, allows a long segment (up to 5–7 cm) for primary reconstruction in most cases. In the left extended hepatectomy, we similarly dissected the right PV bifurcation of the first and even second order. This allows resections of PV segments of at least 4 cm.

The true dilemma on top of the possibility of dissecting the vessel is the one relating to oncological safety. The incomplete reliability of frozen sections, the difficulty in sampling and the differential diagnosis between fibrotic reaction secondary to SEMS and carcinoma are the challenges in these cases. This has prompted our aggressive policy of vascular resections, which yielded satisfactory results.

A technical aspect of major importance is the quality of bile duct mucosa at the resection margin and the safety of the structured anastomosis. Although the ideal approach would be to achieve a resection margin beyond the limit of the stent, this was not always feasible. Instead of extending the margins of the parenchyma resection, risking the loss of functional liver volume, we applied the alternative of transecting the stent below its proximal end and removing the metal mesh wire by wire from the anastomotic biliary stump. This manoeuvre offers the advantage of avoiding excessive mucosa detachment during the stent extraction. Additionally, the resulting, stent-free, extra bile duct length can be resected with safety at the level of less inflammatory mucosa, promoting the quality of the anastomotic tissues.

There was one postoperative death in our series due to hepatic artery and portal vein thrombosis after extended right hepatectomy and combined vascular resection and reconstruction which required immediate relaparotomy. This occurred in the patient with the poorest stent function and also the highest preoperative bilirubin despite the efforts for relief of jaundice and cholangitis with ERCP and PTD prior to surgery. According to the histology report, there was evidence of ascending cholangitis and micro-abscess formation within the liver parenchyma at a distance from the tumour. This finding is in agreement with the reported high risk of postoperative complications in cases with hilar malignancies and unrelieved jaundice prior to resection. However, it might be argued that hepatic artery resection and unrelieved jaundice should be considered as absolute contraindications for any attempt in such complex

cases, especially if a right extended hepatectomy is the necessary type of resection.[12–14]

Operating in the presence of endobiliary metallic stents represents a highly demanding surgical situation. Although not common, the option of revising the initial diagnosis in the presence of SEMS is indeed a reality, which probably will become more frequent in the future due to centralization of the complex hepatobiliary cases in high-volume surgical centres. Although hospital and surgeon procedure volume are well known to be independent factors associated with improved outcomes in complex HPB surgery,[15,16] the importance of the available hospital clinical resources receives constantly more attention in the published literature. Joseph et al.[17] has highlighted recently the interdependent relationship between hospital volume and the available clinical support system in achieving superior results in the field of pancreatic resections.

We consider the exposure of the surgical team in liver transplantation and specifically in living-donor-related transplants (LDLT), equally important in the management of these complex cases. Nguyen et al.[18] demonstrated that volumes of liver transplant and partial hepatectomy procedures are strongly correlated with superior outcomes following liver resections for complex hepatocellular carcinoma cases. In accordance, surgical centres with extensive experience in living donor liver transplantation reported recently significantly improved results in 302 cases of hilar cholangiocarcinoma resected within a 7-year period, implementing the experience of LDLT in the surgical approach of these cases.[19]

The outcome of our case series might raise concerns regarding the benefit and the resource utilisation they demanded. However, despite the recent improvements in survival by non-surgical alternatives, R0 resection remains the mainstay among the therapeutic options with clear superiority regarding long-term outcome.[20,21] We believe that "centralising" these complex surgical cases to high-volume tertiary centres with a dedicated HPB multidisciplinary team, prior to the application of any palliative measures, might result in the reduction of their occurrence.

Our concept is that, in the setting of malignant entities, the presence of metallic stents does not alter the plan of the radical resections needed to achieve a therapeutic outcome. In contrast, their application in benign conditions mimicking inoperable neoplasms upgrades the surgical demands at the level of oncological surgery. Since a significant rate of complications should be anticipated, a detailed multidisciplinary approach at a high level of expertise and resources should be at the basis of the management algorithm. As surgical resection is the only option for curative treatment of such cases, delaying the placement of metallic stents prior to evaluation at a referral surgical centre is the only option for avoiding this scenario.

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ORIGINAL ARTICLE

Treatment of Acute Delayed Visceral Hemorrhage After Pancreatic Surgery From Hepatic Arteries with Covered Stents

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Abstract
Background Delayed visceral hemorrhage following pancreatic surgery is a rare but life-threatening complication. Usually hemorrhage originates from pseudoaneurysms secondary to pancreatic or biliary fistula. Re-laparotomy is often associated with high morbidity and mortality. Endovascular occlusion with metallic coils can stop pseudoaneurysmatic bleeding, but hepatic artery occlusion can result in severe organ damage. Interventional treatment with covered stents is an alternative providing persistent organ perfusion.
Results In our department endovascular stenting for visceral hemorrhage was introduced in November 2008. From November 2008 until October 2009, 303 patients underwent pancreatic surgery at our institution. Among those, four patients were successfully treated with covered stents for delayed visceral hemorrhage. In all four patients bleeding originated from hepatic arteries. Mean onset of hemorrhage was 24 days after surgery. Endovascular stenting was successful in all four patients. None of these patients required re-operation or died during the study.
Conclusion Treatment of delayed visceral hemorrhage from hepatic arteries after pancreatic surgery with covered stents is safe and effective. Endovascular stenting is associated with a lower morbidity than re-laparotomy or coil embolisation. Emergency angiography with endovascular stenting should be considered for all patients with delayed hemorrhage from hepatic arteries after pancreatic surgery.

Keywords Hemorrhage · Hepatic artery · Interventional treatment · Covered stent · Pancreatic surgery

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Background

Over the last two decades advances in surgical technique and improvement of perioperative management have reduced mortality after pancreatectomy in experienced centres clearly below 5%. However, morbidity after pancreatic surgery remains high, reaching 30–40%.[1–3] The most frequent complications include delayed gastric emptying, pancreatic fistula and abscess formation.[4,5] Hemorrhage is a less common, but life-threatening complication, that occurs in the early or the late post-operative phase. The frequency of post-operative hemorrhage varies among different series between 4% and 16%.[6,7]

Early post-operative hemorrhage usually originates from a non-secured vessel requiring re-laparotomy, while the management of delayed hemorrhage remains controversial. Delayed post-operative hemorrhage is defined as a bleeding episode that occurs at least 24 h after the index operation, requiring more than four packed red cells within 24 h.[8]

Delayed visceral hemorrhage after pancreatic surgery usually originates from pseudoaneurysms of main branches of the celiac arteries or the superior mesenteric artery. Usually, pseudoaneurysms form as a result of pancreatic or biliary fistula in combination with local abscess formation. The initial symptoms can be misleading, since patients often present with upper gastrointestinal bleeding in the late post-operative course or even after discharge from the hospital. Diagnostic procedures include endoscopy, abdominal ultrasound, CT scan, angiography and surgery. The rapid onset of massive hemorrhage is often the reason to perform an emergency re-laparotomy, although an identification of the bleeding source cannot always be achieved.

Endovascular embolisation of bleeding vessels with metallic coils is an alternative to surgery. Although coil embolisation achieves bleeding control in most cases, vascular occlusion of the hepatic artery can result in serious organ damage. Endovascular treatment with covered stents is favourable, as it combines the possibility of occlusion of the bleeding pseudoaneurysm with maintenance of organ perfusion.

Among the literature several studies reported on the management of delayed visceral hemorrhage after pancreatic surgery. Surgical treatment of late visceral hemorrhage is associated with high morbidity and mortality, while endovascular coil embolisation of the hepatic artery is often associated with further complications. Therefore alternative treatment options are warranted. Among the literature several case reports described successful application of covered stents for delayed visceral hemorrhage after pancreatic surgery, but only few studies included more than one patient.[9–12] Among these studies patients had different localisations of the side of hemorrhage. To our knowledge there is no study on the successful treatment of late visceral hemorrhage from the hepatic artery after pancreatic surgery with covered stents.

Patients and Methods

In our department endovascular treatment of post-operative hemorrhage with covered stents was initialised in November 2008. All patients undergoing pancreatic surgery from November 2008 until October 2009 were retrospectively reviewed for endovascular treatment with covered stents. Six patients were identified who underwent endovascular stenting with covered stents. Two patients were excluded from the analysis because they did not undergo angiography for acute hemorrhage. One patient underwent elective endovascular stenting of splenic artery pseudoaneurysm secondary to chronic pancreatitis. The other patient had a left renal artery pseudoaneurysm. Endovascular stent application was performed 3 months after pylorus preserving pancreatectomy for pancreatic cancer.

The remaining four patients all had emergency angiography for delayed visceral hemorrhage after pancreatic surgery. Of these patients medical records, radiological reports and images were retrospectively reviewed. All three patients who underwent resection had two flat silicon drains that were routinely placed close to the pancreatic and to the biliary anastomosis. Anastomotic leakage was diagnosed as high amylase level (>3 times the upper normal serum value) or bile contents in abdominal drains after five post-operative days. Delayed visceral hemorrhage was defined as bleeding episode more than 24 h after the index operation, including clinical impairment (tachycardia, hypotension and shock), requiring blood transfusion and further treatment for hemodynamic stabilisation.

Interventional Management

All patients had an emergency abdominal CT scan with the diagnosis of a great post-operative hemorrhage. After hemodynamic stabilisation emergency angiography using standard Seldinger technique via femoral access was performed in all four patients. One patient had severe celiac artery stenosis requiring additional angiographic sessions via brachial access. Abdominal, celiac and superior mesenteric artery angiography was performed. After identification of the side of hemorrhage the diameter of the bleeding artery was measured. The appropriate stent graft was chosen and then placed in the centre of the side of hemorrhage. All transcatheter arterial stent placement procedures were performed by an experienced radiologist.

Outcome Parameters

Successful endovascular stenting was defined as cessation of hemorrhage without further transfusion requirements, hemodynamic stabilisation and persistent organ perfusion. Hemodynamic stabilisation was ensured by regular heart rage <100 bpm and mean arterial blood pressure >70 mmHg. Mortality was defined as 30-day mortality after endovascular stenting. Major complications were defined as need for surgical intervention, pneumonia, hepatic failure or abscess formation, biliary fistula, myocardial infarction, stroke or any complication that prolonged in hospital stay.

Results

Patients` Characteristics

Among all 303 patients undergoing pancreatic surgery from November 2008 until October 2009 four patients met the inclusion criteria of acute hepatic artery hemorrhage. All patients were men with a mean age of 58 years. The indication

for surgery included two patients with chronic pancreatitis, one patient with pancreatic cancer and one patient with distal bile duct cancer. Three patients underwent pylorus preserving pancreatectomy. These three patients underwent standard lymphadenectomy excluding para-aortal lymph node dissection. One patient with chronic pancreatitis and suspicion of pancreatic cancer did not undergo surgery at our institution. He was transferred from an external hospital with late visceral hemorrhage 11 days after surgery. This patient underwent an attempt of pancreatic head resection, but during the operation a pancreatic head resection was considered impossible secondary to the severity of chronic inflammation (Demographic data; Table 1).

Bleeding Details

The mean onset of visceral hemorrhage was 24 days after surgery (range 11–36 days). The initial work up included abdominal CT scans for all patients. In two patients the CT scan identified acute hemorrhage from pseudoaneurysmatic transformation of the right hepatic artery (Fig. 1). The other CT scan showed acute bleeding from a pseudoaneurysm of the proper hepatic artery, while the last CT scan could not exactly localise the bleeding vessel. The mean hemoglobin value before angiography was 8.7 g/dl (range 7.7–9.4 g/dl). The mean transfusion requirement was four red packed cells (range 3–5). Mean intensive care unit stay was 2 days (range 1–3). In three patients initial signs of bleeding occurred after discharge from the hospital, while one patient was transferred to our institution with delayed visceral hemorrhage.

All patients who underwent resection showed infectious complications. One patient developed hepatic bilioma requiring CT drainage. Drainage fluid showed infection with *Enterococcus* and *Enterobacter*, requiring further antibiotic treatment. In another patient intra-operative bile duct cultures revealed *Klebsiella*. Post-operative wound infection grew the same bacteria, requiring isolation and antibiotic treatment. Bile duct cultures of the last patient showed infection with *Escherichia coli* requiring further antibiotic treatment. The patient who was transferred with visceral hemorrhage from the external hospital had no signs of infection on CT scan, although laboratory analysis revealed an augmented CRP value, as showed all other patients with late hemorrhage. The mean CRP value was 122 mg/l (range 96–142 mg/l), (Bleeding details; Table 2). No pancreatic fistula could be verified, but all four patients had their drainages removed before the onset of hemorrhage.

Angiographic Details

All patients had angiography via right femoral access. In all four patients the initial angiography was able to identify the bleeding source. Three patients showed bleeding from pseudoaneurysmatic transformation of the right hepatic artery. In two of these patients the right hepatic artery originated from the superior mesenteric artery (Figs. 2, 3 and 4). In one patient angiography revealed bleeding from the crossing of the proper hepatic artery with the gastroduodenal artery. This patient required angiography via brachial access secondary to a stenosis of the celiac arteries. Initially this patient underwent vascular occlusion of the distal gastroduodenal artery by coil embolisation. Stenting of the proper hepatic artery then resulted in complete occlusion of the pseudoaneurysmatic hemorrhage. Another covered stent (7/37 mm) was placed after dilation of the stenosis of the celiac arteries (Angiographic details; Table 3).

Outcome

Abdominal CT scans were performed in all patients 5 to 7 days after stent placement, showing regular hepatic perfusion without remaining pseudoaneurysms. Mean hospital stay after endovascular stenting was 13 days (range 9–23 days). None of the included patients developed serious complications or died during the study. One patient with wound infection required antibiotic treatment and isolation, but no further complication occurred. Three patients had clinical follow-up in hospital after discharge. Two patients had additional CT scans 2 and 11 months after stent placement showing regular hepatic perfusion through the covered stent.

Discussion

In experienced centres mortality after pancreatic surgery has significantly decreased over the last two decades. However,

Table 1 Demographic data

Patient	Age	Gender	Indication for surgery	Surgery	Histology
1	58	M	Chronic pancreatitis	Whipple procedure	Chronic pancreatitis
2	58	M	Pancreatic cancer	Whipple procedure	pT3, pN1(10/48), pM1 (hep)
3	35	M	Chronic pancreatitis	Exploration of the Pancreas with multiple biopsies	Chronic pancreatitis
4	79	M	Distal bile duct cancer	Whipple procedure	pT3, pN1 (15/38), pM0

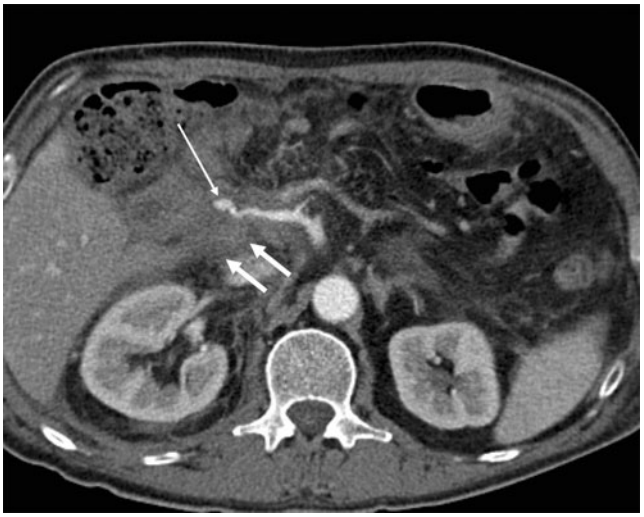

Fig. 1 Contrast CT scan shows a retroperitoneal hematoma (*large arrows*) secondary to hemorrhage from a *right* hepatic artery pseudoaneurysm (*small arrow*)

pancreatic surgery remains associated with high morbidity, even in centres of excellence. Although rare, delayed visceral hemorrhage is a life-threatening complication that requires immediate diagnostic workup. Since re-laparotomy is associated with high morbidity and mortality, minimal invasive treatment approaches, including endovascular coil embolisation and endovascular stenting, promise better results.

Successful coil embolisation for delayed visceral hemorrhage after pancreatic surgery has been reported by several groups.[13,14] Using endovascular coil embolisation, the bleeding can be successfully stopped, but vascular occlusion can result in severe organ damage, requiring further surgical treatment.[15]

Acute hemorrhage from the splenic artery can be managed by endovascular coil embolisation without severe morbidity, since spleen function is not required for survival in the early post-operative phase. Although endovascular stenting is favourable because organ function is preserved, pancreatic fistula often requires re-laparotomy with resection of the pancreatic remnant and the spleen.[16,17]

Occlusion of the hepatic artery should be avoided because an immediate interruption of normal organ perfusion can lead to liver cell necrosis, abscess formation, organ failure and impaired healing of the hepatico-jejunostomy, even if portal vein blood flow is maintained.[18–20] Although technically demanding, serious complications after endovascular stenting are less frequent.[9,21] Therefore the endovascular treatment with covered stents, especially for massive hepatic artery hemorrhage, offers several advantages.

Endovascular treatment uses an approach from a non-infected side. Even if emergency surgery can stop the bleeding, persistence of local infection can erode suture material and further bleeding episodes may occur. Theoretically stent graft material may also become colonised by micro-organisms leading to septic complications with need for stent removement. But to our knowledge there is not report on prosthetic infection after treatment with covered stents for delayed visceral hemorrhage.

Covered stents can be placed over the bleeding aneurysmatic neck of the injured vessel with hemodynamic stabilisation and persistent organ perfusion. Using this minimal invasive procedure emergency surgery can be avoided. After bleeding control, re-laparotomy can be performed in hemodynamically stable patients, if necessary. This is certainly one reason for the lower morbidity after interventional treatment.[21]

Patients undergoing endovascular treatment for delayed visceral hemorrhage usually require less blood transfusion. The mean transfusion requirements for patients with delayed visceral hemorrhage varies between 7.7 (range 3–12) and 12.5 (range 3–37) units of red packed cells for patients undergoing endovascular treatment and for patients undergoing surgery or endovascular procedures.[15,20] Among our series the mean hemoglobin at the onset of

Table 2 Bleeding details

Patient	Onset of hemorrhage	Abdominal CT scan	Hb (g/dl)	Tranfusion requirements	Bleeding after discharge	Infectious complication	Intensive care unit stay	CRP (mg/l)
1	Day 15	Pseudoaneurysmatic hemorrhage from the right hepatic artery	7.7	3 units of blood	Yes	Bacteribilia	1 day	139
2	Day 33	Pseudoaneurysmatic hemorrhage from the hepatic artery	9.4	5 units of blood	Yes	Bacteribilia, bilioma	2 days	142
3	Day 11	Hemorrhage of celiac axis	9.2	4 units of blood	No	No	1 day	112
4	Day 36	Pseudoaneurysmatic hemorrhage from the right hepatic artery	8.5	4 units of blood	Yes	Bacteribilia	3 days	96

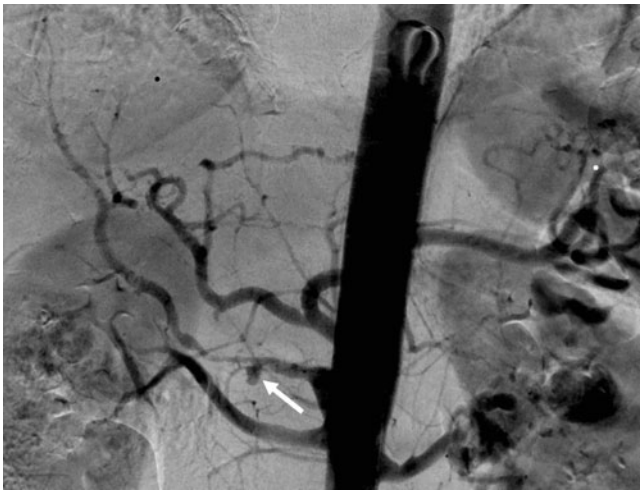

Fig. 2 Angiography of the abdominal aorta shows pseudoaneurysmatic bleeding from the *right* hepatic artery (*arrow*), which originates from the superior mesenteric artery

hemorrhage was 8.7 g/dl. The mean transfusion requirements was four red packed cells, while none of the included patients needed transfusion of platelets or fresh frozen plasma. The reason for the low number of transfusion requirements among our series might be the immediate success of emergency angiography in all four patients, while emergency surgery is probably responsible for the high transfusion requirements among other studies.

The aetiology of delayed visceral hemorrhage after pancreatic surgery is usually explained as a result of inflammatory erosion of the arterial vessel wall secondary to bile and pancreatic juice or local infection. Among our series we could not identify a pancreatic fistula, but the presence of pancreatic leakage cannot be excluded because all patients had their drainages already removed when hemorrhage occurred. One patient had post-operative CT drainage for hepatic bilioma before the onset of delayed visceral hemorrhage. Although the other patients did not show intra-abdominal abscess formation on CT scan prior to angiography, the mean CRP value was 122 mg/l, indicating local infection. Local bacteraemia in patients with malignancies can be affected by preoperative biliary drainage since bile duct stenting results in bacteribilia.[22] This is especially important if resistant bacteria are present. Among our series all three patients who underwent resection at our institution had undergone preoperative endoscopic biliary drainage, resulting in bile duct infection. In two patients intra-operative bile duct cultures grew resistant bacteria. The presence of these more virulent species might contribute to vascular vessel wall erosion with subsequent late visceral hemorrhage in these patients. It is remarkable that 50% (2/4) of patients had a replaced right hepatic artery. These vessels might have been more prone to injury during dissection. One study analysed this problem, but the authors did not find a higher postoperative morbidity in patients with a replaced right hepatic artery. In this analysis the number of included patients was only 135 which might have been too low to find a significant difference.[23]

Since healthy peri-vascular tissue is often removed in patients with pancreatic cancer during lymphadenectomy, these patients have a higher risk for visceral hemorrhage. Among the literature the frequency of post-operative hemorrhage after operations for malignant tumours is 3.2% vs. 1.9% after operations for benign diseases.[20] Among our series two patients had lymph node positive malignancies, while one patient underwent resection for

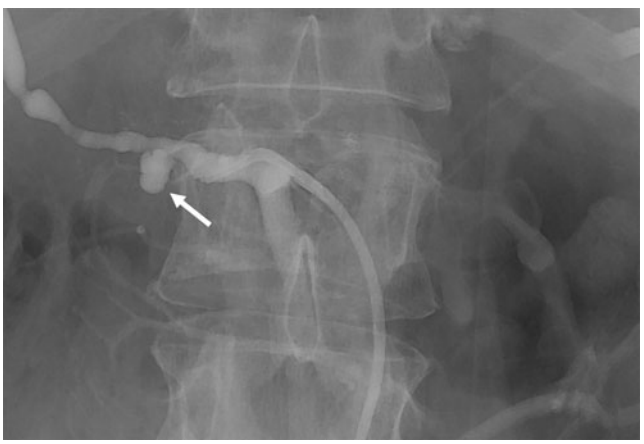

Fig. 3 Selective angiogram of the *right* hepatic artery shows extravasation of contrast medium from the pseudoaneurysm during angiography (*arrow*)

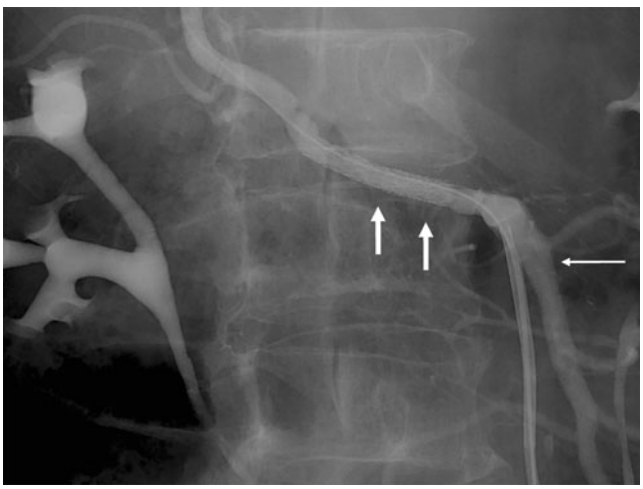

Fig. 4 Post-interventional angiography shows good flow through the covered stent (*large arrows*) without perfusion of the pseudoaneurysm or contrast medium extravasation. The *right* hepatic artery originates from the superior mesenteric artery (*small arrow*)

Table 3 Angiographic details

Patient	Number of angiographic sessions	Side of hemorrhage	Anatomic variation	Number of stents	Stent application	Hemodynamic stabilisation	Further intervention
1	1	Right hepatic artery	Right hepatic artery originating from superior mesenteric artery	1	5.5×15×80 mm	Yes	No
2	1	Right hepatic artery	No	1	5.5×38×80 mm	Yes	No
3	3	Proper hepatic artery/ gastroduodenal artery	Stenosis of the celiac axis	2	7×22 mm, 7×37 mm	Yes	Coil embolisation
4	1	Right hepatic artery	Right hepatic artery originating from superior mesenteric artery	2	7×38 mm, 6×38 mm	Yes	No

suspected pancreatic cancer. Final histology showed chronic pancreatitis including 36 lymph nodes. One patient developed pseudoaneurysmatic hemorrhage 11 days after an unsuccessful attempt of pancreatic head resection at an external institution with a bleeding pseudoaneurysm at the crossing of the gastroduodenal artery with the hepatic artery. The "early" onset of pseudoaneurysmatic bleeding suggests a different mechanism of pseudoaneurysm formation. All other three patients underwent resection and had their bleeding episode 28 days after surgery from true hepatic artery pseudoaneurysms. Among most other studies the mean time until the onset of delayed hemorrhage was 24 days after surgery.[9,20] Furthermore only the patient with the "early" visceral hemorrhage had sentinel bleed before the onset of major hemorrhage.[24]

Many studies analysing the therapy of post-operative hemorrhage after pancreatic surgery included patients who underwent re-laparotomy, coil embolisation or combination of both procedures as first line treatment. Studies on endovascular treatment with covered stents are less frequent and many patients underwent endovascular stenting after failure of emergency surgery.[15,20,21] Among our series emergency angiography was considered first line treatment. Only few other studies initially used covered stents to treat visceral hemorrhage. Hankins et al. and Pasklinsky et al. both included two patients with spleen and hepatic artery pseudoaneurysms, who underwent successful endovascular stenting after pancreatic surgery.[10,12] Rami et al. performed successful endovascular stenting of pseudoaneurysmatic bleed from the hepatic artery in four patients, but these patients did not suffer from hemorrhage after pancreatic surgery.[25] Heiss et al. included four patients after pancreatic resection. Although all patients underwent duodenohemipancreatectomy, one patient required additional re-laparotomy for persistent hemorrhage. Two patients underwent endovascular stenting for spleen artery pseudoaneurysm, one of them in a prophylactic manner without active hemorrhage.[9] Stoupis et al. included five patients who underwent endovascular stenting for gastroduodenal stump artery hemorrhage after pancreatic surgery. Although endovascular stenting was able to stop the bleeding in all five patients, three patients died between the second and the tenth post-interventional day.[11]

Although our data represent only a small series of selected patients, they show that interventional treatment with covered stents is safe and effective for patients with bleeding pseudoaneurysms of the hepatic artery after pancreatic surgery. Among our study emergency angiography was able to localise the hepatic artery as bleeding source and endovascular stenting was successful in all four patients. None of the included patients required emergency re-laparotomy. Post-interventional CT scans revealed regular hepatic perfusion through the covered stent without pseudoaneurysms in all four patients. Until April 2010 none of the included patients required further interventional treatment or surgery.

Conclusion

The management of delayed hemorrhage after pancreatic surgery from the hepatic artery should include the treatment with covered stents. Endovascular stenting is safe and effective. The morbidity and the mortality after endovascular stent application is low compared to coil embolisation or surgery. Therefore emergency angiography should be considered in every patient with delayed visceral hemorrhage from the hepatic artery. Further investigations should also include long-term results.

Conflicts of Interest No conflicts of interest are to be stated by the authors. All authors certify that they do not have any commercial association conflicting with the manuscript presented.

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ORIGINAL ARTICLE

High-Throughput Mutation Profiling in Intraductal Papillary Mucinous Neoplasm (IPMN)

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Abstract
Background Specific mutations leading to the development of various histological grades of intraductal papillary mucinous neoplasm (IPMN) have been partially characterized.
Methods Analysis of 323 oncogenic mutations in 22 tumor-related genes was conducted, using a chip-based matrix-assisted laser desorption time-of-flight mass spectrometer of DNA extracted from microdissected cells of low-grade (n=14), borderline (n=6), and invasive IPMN (n=7). Additional assays were performed on the DNA extracted from dyplastic cells found in the background of the adenocarcinoma.
Results We identified 9 K-ras mutations (low grade, 2/14; borderline, 1/6; invasive, 6/7), 3 p53 mutations (low grade, 1/14; invasive 2/7), and 2 PIK3CA mutations (low grade, 1/14; invasive, 1/7). K-ras, p53, and PIK3CA mutations present in the invasive cancer were absent in the adjacent precursor cells in 50% of the cases. In one patient, K-ras mutation was present in the precursor lesion and absent in the adjacent invasive lesion.
Conclusions Of the 22 screened tumor-related genes, only K-ras, p53, and PIK3CA mutations were found in IPMN. K-ras mutations are more prevalent in invasive than premalignant IPMN. The variable coexistence of mutations in the invasive cancer and in the adjacent precursor cells may point to the heterogeneous nature of this tumor.

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Keywords IPMN · Mutations · High throughput

Introduction

Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is a relatively new clinicopathological entity, first reported in 1982 by Ohhashi et al.[1] and recently introduced into the World Health Association (WHO) classification.[2] These tumors are characterized by papillary projections of duct epithelium, mucin production, and dilatation of the pancreatic duct. Histologically, IPMN is distinguished by replacement of normal ductal epithelium by mucinous metaplasia as well as a broad spectrum of pathological disorders, including simple hyperplasia (adenoma), cell atypia (borderline tumor), carcinoma in situ, and invasive adenocarcinoma that displays invasion of malignant cells into the pancreatic tissue surrounding the ducts. At the time of diagnosis, approximately 40–60% of the tumors have a component of invasive adenocarcinoma.[3–5] In addition, IPMN is found in the background of 10% of pancreatic

adenocarcinoma cases, suggesting that it had been the initial lesion leading to cancer in these patients.[6]

The histological variety of IPMN and the presence of premalignant dysplastic cells in the pancreatic tissue surrounding the invasive component are an excellent system in which to study the genetic alterations involved in tumor development. The specific mutations leading to the development of various histological grades of IPMN (hyperplasia, atypia, invasive cancer) have been partially characterized in previous studies. Reported genetic alterations identified in IPMN include mutations in K-ras,[7] PIK3CA,[8] and BRAF[9] genes and overexpression of the TP53 and ERBB2 proteins.[10,11] The role and the timing of specific oncogenic mutations in the gradual progression of adenoma to carcinoma in IPMN, however, have not been clearly elucidated. Attempts to characterize the oncogenic mutations in the different grades of IPMN within the same tumor by using techniques of tissue microdissection[12,13] have demonstrated early polyclonal epithelia gradually replaced by monoclonal neoplastic cells and gaining K-ras mutations as the tumor progresses.

The aim of our current study was to explore the genetic alterations responsible for tumor development in IPMN by screening the different histological grades of IPMN for the presence of oncogenic mutations and by evaluating whether genetic alterations present in invasive IPMN coexist in the adjacent precancerous cells. Towards this end, we based our methods on the hypothesis that gain-of-function mutations do not occur randomly in most known oncogenes and tumor suppressor genes. Instead, changes affecting a small number of "hotspot" codons often account for the majority of somatic mutations. Therefore, a limited number of genetic assays could effectively deal with a large proportion of known mutations. A high-throughput genotyping analysis could provide an effective means to screen for major known cancer mutations in IPMN tissue. Here, we performed a high-throughput analysis of multiple oncogenic mutations in the different histological grades of IPMN using a chip-based matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometer (Sequenom, San Diego, CA, USA).

Materials and Methods

Patients and Tissue Samples

We used a prospective database of all patients identified as having IPMN in our institutional database between February 2002 and May 2008. Additionally, all pathological results of pancreatic resections performed in our department from 1995 to 2002 (350 specimens) were reviewed. These latter patient data were derived from pathology reports and therefore subject to the pathologist's diligence. We are aware that some IPMN cases that were operated before February 2002 were probably missed and therefore not included in our analysis. A pathologist experienced in pancreatic pathology (E.B.) reviewed the histological slides in all cases suspected of being IPMN according to the pathological report. The selected cases were reviewed again by another pathologist (S.M.), and following confirmation of a diagnosis of IPMN, the specimens were entered into the study. Histological typing of the tumors was performed according to the classification recommended in the revised WHO classification in 2000[2] as having tall, columnar, mucin-containing epithelium with or without papillary proliferations and involving the pancreatic ducts. Tumors were graded as low-, moderate-, and high-grade dysplasia and as invasive carcinomas. High-grade dysplasia was differentiated from invasive carcinoma according to the presence of stromal invasion.

DNA Samples

Paraffin-embedded tumor samples were reviewed by one pathologist (S.M.) who marked tissue margins of invasive and noninvasive tumor and also confirmed that more than 90% of cells in the marked area were of tumor origin. Microdissection was then performed by hand. Eight micrometer-thick sections were cut from the paraffin-embedded tumor block and transferred onto glass slides, one of which was stained with hematoxylin and eosin, and the tissue margins were marked. The tumor within the marked margins was carefully removed by means of a sharp scalpel. The retrieved material was then transferred in a 1.5-mL Eppendorf tube. Eight to 12 sections were harvested for each case. Deparaffinization was performed using xylene. Genomic DNA was extracted using a classical DNA extraction technique.[14]

Selection of Oncogenic Mutations

Selection of oncogenic mutations was based on the methods of Thomas et al.[15] who designed a245 genotyping assays of 238 somatic mutations in 17 human oncogenes. We added more mutations after we conducted searches of two databases of known somatic oncogenic mutations, Cosmic (catalogue of somatic mutations in cancer) and PubMed. The resulting list (supplementary Table 1) contained 323 genotyping assays of known somatic mutations involving 22 human oncogenes and tumor suppressor genes. Mutations with high prevalence in other cancers (e.g., P53, BRAF, K-ras family mutations) and genes with proven clinical implications (e.g., *KIT* and *EGFR*) were given preference.

Table 1 Clinical and molecular findings in patients with intraductal papillary mucinous neoplasm of the pancreas

Case	Sex	Age (years)	Grade	Differentiation of cancer	Operation	Surgical margin	Outcome	F/U interval (months)	K-ras	p53	PIK3CA
1	F	80	Low		Distal pancreatectomy	LG IPMN	NED	48			
2	M	65	Low		Whipple operation	LG IPMN	Rec LG IPMN	49			
3	M	61	Moderate		Total pancreatectomy	NR	NED	48			
4	F	74	Low		Total pancreatectomy	NR	NED	24			
5	M	57	Low		Whipple operation	IPMN	NED	21			
6	F	70	Low		Total pancreatectomy	NR	NED	27			
7	M	71	Invasive	Poor	Whipple operation	Normal	NED	35	G12V		
8	F	59	Invasive	Poor	Whipple operation	Normal	Rec invasive cancer	34	G12V	R248Q	
9	F	53	Low		Total pancreatectomy	NR	NED	6			
10	M	66	Invasive	Poor	Whipple operation	Normal	Deceased d/t dis	9	G12V		
11	M	69	Low		Distal pancreatectomy	Normal	NED	13			
12	F	73	Low		Total pancreatectomy	NR	Deceased w/o dis	8		C176Y	H1047R
13	M	77	Invasive	Moderate	Whipple operation	Normal	Deceased d/t dis	9	G12V		
14	F	69	Invasive	Well	Whipple operation	LG IPMN	NED	29	G12V		
15	M	83	Invasive	Poor	Total pancreatectomy	NR	Deceased d/t dis	13			H1047R
16	M	58	Moderate		Total pancreatectomy	NR	Perioperative death	0			
17	M	57	Moderate		Whipple operation	Normal	NED	72	G12V		
18	F	79	Moderate		Whipple operation	Normal	NED	98			
19	F	71	Low		Total pancreatectomy	NR	NED	44	G12V		
20	F	77	Low		Distal pancreatectomy	Normal	NED	25			
21	F	70	Low		Total pancreatectomy	NR	NED	33			
22	F	79	Invasive	Poor	Whipple operation	Normal	Deceased d/t dis	6	G12D	R248Q H179R	
23	F	64	Low		Distal pancreatectomy	Normal	NED	27	G12V		
24	M	66	Low		Whipple operation	Normal	NED	78			
25	F	78	Moderate		Distal pancreatectomy	LG IPMN	Rec LG IPMN	84			
26	F	74	Low		Whipple operation	Normal	NED	62			
27	M	49	Moderate		Whipple operation	Normal	NED	90			
27	M	49	Moderate		Whipple operation	Normal	NED	90			

F female, *M* male, *LG* low grade, *NR* not relevant, *NED* no evidence of disease

MALDI-TOF Assay Outline

Genotyping assays were designed using the Sequenom MassARRAY Assay Design 3.0 software with a maximum of six multiplexed assays per well. Assays were designed manually for complex mutations. Specific primers flanking the mutation site and extension primers that bind adjacent to the mutation site were designed. A primer extension reaction was carried out following the amplification of the region of interest. The extension reaction included sequence-specific hybridization and sequence-dependent termination that generated different products for the mutated and wild-type alleles, each with its unique mass values. Genotyping was terminated by spotting the extension products onto silicone chips preloaded with proprietary matrices (SpectroChip; Sequenom) that were subsequently read by the MALDI-TOF mass spectrometer (Figs. 1, 2, 3, and 4).

Mass Spectrometric Genotyping

PCR amplifications were carried out in standard 384-well plates in a 5-μL final volume containing 40 ng of template DNA, 0.1 U of Taq polymerase (Hotstar Taq, Quiagen, Valencia, CA, USA), 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 200 nmol of each primer, 1 mM $MgCl_2$, and Hotstar buffer. The primers are listed in Supplementary Table 2. The PCR thermal cycling was carried out in an ABI-9700 instrument with the following conditions: 15 min at 95°C, 30 s at 65°C, and 60 s at 72°C; and 38 cycles of 20 s at 95°C, 30 s at 53°C, and 60 s at 72°C. The PCR products were incubated with shrimp alkaline phosphatase (0.3 U in a total volume of 7 μL; 20 min at 37°C and 5 min at 85°C) in order to remove the non-incorporated dNTPs. The massEXTEND® (Sequenom) analysis was then conducted in a total volume of 9 μL containing 1 μL extension primer (Supplementary Table 2), 0.2 μL termination mix (list of specific termination mix for each assay in Supplementary Table 2), and 1.25 U ThermoSequenase (Sequenom) in 0.22× PCR buffer. The cycling conditions were: incubation for 2 min at 94°C followed by 99 cycles of 5 s at 94°C, 5 at 52°C, and 5 s at 72°C. Following this step, 6 mg of massEXTEND® cleanup resin (Sequenom) and 25 μL of H_2O were added to remove extraneous salts. A Samsung nanodispenser was used to apply 15 nL of the extension products from each well of the sample plate onto the SpectroChips. Mass spectra were recorded on a Bruker Biflex MALDI-TOF mass spectrometer operated on the linear mode and finally analyzed by MassARRAY Typer Software (Sequenom).

Additional Assays

All assays found positive for mutation in the multiplex assay were repeated using a singleplex assay for the identified mutation. Singleplex assays for mutations found in adenocarcinoma specimens were also performed on the DNA extracted from microdissected IPMN dysplastic cells found in the background of the adenocarcinoma. The protocol followed for the singleplex assay was similar to that of the multiplex assay, with different reagent concentrations for the first PCR reaction (supplementary Table 2). Three milligrams of massEXTEND® cleanup resin (Sequenom) was used with 16 μL of H_2O for salt removal.

Sequencing of K-ras Oncogene

Sequencing of the K-ras exon 2 was performed in 18 specimens in order to verify the results of the Sequenom assays. Following DNA extraction, genomic DNA (60 ng per sample) was amplified with primers covering the

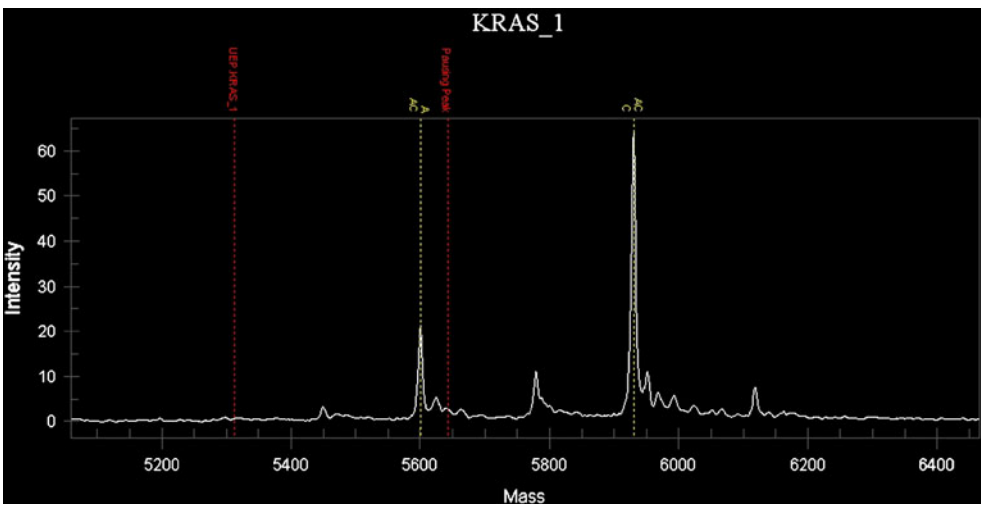

Fig. 1 MALDI-TOF verification singleplex assay demonstrating concurrent wild-type and mutant K-ras (G12V mutation)

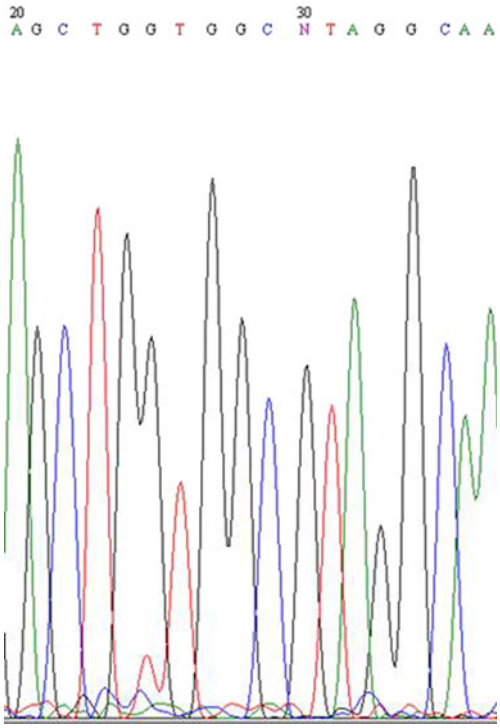

Fig. 2 Direct sequencing of the same patient of Fig. 1 demonstrating concurrent wild-type and mutant K-ras (G12V mutation)

coding region and exon/intron border. DNA sequencing was performed with ABI's automated sequencer at a commercial laboratory. The results were blindly compared with the results obtained from the Sequenom assays.

Ethical Considerations

The study protocol was approved by the Human Ethics Review Committee of the Ministry of Health and of the Tel Aviv Sourasky Medical Center.

Results

The 323 assays of 22 tumor-related genes were performed on 27 specimens, including 14 low-grade IPMNs, 6 moderate-grade IPMNs, and 7 invasive cancers arising on the background of IPMN. The clinical and molecular findings are summarized in Table 1. Additional singleplex assays of 2 K-ras mutations in codon 12 (G12D, G12V) were performed in another 7 specimens of invasive IPMN.

K-ras Mutations

K-ras mutations in codon 12 were found in 2 of 14 patients with low-grade IPMN (14.3%), in 1 of 6 patients with moderate-grade IPMN (14.3%), and in 6 of 7 patients with invasive IPMN (85.7%). No other mutations in K-ras oncogene were found. The two singleplex K-ras codon 12 mutation assays performed on two additional specimens of invasive IPMN revealed another two mutations. In the eight patients with invasive IPMN and a K-ras mutation, analysis of the background noninvasive IPMN demonstrated the same K-ras mutation in four patients, and no K-ras mutation in the other four patients (Table 2). A single patient with invasive carcinoma negative for K-ras mutation had a codon 12 mutation in the background IPMN. The other five patients with invasive IPMN and no K-ras mutation had no mutation in the background IPMN. The Sequenom results were fully matched with the direct sequencing results, including six assays with K-ras mutations and seven assays negative for K-ras mutations.

P53 Mutations

P53 mutations were found in three patients, including two patients with invasive IPMN (28%) and one patient with low-grade IPMN (5%). One patient with invasive IPMN

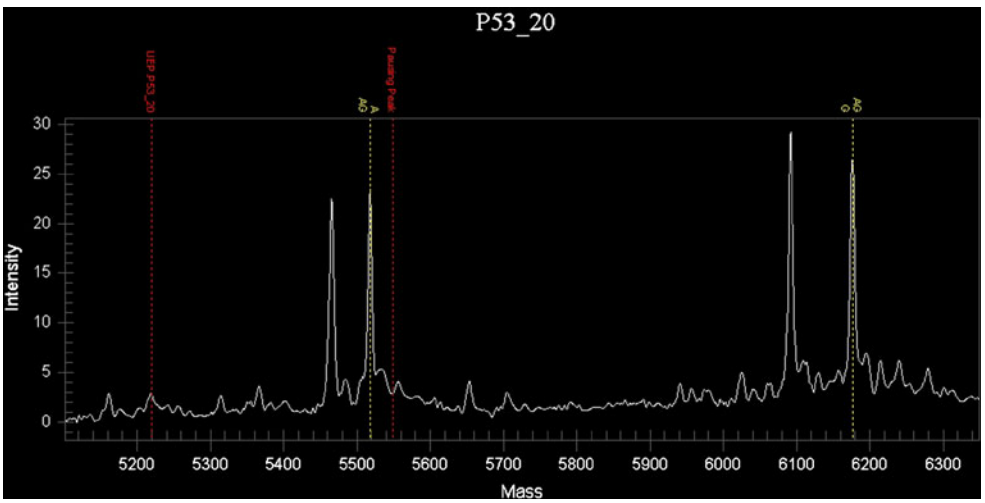

Fig. 3 MALDI-TOF multiplex assay demonstrating concurrent wild-type and mutant p53 mutation (R248Q)

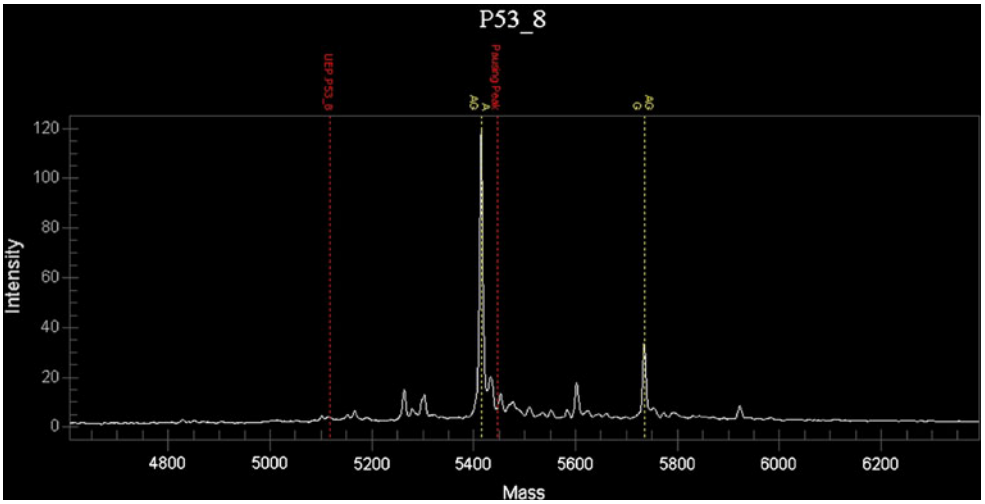

Fig. 4 MALDI-TOF singleplex assay demonstrating concurrent wild-type and mutant p53 mutation (H179R)

had two mutations in the p53 gene. Analysis of the DNA from the adjacent noninvasive IPMN cells demonstrated the presence of one of these mutations (p53_20), but not the other (p53_8). The other patient with invasive IPNM had the same p53 mutation (p53_20) present in the background noninvasive IPMN (Table 2). The patient with noninvasive IPMN and p53 mutations had additional mutation in the PIK3CA gene.

PIK3CA Mutations

PIK3CA mutations were found in two patients, one of whom had noninvasive IPMN and another who had invasive IPMN. The patient with noninvasive IPMN had additional mutation in the p53 gene. Analysis of the DNA from the adjacent noninvasive IPMN cells failed to demonstrate any PIK3CA mutation in the patient with invasive IPMN (Table 2).

Patient Outcome

The relevant clinical data and long-term outcome of the patients are listed in Table 1. All patients with invasive cancer had at least one mutation in either K-ras (n=6), PIK3CA (n=1), or p53 (n=2) genes. Only patients with K-ras mutations and no additional or other mutations experienced long-term disease-free survival. Five of the patients had positive surgical margins, all five with benign IPMN. The pathology of the main specimen of these patients was low-grade IPMN (n=3), borderline IPMN (n=1), and malignant IPMN (n=1). Two of these patients experienced recurrence of IPMN and both had recurrence of noninvasive IPMN. Both of these patients did not have an oncogenic mutation. No clear clinical–molecular correlations could be demonstrated, possibly due to the small number of patients included in this study.

Discussion

We performed a high-throughput analysis of 323 hotspot mutations in 22 tumor-related genes in order to characterize the specific mutations in various grades of IPMN. Much of the comparative analysis depends on tumor differentiation determined by microscopic evaluation, which is subjective and user-dependent. We attempted to minimize this drawback by ensuring that all slides were re-evaluated by two independent pathologists who confirmed the diagnosis of IPMN as well as the specific grades of all the included slides. We identified 9 K-ras mutations (low grade in 2/14, borderline in 1/6, and invasive in 6/7), 3 p53 mutations (low grade in 1/14 and invasive in 2/7), and 2 PIK3CA mutations (low grade in 1/14 and invasive in 1/7). We based our mutation screening assays on a recently described MALDI-TOF-based assay of 238 mutations,[15] to which we added 85 other mutations. The assay involves PCR amplification of the region containing

Table 2 Mutations in malignant intraductal papillary mucinous neoplasm (IPMN) and adjacent noninvasive IPMN

Mutation	IPMN (−) carcinoma (±)	IPMN (±) carcinoma (−)	IPMN (±) carcinoma (±)
Kras_G12V	2	1	3
Kras_G12D	2	0	1
P53_H179R	1	0	0
P53_R248Q	0	0	2
PIK3CA_H1047R	1	0	0

the mutation, a primer extension reaction through the mutation site, generation of allele-specific extension products, and analysis using the MALDI-TOF mass spectrometer. This assay was compared to both Sanger sequencing and a highly sensitive pyrosequencing by a synthesis method[15] for EGFR and K-ras mutations and was proved to be highly sensitive for mutation detection. In previous studies, we have shown that this method is capable of mutant detection down to 1% mutant allele for JAK2 mutations,[16] and BCR ABL kinase domain mutations.[17] In the current study, we also verified the assay's results for the K-ras mutations using direct sequencing, and all assays that were found to be positive for mutations were repeated using singleplex assays.

The progression of pancreatic adenocarcinoma is strongly associated with the presence of K-ras mutations found in close to 100% of lesions.[18–20] K-ras mutations have been shown to occur at a relatively early stage of carcinogenesis in the pancreas.[21,22] The frequency of K-ras mutations in pancreatic adenocarcinoma evolving from IPMN is lower, reportedly between 14% and 86%.[7,23–25] We found K-ras mutations significantly less frequently in early lesions than in invasive IPMN cancers. It is hypothesized that IPMN is a precursor lesion of pancreatic carcinoma and that a sequenced grade-continuum from low-grade dysplasia exists, leading eventually to invasive cancer development. To date, there are no reliable tools to enable a clear identification of invasive component or to predict the time frame for cancer transformation in IPMN patients. This results in delayed cancer diagnoses as well as unnecessary operations performed for noninvasive benign tumors. According to the consensus guidelines, patients with small asymptomatic branch type IPMN are not offered surgery due to the low risk of having invasive cancer.[26] However, the long-term outcome of these patients is unknown, and some of them may eventually develop invasive cancer. Moreover, a reliable surveillance protocol to diagnose early cancer or imminent transformation within IPMN does not exist. Previous studies have demonstrated that the presence of K-ras mutation in IPMN cyst fluid does not assist in distinguishing benign from malignant IPMN.[27] Our data demonstrate that most patients with invasive IPMN have a K-ras mutation, whereas only 15% of patients with benign IPMN patients have this mutation. The p53 and PIK3CA mutations were also more common in invasive IPMN. This may imply that patients that have noninvasive IPMN with K-ras mutation (and perhaps p53 and PIK3CA mutations as well) will develop invasive cancer faster than patients without these mutations. More studies are needed to evaluate whether the presence of specific oncogenic mutations is associated with higher likelihood for future malignant transformation in patients with small, branch duct IPMN treated conservatively according to current guidelines.

Higher rates of K-ras mutations were found in studies that examined multiple sections from the same subject,[7,23] possibly pointing to a heterogeneous nature of IPMN cells. Analysis of the invasive cancer cells and the adjacent noninvasive IPMN tissue in our patients with invasive IPMN demonstrated a lack of uniformity of the mutational status of the K-ras, PIK3CA, and p53 genes (Table 2). These results may also be explained by a heterogeneous precursor lesion that contains different clones of neoplastic cells, only some of which continue and develop into malignant cells as they acquire additional mutations. These results are different from those of a previous report by Wada et al.,[12] which showed an identical K-ras sequence in the precursor and invasive lesions in most cases of IPMN.

Much attention has been recently given to the significance of the PIK3CA gene mutations identified in several human cancers.[13] Mutations usually occur in exons 9 and 20, affecting functionally important domains of this protein.[13] While mutations in the PIK3CA gene are not infrequent in several types of cancers, e.g., colon, gastric, breast, brain, ovarian, and lung,[13,28–30] no mutations have been described in pancreatic cancer, and a negative finding was reported in at least two studies.[13,31] In contrast, Schönleben et al.[8] reported that 11% (4/36) of the IPMNs they evaluated had PIK3CA mutations. One of these mutations was a previously described missense hotspot mutation in exon 20 H1047R,[13] and the others were novel mutations. They observed that mutations seemed to be a rather late event in the development of IPMN cancer. They found mutations in borderline tumor (n=1), in situ cancer (n=1), and invasive cancer (n=2). The H1047R mutation was found in invasive cancer. This is the first gene that had been found to be mutated in IPMN but not reported in ductal adenocarcinoma. In the present study, two of the 27 specimens contained an H1047R mutation of the PIK3CA gene. This mutation was found in one patient with invasive cancer and another patient with low-grade IPMN (which also had a p53 mutation). This finding is compatible with that of the previous study[8] and confirms a 10% mutation rate for the PIK3CA gene in IPMN.

Several studies have demonstrated overexpression of p53 among IPMNs, with an increasing expression level during progression from adenoma to invasive cancer.[32–35] Abe et al.[34] reported overexpression of p53 on immunohistochemical analysis in 0% of cases in low-grade adenoma and in 23% of cases of carcinoma in situ. This was significantly lower than the overexpression rate in PanIN lesions. Sasaki et al.[35] reported nuclear p53 expression in 38% of invasive IPMN tissue, but not in premalignant low-grade or borderline tissues. The profile of specific p53 mutations and their role in the carcinogenesis of IPMN have not been studied. We found p53 mutations in

one patient with low-grade IPMN and in two patients with invasive IPMN, one of whom had two concurrent mutations. Both patients with invasive IPMN had the hotspot R248Q mutation, which is the most common p53 mutation reported in human cancer, and the second most common mutation in pancreatic cancer (Universal Mutation Database, www.umd.necker.fr). Interestingly, the same mutation was found in the adjacent nonmalignant IPMN in both of these cases. The R248Q mutation was present while the other (H179R) was not present in the adjacent premalignant tissue in the patient with two p53 mutations. This may point to a dominant role of the specific R248Q mutation in IPMN tumor evolution. Larger studies are needed to explore the possible role of specific p53 mutations on tumor evolution in IPMN, as well as their prognostic significance.

In summary, the MALDI-TOF-based assay is an effective means by which to screen neoplastic tissue for a large number of oncogenic mutations. Of the 22 screened tumor-related genes, only K-ras, p53, and PIK3CA mutations were found in IPMN. Oncogenic mutations, especially in K-ras, are significantly more prevalent in invasive IPMN than in low-grade and borderline IPMN. The variable existence of mutations present in the invasive cancer in the adjacent precursor cells and the presence of a K-ras mutation in the precursor lesion but not in the adjacent malignancy in one patient may point to a heterogeneous nature of this tumor. More studies are needed to determine the role of specific oncogenic mutations as molecular markers for future progression in non-resected tumors and their value as prognostic markers in resected tumors.

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ORIGINAL ARTICLE

Peripancreatic Fat Invasion Is an Independent Predictor of Poor Outcome Following Pancreaticoduodenectomy for Pancreatic Ductal Adenocarcinoma

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Abstract
Background Following pancreaticoduodenectomy for pancreatic ductal adenocarcinoma (PDAC), identification of peripancreatic fat tumor invasion promotes a tumor to stage T3. We sought to understand better the impact of histological peripancreatic fat invasion on prognosis and site of recurrence in a cohort of patients with PDAC.
Methods We analyzed the patient demographics, outcome, and recurrence data that had been prospectively collected in 189 consecutive PDAC undergoing potentially curative pancreaticoduodenectomy between 1996 and 2009. Pathological features were reassessed for all patients. Survival outcome was compared using Kaplan–Meier/Cox proportional hazards analysis. The primary site of recurrence was defined as either locoregional or distant metastases.
Results The median survival of this PDAC cohort was 18.9 months (95% confidence interval (CI) 15.7–22.2). Histological peripancreatic fat invasion was evident in 51 (27%) patients and was associated with lymph node metastases ($p=0.004$) and larger tumor size ($p=0.015$). The presence of peripancreatic fat invasion was associated with reduced overall survival following resection (12.4 months [95% CI 9.9–15.0]) when compared to those patients with no evidence of fat invasion (22.6 months [95% CI 18.5–26.7]; $p<0.0001$). By multivariate survival analysis, independent predictors of overall survival included tumor grade ($p=0.002$), lymph node involvement ($p=0.025$), resection margin status ($p=0.003$), venous invasion ($p=0.045$), and peripancreatic fat invasion ($p=0.007$). Invasion into the pancreatic fat was significantly associated with the primary site of recurrence being locoregional failure ($p=0.002$).
Conclusions Peripancreatic fat invasion was identified as being an independent predictor of poor outcome following pancreaticoduodenectomy for PDAC. Additionally, the presence of peripancreatic fat invasion was associated with locoregional disease as the primary site of recurrence. This may have implications for the staging of PDAC and potentially require incorporation into future staging systems to improve outcome stratification.

Keywords Pancreatic cancer · Peripancreatic fat invasion · Pancreaticoduodenectomy · Prognosis

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is undoubtedly one of the most lethal and aggressive of all solid malignancies. This disease is characterized by early locoregional spread and distant metastasis, and as a result, most tumors (85%) are unresectable at the time of diagnosis. Even for patients with localized, surgically resectable disease, long-term survival

after treatment for pancreatic adenocarcinoma is poor with 5–10% 5-year survival in even the largest tertiary referral centers.[1]

It is accepted that various pathological factors including tumor grade, tumor size, lymph node status, perineural invasion, and resection margin involvement influence outcome following PDAC resection.[2–7] More recently, refinement of pathological assessment has provided further prognostic tools following pancreaticoduodenectomy (PD) including lymph node ratio,[8] actual number of positive lymph nodes,[9] and site of margin involvement.[10] For carcinoma of the exocrine pancreas, the tumor–node–metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) describes that following PD for pancreatic cancer, extension of the tumor beyond the pancreas to the surrounding soft tissues including the duodenum (and ampulla), the biliary tract, and the peripancreatic fat promotes a tumor from T2 to T3 status. There has as yet been no specific investigation of the prognostic influence of the individual components of the determinants of T3 disease, including spread to the duodenum, common bile duct, or peri-pancreatic fat invasion following resection for PDAC.

Accurate assessment of pathological prognostic factors has important implications beyond simple prognostic stratification. Currently, stratification within the setting of randomized control trials for adjuvant therapy is based upon pathological factors that are determined following resection and include the presence of lymph node metastases and resection margin status. Preoperative identification of patients with a poor prognosis is desirable to aid appropriate clinical decision making and help realize the true potential of existing and novel therapies. This has the additional benefit of maximizing long-term survival while minimizing treatment-related toxicity.

Despite the limited survival benefit associated with resection, further management challenges result from a high local failure rate that can reach 80%.[11,12] Adjuvant chemoradiotherapy has been proposed as a means to reduce the risk of local recurrence toward 20%.[13,14] However, evidence is lacking to support the routine use of chemo-radiation. The focus of outcome measures following PD for PDAC remains overall survival, with only limited data describing the pattern of recurrence[11,12,15,16] or the factors associated with local recurrence. To date, only resection margin involvement and lymph node status have been compared to the pattern of failure,[15,17] with no consideration made of association between the pattern of failure and the presence of venous invasion, perineural invasion, lymphatic invasion, or peripancreatic invasion. Spread into the surrounding adipose tissue could result in the presence of residual tumor in the pancreatic bed and hence negatively influence survival and be associated with an increased rate of local recurrence.

In this study, we report the outcome following PD in 189 patients with PDAC. We sought to identify not only the influence of peripancreatic fat invasion on survival but furthermore the influence of various clinicopathological factors including peripancreatic fat invasion on the pattern of primary recurrence.

Patients and Methods

All patients underwent surgery in the West of Scotland Pancreatic Unit, Glasgow Royal Infirmary, Glasgow, UK, during a 13-year period (1 January 1996 to 31 June 2009). All patients underwent either classical or pylorus-preserving PD, performed by a team of surgeons. Surgical death was defined as in-hospital mortality. This analysis was limited to patients undergoing PD for resection of PDAC. Other lesions (e.g., ampullary, duodenal or distal bile duct adenocarcinomas, mucinous cystadenocarcinomas, or intraductal papillary mucinous neoplasms) were excluded.

The decision to perform resection was made by a multi-disciplinary team (MDT) including surgeons, oncologists, radiologists, and pathologists. The criteria for resectability were (a) computed tomography (CT) evidence of localized tumor in the head of the pancreas, (b) no evidence of superior mesenteric vein (SMV) occlusion or significant narrowing by tumor, and (c) no overt arterial involvement.[18] Patients (n=31) with localized lesions adjacent to either SMV or portal vein underwent resection of the involved vessel segment with vascular reconstruction.

Operative Procedure

The “standard” PD within this study begins as the lesser sac is entered by raising the greater omentum off the colon and following this plane to the right. Division of the peritoneal reflection and caudal mobilization of the hepatic flexure of the colon exposes Gerota’s fascia covering the right kidney. The peritoneum to the lateral aspect of the second part of the duodenum is divided in line with the right side of the inferior vena cava entering the vascular plane, mobilizing head of pancreas to expose the surface of the vena cava craniocaudally from the liver down to the right gonadal vein and across medially exposing the left renal vein, and the anterior aspect of aorta above the level of the inferior mesenteric artery. By following the right-sided colonic mobilization plane medially, the groove between the mesocolon anteriorly and the anterior aspect of the pancreas and duodenum posteriorly is developed to expose the

superior mesenteric pedicle, the vein lying anterior and to the right of the superior mesenteric artery (SMA) at this level. The anterior, duodenal, and posterior aspects of the pancreatic head are fully mobilized back to the midline, leaving the medial lymphovascular structures intact.

The transection phase requires division of the jejunum, small bowel mesentery, bile duct, pancreas, mesopancreas (medial transection margin), and distal stomach/duodenum (± vein resection) to allow resection completion. On the right side, the peritoneum overlying the SMV is opened at the level of the third part of duodenum and the perivascular plane followed caudally toward the neck of the pancreas exposing the SMV as it emerges under the neck of the pancreas. In the infracolic compartment, the pre-aortic dissection is followed in a cranial direction, anterior to the left gonadal vein and medial to the inferior mesenteric vein (IMV) clearing the left renal vein. The IMV is variable but usually enters either the splenic or SMV anterior to the SMA so this is exposed where it is crossed by the IMV. The SMA is then followed inferiorly to its root emerging above the left renal vein taking care not to denude more than 180° of the circumference. Transection margin frozen section analyses are performed to establish the presence of residual disease, with further pancreatic body resection undertaken until negative histopathological status is obtained. The majority (95%) of patients had classical pancreaticoduodenectomy with reconstruction by a four layer, duct to mucosa pancreaticojejunostomy.

The extent of resection remained constant for the duration of this study, although the precise order in which individual steps were undertaken would vary between procedures, to facilitate the early identification of locoregional inoperability. Lesions within the uncinate process or those sited medially would undergo an "artery first" exploration to ensure the absence of arterial involvement, whereas lesions at the neck undergo an early dissection of the hepaticoduodenal ligament to ensure proximal clearance. Short segment (<180°) venous involvement was managed by en bloc resection and primary anastomosis. Arterial involvement was considered a contraindication to resection.

Postoperatively, all patients were considered for adjuvant therapy at the MDT meeting. In the earlier years of the study, patients were considered for the European Study of Pancreatic Cancer (ESPAC-1) randomization; in the later years, they were considered for ESPAC-3 randomization. For those patients receiving adjuvant therapy (n=78), there was a range of five treatment options from both these studies, with 40 (51.3%) patients receiving 5-FU with folinic acid, 32 (41.0%) receiving gemcitabine, three (3.8%) receiving radiotherapy alone, and three (3.8%) receiving 5-FU with radiotherapy. Of those who did not receive adjuvant therapy (n=111), 11 (9.9%) were randomized to the observation arm of the ESPAC study; four (3.6%) patients were commenced on adjuvant chemotherapy, however received only one cycle before suffering from complications; two (1.8%) patients had a previous malignant diagnosis (breast and colorectal) and so were not eligible for entry to a chemotherapy trial; 55 (49.5%) were considered unsuitable for randomization on the basis of poor performance status, prolonged hospitalization following resection, or persistent pancreatic fistula; and the remaining 39 (35.1%) patients declined randomization.

Follow-up comprised a standardized protocol of outpatient reviews. CT scans were performed whenever local recurrence or metastatic disease was suspected. In patients with CT confirmed recurrent disease, the patient was considered for chemotherapy if oncologically naïve, or for re-challenge if they had received previous adjuvant chemotherapy.

The first site or sites of disease recurrence were classified as distant or locoregional. Local recurrence was defined as recurrence in the region of the pancreatic bed and the root of the mesentery while regional recurrence was defined as recurrence in the soft tissues or lymph nodes beyond the pancreatic bed or within the peritoneal cavity (including ascites and/or the presence of wound recurrence). Distant recurrence was defined as recurrence in the liver, lungs, or other distant organs. Radiographic findings consistent with recurrent disease were considered adequate proof of recurrence while only occasionally was tissue evidence obtained. Only the first site of recurrence at presentation was considered for analysis.

Pathology Reporting

The pathology reports from all patients identified as undergoing PD for PDAC between 1996 and 2009 were reviewed. During the study period, the resection specimens have been assessed by senior pathologists (including AKF and KO). AKF has led the local standardization of "taking in" procedures and is a co-author of the widely accepted Royal College of Pathologists (RCPath) National pancreatic specimen reporting guidelines.[7,10,19–21] Microscopic assessment and reporting include maximum tumor diameter and extent and location of local spread; tumor grade; perineural, venous, and lymphatic invasion; total of lymph nodes examined; and number positive and the presence of peripancreatic fat invasion (Fig. 1). The original hematoxylin-and-eosin (H&E) slides for the entire cohort were reassessed with the specific aim of identification of peripancreatic fat invasion (performed by NBJ). TNM staging is performed in accordance with the UICC/AJCC staging system[22] which corresponds to the RCPath guidelines.[19] For this study, tumor grade is categorized into high for poorly differentiated tumors and low for moderately and well-differentiated tumors.[23] R1 status was assessed according to the RCPath criteria. The guidelines define margin positivity as the presence of tumor at or ≤1 mm from a margin when

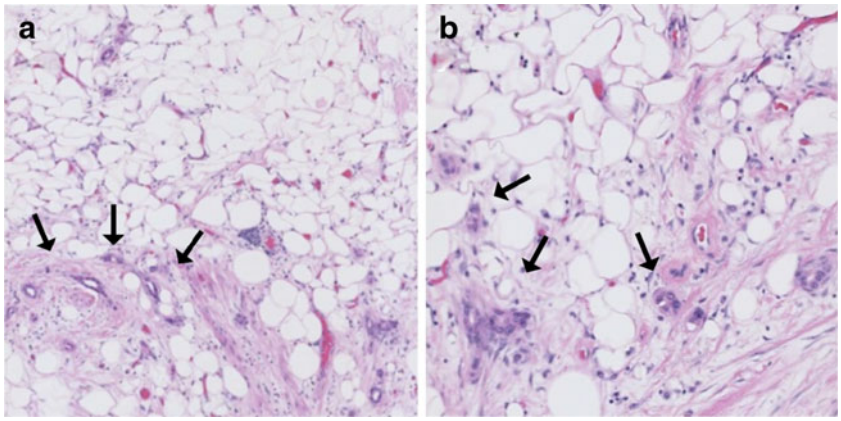

Fig. 1 Illustrations of pancreatic ductal adenocarcinoma invading into the peripancreatic fat. **a** Low power image of fibro-fatty tissue containing infiltrating adenocarcinoma (*black arrows*). **b** Higher power image of individual infiltrating ductal structures (*black arrows*; both hematoxylin and eosin)

assessed by microscopy of a H&E-stained slide.[19] Marginal status is further categorized as direct extension when directly infiltrating tumor was present at or ≤1 mm from a resection margin or locoregional extension when there is perineural, venous, or lymphatic infiltration or tumor within a lymph node ≤1 mm from a margin.

Statistical Analysis

Categorical variables were compared using the χ^2 test. The Mann–Whitney test was used to compare continuous variables. The principal outcome measure was length of survival as measured from the time of the original surgery. Length of survival following surgery and cause of death were obtained from our database and validated using the NHS Scotland Information Services Department (http://www.isdscotland.org). Kaplan–Meier survival analysis was used to analyze the overall survival from the time of surgery. Patients alive at the time of follow-up point were censored. The last follow-up period for patients still alive was March 2010. To compare the length of survival between curves, a log-rank test was performed. A Cox proportional hazards model was used for univariate analysis to adjust for competing risk factors, and the hazard ratio (HR) with 95% confidence intervals (CIs) was reported as an estimate of the risk of disease-specific death. Variables that were found to be significant on univariate analysis at $p<0.10$ were included in multivariate analysis in a backward stepwise fashion. Statistical significance was set at a p value of ≤0.05. All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinicopathological Characteristics of the Patient Cohort

From a total of 375 PD performed between 1996 and 2009, 201 patients were identified as having had a PD for PDAC. The in-hospital mortality was 5.9% (12 patients), with ten (4.9%) dying within 30 days of operation. These 12 patients were excluded from analysis, as pathological tumor-associated factors including peripancreatic fat invasion did not affect postoperative survival, leaving 189 patients in the study. The characteristics of the cohort are summarized in Table 1. Note that all patients were tumor stage T2 or T3. There were two instances of postoperative, non-cancer-related mortality that occurred as a result of pneumonia and cerebrovascular vascular accident, occurring at 14 and 44 months of follow-up, respectively.

Table 1 Demographic, operative, pathological, and treatment characteristics of 189 patients undergoing pancreaticoduodenectomy for pancreatic ductal adenocarcinoma

Characteristic	Number
Demographic	
Gender (F/M)	86/103
Age (<65/≥65)	104/85
Pathological	
Tumor stage (T2/T3)	18/171
Duodenal invasion (absent/present)	60/129
Bile duct invasion (absent/present)	103/86
Peripancreatic fat invasion (absent/present)	138/51
Lymph node metastasis (absent/present)	37/152
Tumor size (≤30/>30 mm)	98/91
Tumor grade (low/high)	127/62
Perineural invasion (absent/present)	16/173
Venous invasion (absent/present)	94/95
Lymphatic invasion (absent/present)	131/58
Resection margin status (R0/R1)	51/138
Operative, treatment, and outcome	
Vascular resection (no/yes)	158/31
Adjuvant chemotherapy (no/yes)	114/75
Survival (months; median/mean)	18.9/28.2

Peripancreatic Fat Invasion and Relationship with Clinicopathological Characteristics

Detailed review of pathology specimens revealed that 51 (26.9%) patients had histological involvement of the peripancreatic fat. During the study period, the rate of peripancreatic fat invasion did not vary significantly being 28.9% prior to 2002 and 25.1% following this time. The relationship between the demographic, operative, pathological, and treatment characteristics of the cohort according to presence or absence of peripancreatic fat invasion is shown in Table 2. Excluding T stage, the only characteristics significantly associated with peripancreatic fat invasion were the presence of larger tumor size and lymph node metastasis. There was no significance difference in rate of peripancreatic fat invasion based on the presence of resection margin involvement. Of the 51 resections without evidence of resection margin involvement, 11 (21.5%) patients had histological evidence of peripancreatic fat invasion. For those patients identified as having peripancreatic fat invasion, 15 specimens (29.4%) showed evidence of widespread adipose tissue invasion present at two or more locations. In 15 specimens (29.4%), it was present at the anterior or inferior aspect of the pancreas (six of which had peripancreatic fat invasion adjacent to the common bile duct or ampulla), while 11 specimens (21.6%) had fat invasion near the medial/SMV margin or the pancreatic transection region. In the remaining ten specimens (19.6%), it was present at posterior or superior aspects.

Table 2 Demographic, operative, pathologic, and treatment characteristics stratified by peripancreatic fat invasion in 189 patients undergoing resection for pancreatic ductal adenocarcinoma

		Peripancreatic fat invasion		
	Total 189	Absent n=138 (%)	Present n=51 (%)	p value
Patient-related factors				
Age	<65	77 (56)	26 (51)	0.621
	≥65	61 (44)	25 (49)	
Gender	Female	64 (47)	21 (41)	0.513
	Male	74(53)	30 (59)	
Tumor-related factors				
Tumor stage	T2	18 (13)	0 (0)	0.001
	T3	120 (87)	51 (100)	
Tumor size	<30 mm	79 (57)	19 (37)	0.015
	≥30 mm	59 (43)	32 (63)	
Tumor grade	Low	93 (67)	34 (67)	0.925
	High	45 (33)	17 (33)	
Lymph node status	Absent	34 (25)	3 (6)	0.004
	Present	105 (75)	48 (94)	
Margin involvement	R0	40 (29)	11 (22)	0.359
	R1	98 (71)	40 (78)	
Perineural invasion	Absent	13 (9)	3 (6)	0.564
	Present	125 (91)	48 (94)	
Venous invasion	Absent	74 (54)	21 (41)	0.129
	Present	64 (46)	30 (59)	
Treatment-related factors				
Vein resection	No	117 (85)	41 (80)	0.469
	Yes	21 (15)	10 (20)	
Adjuvant chemotherapy	No	78 (57)	35 (69)	0.145
	Yes	59 (43)	16 (31)	

Survival and Relationship with Clinicopathological Characteristics

The overall median survival for the 189 patients was 18.9 months (95% CI 15.7–22.2). Univariate analysis using log-rank tests of the clinicopathological characteristics in relation to survival is shown in Table 3. The factors significantly associated with poorer overall survival (p<0.05) were higher tumor (T) stage, tumor size >30 mm, lymph node metastasis, high tumor grade, venous invasion, perineural invasion, R1 margin status, no adjuvant chemotherapy, and peripancreatic fat invasion.

Relationship Between Survival and Determinants of T3 Status Including Peripancreatic Fat Invasion

The presence of duodenal invasion (including spread to the ampulla) was not associated with a significant reduction in survival as shown in Table 3. The 86 (45.5%) patients with evidence of bile duct invasion had a shorter median survival compared to the 103 (54.5%) patients with no invasion, the median survival being 16.8 months (95% CI 13.1–20.4) and 23.1 months (95% CI 16.3–29.3), respectively (p=0.049). The 51 (26.9%) patients with peripancreatic fat invasion had a significantly shorter overall survival compared to the 138 (73.1%) patients with no fat invasion, the median survival being 12.4 months (95% CI 9.9–15.0) and 22.6 months (95% CI 18.5–26.7), respectively (p<0.0001; Fig. 2).

Relationship Between Peripancreatic Fat Invasion, Lymph Node Status, Tumor Size, and Survival

As the presence of peripancreatic fat invasion was related to lymph node involvement and more frequently present in larger tumors, we assessed survival according to both of these established prognostic markers stratified by the presence of peripancreatic fat invasion (Fig. 3a, b). The presence of peripancreatic fat invasion had a significant negative impact on overall survival both for patients with lymph node involvement with a median survival of 20.7 months (95% CI 17.4–23.9) vs 13.3 months (95% CI

Table 3 Survival and relationship with clinicopathological characteristics in 189 patients undergoing pancreaticoduodenectomy for pancreatic ductal adenocarcinoma: univariate model identifying significant prognostic factors

Prognostic variable	No. of patients	Median survival (months)	95% CI	*p* value
Overall	189	18.9	15.7–22.2	–
Gender				
Female	86	20.4	16.1–24.7	0.072
Male	103	17.8	13.5–22.2	
Age (years)				
≤65	104	18.2	14.8–21.6	0.081
>65	85	21.9	14.9–29.1	
Tumor stage				
T2	18	36.2	17.5–54.9	0.002
T3	171	17.8	15.0–20.7	
Peripancreatic fat invasion				
Absent	138	22.6	18.5–26.7	0.0001
Present	51	12.4	9.9–15.0	
Duodenal invasion				
Absent	60	22.3	15.4–29.8	0.155
Present	129	17.8	14.2–21.4	
Bile duct invasion				
Absent	103	23.1	16.3–29.3	0.049
Present	86	16.8	13.1–20.4	
Lymph node status				
N0	37	35.9	13.7–58.1	0.002
N1	152	18.4	15.6–21.1	
Tumor size (mm)				
≤30	98	21.8	15.8–27.8	0.022
>30	91	16.2	11.7–20.6	
Tumor grade				
Low	127	21.8	16.8–26.8	0.028
High	62	13.1	9.0–17.2	
Perineural invasion				
Absent	16	18.2	13.5–22.9	0.023
Present	173	16.7	14.0–19.5	
Venous invasion				
Absent	94	24.7	18.3–31.1	0.001
Present	95	15.6	12.9–18.2	
Resection margin status				
R0	51	27.5	23.8–31.2	0.0001
R1	138	16.2	13.0–19.3	
Vascular resection				
No	158	19.8	16.1–23.5	0.056
Yes	31	13.4	7.02–19.9	
Adjuvant chemotherapy				
No	114	14.8	9.7–19.8	0.021
Yes	75	21.9	16.9–26.9	

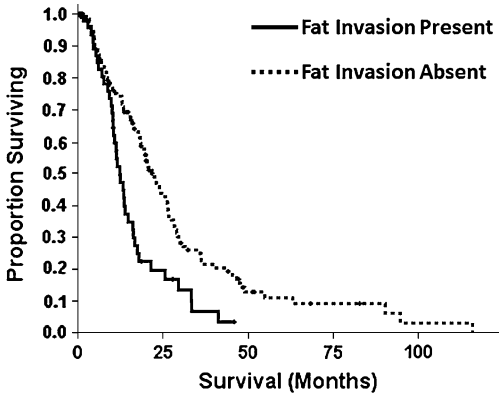

Fig. 2 Kaplan–Meier survival curves for patients following pancreaticoduodenectomy for pancreatic ductal adenocarcinoma. Illustration of the survival benefit associated with an absence of peripancreatic fat invasion in contrast to a resection with evidence of pancreatic fat invasion. The median survival for the 138 patients with no peripancreatic fat invasion was 22.6 months compared to 12.4 months for the 51 patients with fat invasion (log-rank test, $p=0.0001$)

10.4–16.2; $p=0.012$), and for those without lymph node metastases at resection, the median survival was 36.6 months (95% CI 13.8–59.5) vs 10.1 months (95% CI 1.9–17.1; $p=0.035$). Likewise peripancreatic fat invasion significantly negatively influenced the overall survival for patients with a tumor size greater than 30 mm with a median survival of 20.0 months (95% CI 14.1–25.9) vs 11.3 months (95% CI 6.1–16.5; $p=0.036$) and for those with tumors smaller in size with the median survival being 25.8 months (95% CI 19.9–31.8) vs 13.3 months (95% CI 11.0–15.6; $p=0.014$). While there was a trend toward peripancreatic fat invasion at the medial/SMV margin and transection margin being associated with a worse prognosis than other sites, sample size prevented more detailed analysis.

Relationship Between Peripancreatic Fat Invasion and Adjuvant Chemotherapy

While there was a trend toward adjuvant chemotherapy being used less frequently in those patients with no peripancreatic fat invasion, this was not significant ($p=0.15$). Adjuvant radiotherapy was only used in the management of three patients, as its routine use was not supported by the outcome of the original ESPAC-1 study.[18] Certainly adjuvant chemotherapy in any form provides a significant survival benefit within this cohort of PDAC (Table 3; $p=0.021$). For the 138 patients without peripancreatic fat invasion, when all chemotherapy regimens were combined, there was no significant improvement in outcome ($p=0.41$). Subsequent analysis revealed that those patients receiving adjuvant gemcitabine ($n=27$) did survive significantly longer (median survival 27.6 months [CI 21.3–33.4]) than those receiving 5-FU combinations (22.6 months [CI 15.9–29.2]) or no adjuvant therapies

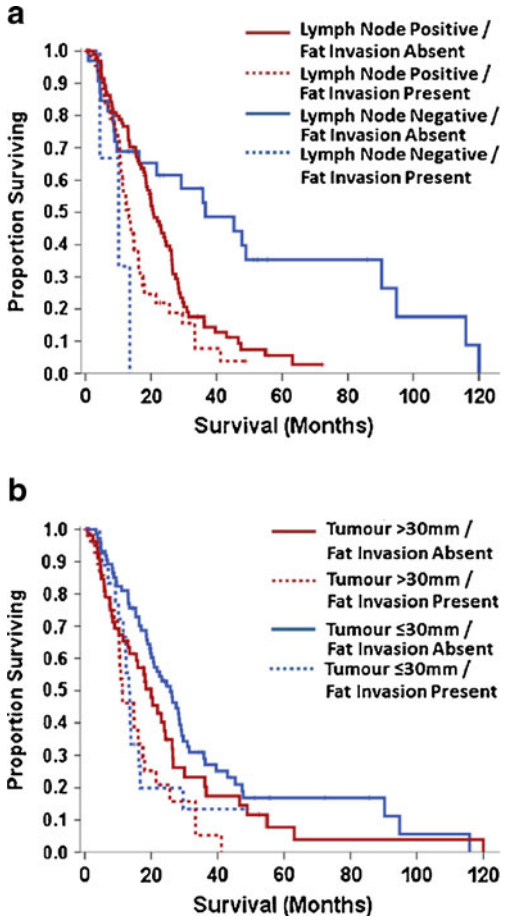

Fig. 3 Kaplan–Meier survival curves for patients following pancreaticoduodenectomy for pancreatic ductal adenocarcinoma demonstrating the prognostic influence of **a** lymph node status stratified by peripancreatic fat invasion with peripancreatic fat invasion significantly reducing the survival of patient with lymph node negative resections (log-rank test, $p=0.035$) and **b** tumor size stratified by peripancreatic fat invasion with peripancreatic fat invasion significantly reducing survival even when tumor is less than 30 mm in size (log-rank test, $p=0.014$)

(19.8 months [CI 17.9–21.6]; $p=0.046$). Adjuvant chemotherapy (both 5-FU and gemcitabine regimens) did significantly improve overall survival when employed in patients with peripancreatic fat invasion (median survival for patients receiving chemotherapy 16.2 months [CI 11.7–20.7] vs 11.6 months [CI 9.3–13.8] for those without adjuvant therapy; $p=0.015$) suggesting that the presence of peripancreatic fat invasion may provide predictive information regarding chemotherapeutic allocation.

Relationship Between Prognostic Factors and Survival by Multivariate Analysis

Covariates that affected survival at the $p<0.1$ level of significance were included in a multivariate Cox proportional hazards model (Table 4). Factors that independently adversely affected overall survival were high tumor grade (HR=1.80; 95% CI 1.25–2.61), higher tumor stage (HR=2.45; 95% CI 1.30–4.62), lymph node involvement (HR=1.89; 95% CI 1.11–3.31), venous invasion (HR=1.42; 95% CI 1.01–2.08), resection margin involvement (HR=1.91; 95% CI 1.24–2.92), and the histological presence of peripancreatic fat invasion (HR=1.93; 95% CI 1.18–3.45). Adjuvant chemotherapy was associated with prolonged survival following resection (HR=0.61; 95% CI 0.41–0.90).

Multivariate survival analysis was repeated including only the 171 T3 tumors (Table 5). Within this model peripancreatic fat invasion again independently negatively influenced survival (HR=1.93; 95% CI 1.18–3.45) as did high tumor grade (HR=1.89; 95% CI 1.29–2.79), venous invasion (HR=1.49; 95% CI 1.03–2.17), and resection margin involvement (HR=1.86; 95% CI 1.19–2.87). Although adjuvant therapy continued to provide independent survival benefits following resection for the T3 only cohort, lymph node involvement was no longer an independent predictor of poor outcome (HR=0.61; 95% CI 0.40–0.95).

Impact of Clinicopathological Factors on Disease Recurrence

The median follow-up for censored patients was 25.8 months (95% CI 19.0–32.5) and for all patients including those who had died was 21.4 months (95% CI 17.2–23.7). During the study period, recurrent disease occurred in 144 of 189 patients (76.2%). Distant metastases (including liver and lung) occurred in 78 patients (54.2%) with 66 (45.8%) developing locoregional recurrence. According to a multivariate analysis T3 stage, peripancreatic fat invasion, high tumor grade, tumor size ≥30 mm, resection margin involvement, and venous invasion were associated with recurrence at any site ($p<0.05$). Among the entire cohort, univariate analysis revealed that lymph node status and peripancreatic fat invasion were associated with local recurrence (Table 6). By multivariate analysis, only peripancreatic fat invasion (HR 2.95, $p<0.001$) remained independently associated with local recurrence. Further χ^2 test analysis revealed that recurrent disease was identified in 105 (76.1%) of 138 patients who had no evidence of peripancreatic fat invasion and in 39 (76.5%) of 51 patients who had evidence of peripancreatic fat invasion following resection (Table 7). Peripancreatic fat invasion affected the site of first recurrence; with 50.9% (26 out of 51) of patients with locoregional recurrence in those tumors exhibiting peripancreatic fat invasion present representing a significantly greater proportion than in the 28.9% (40 out of 138) of patients whose tumors had no evidence of peripancreatic fat invasion ($p=0.002$). High tumor grade was associated with

Table 4 Predictors of survival in all 189 patients following pancreaticoduodenectomy using multivariate Cox regression analysis

		Overall survival	
		HR (95% CI)	*p* value
Patient-related factors			
Age (years)	<65/≥65	0.88 (0.73–1.06)	0.170
Gender	Female/male	1.19 (0.82–1.75)	0.363
Tumor-related factors			
Tumor stage	T2/T3	2.45 (1.30–4.62)	0.006
Peripancreatic fat invasion	Absent/present	1.93 (1.18–3.45)	0.007
Bile duct invasion	Absent/present	1.11 (0.78–1.59)	0.542
Tumor size (mm)	<30/≥30	1.29 (0.89–2.15)	0.172
Lymph node status	Absent/present	1.89 (1.11–3.31)	0.025
Tumor grade	Low/High	1.80 (1.25–2.61)	0.002
Perineural invasion	Absent/present	1.27 (0.53–3.04)	0.586
Venous invasion	Absent/present	1.42 (1.01–2.08)	0.045
Margin involvement	R0/R1	1.91 (1.24–2.92)	0.003
Treatment-related factors			
Vein resection	No/yes	0.96 (0.57–1.63)	0.906
Adjuvant therapy	No/yes	0.61 (0.41–0.90)	0.014

distant metastases being the primary site of recurrence, with distant metastases developing in 53.2% (33 out of 62) of those with high-grade tumors compared to 38.1% (48 out of 126) of those with low-grade tumors. Resection margin status, perineural invasion, venous invasion, lymphatic invasion, tumor size, or use of adjuvant chemotherapy failed to impact on the pattern of recurrence following PD.

Discussion

It is accepted that various pathological factors including resection margin status, tumor grade, lymph node status, and perineural invasion influence outcome following PDAC resection. [3,4,6,24] While spread of tumor to the peripancreatic tissue including adipose tissue upgrades the lesion from T2 to T3 disease, the individual prognostic influence of peripancreatic fat invasion has not previously been investigated following PD for PDAC. This is of particular interest as recently there has been a great deal of progress made toward the redefinition of the surgical pathology terminology associated with pancreatic resection margins and retroperitoneal spread.[7,25,26] We sought therefore to determine the relationship of peripancreatic fat invasion with prognosis and assess its influence on the pattern of failure.

Table 5 Predictors of survival in 171 T3 patients following pancreaticoduodenectomy using multivariate Cox regression analysis

		Overall survival	
		HR (95% CI)	*p* value
Patient-related factors			
Age (years)	<65/≥65	0.88 (0.73–1.06)	0.170
Tumor-related factors			
Peripancreatic fat invasion	Absent/present	1.61 (1.11–2.58)	0.009
Bile duct invasion	Absent/present	1.09 (0.76–1.58)	0.625
Tumor size (mm)	<30/≥30	1.43 (0.99–2.08)	0.056
Lymph node status	Absent/present	1.45 (0.89–2.81)	0.102
Tumor grade	Low/high	1.89 (1.29–2.79)	0.001
Venous invasion	Absent/present	1.49 (1.03–2.17)	0.033
Margin involvement	R0/R1	1.86 (1.19–2.87)	0.006
Treatment-related factors			
Vein resection	No/yes	0.94 (0.55–1.61)	0.824
Adjuvant therapy	No/yes	0.63 (0.40–0.95)	0.038

Table 6 Factors associated with local recurrence following pancreaticoduodenectomy in 189 patients: univariate and multivariate analysis

Variable	Univariate			Multivariate		
	Hazard ratio	95% CI	*p* value	Hazard ratio	95% CI	*p* value
Tumor stage						
T2		–				
T3	1.95	0.77–4.89	0.155	–	–	–
Peripancreatic fat invasion						
Absent		–				
Present	3.31	1.92–5.70	<0.001	2.95	1.71–5.10	<0.001
Duodenal invasion						
Absent		–				
Present	1.04	0.61–1.75	0.884	–	–	–
Bile duct invasion						
Absent		–				
Present	1.20	0.72–1.99	0.472	–	–	–
Lymph node status						
N0		–				
N1	2.29	1.04–5.02	0.038	1.63	0.71–3.74	0.235
Tumor size (mm)						
≤30		–				
>30	1.46	0.88–2.43	0.137	–	–	–
Tumor grade						
Low		–				
High	0.86	0.48–1.54	0.616	–	–	–
Perineural invasion						
Absent		–				
Present	0.43	0.13–1.40	0.163	–	–	–
Venous invasion						
Absent		–				
Present	1.47	0.87–2.47	0.144	–	–	–
Lymphatic invasion						
Absent		–				
Present	1.10	0.62–1.94	0.739	–	–	–
Resection margin status						
R0		–				
R1	1.49	0.82–2.73	0.192	–	–	–
Adjuvant chemotherapy						
No		–				
Yes	0.84	0.49–1.42	0.510	–	–	–

Table 7 Pattern of recurrence according to the presence of peripancreatic fat invasion and tumor grade

	Peripancreatic fat invasion			Tumor grade		
	Absent *n*=138 (%)	Present *n*=51 (%)	*p* value	Low *n*=126 (%)	High *n*=62 (%)	*p* value
Site of first recurrence						
Liver/distant metastases	65 (47.1)	13 (25.5)	0.002	48 (38.1)	33 (53.2)	0.041
Locoregional	40 (28.9)	26 (51.0)		47 (37.3)	16 (25.8)	
No recurrence	33 (24.0)	12 (23.5)	0.889	32 (24.6)	13 (20.9)	0.813

In the present study, peripancreatic fat invasion was evident in 51 (26.9%) tumors from a total of 189 PDACs resected by PD during a 13-year period. The presence of peripancreatic fat invasion was associated significantly with the larger tumors and the presence of lymphatic invasion. However, the presence of pancreatic fat invasion was a rare finding in the absence of lymph node metastases. There was no relationship with gender, age, resection margin involvement, tumor grade, venous invasion, or perineural invasion. We demonstrated that peripancreatic fat invasion was significantly associated with poorer survival following resection (12.4 versus 22.6 months), and this effect was independent of other clinicopathological and treatment factors when tested in a multivariate Cox regression model. The two other determinants of T3 disease are duodenal and common bile duct spread, and while there was a trend toward poor survival in the latter group, this was not an independently prognostic factor. While the majority of tumors resected for PDAC are T3,[4] a figure supported by the current study, we have demonstrated that the T3 categorization has a spectrum of outcomes based upon the site of peripancreatic spread. Despite the association of peripancreatic fat invasion and lymph node involvement, we demonstrated that even in the few cases where adipose invasion was identified in the lymph node negative group, this was associated with a significant reduction in survival. Likewise, despite stratification of patients by tumor size, the presence of peripancreatic fat invasion was associated with a significantly reduced survival following resection suggesting that the negative impact on survival associated with resection was not merely the result of lymph node involvement or larger tumor size. If these results are confirmed, then reclassification of the current pathological staging system (T3a and T3b) to account for this powerful prognostic factor may be appropriate.

Data on both patterns of failure and factors associated with disease recurrence following PD remain poorly defined. Distant recurrence is presumed to occur in the majority following potentially curative resection,[15,17] and this fact combined with the overall poor survival results in the issue of local recurrence being largely ignored. Locoregional recurrence can have important clinical implications notably severe pain along with obstruction of biliary and gastrointestinal tract. Both the incidence and factors associated with local recurrence are important. Indeed, when the pattern of recurrence was investigated in a cohort of advanced PDAC according to a protocol of immediate autopsy with the intent of obtaining high-quality primary and metastatic tissue, 12% were shown to have no evidence of metastatic disease at the time of death.[27]

The incidence of local recurrence varies greatly in the literature. In terms of the pattern of failure in the present study, there was a slight excess of the first site of failure being distant metastases including liver (54%) compared to locoregional failure (46%). Some report, as we have shown, locoregional recurrence rates from 50% to 80%. In contrast, other studies in which the majority of patients received adjuvant chemoradiotherapy have noted a lower risk of local recurrence.[13,15,17,28–30] In particular, those studies that have utilized radiotherapy as part of the management algorithm have noted locoregional recurrence rates of 10–40%. In the present study although 74 patients received adjuvant therapy as part of the ESPAC-1 and ESPAC-3 trials, only two patients received adjuvant radiotherapy.

Identification of patients who are at higher risk of local recurrence may be important. Of previous factors that have been correlated with pattern of disease recurrence in patients with PDAC, a high degree of lymph node disease burden was associated with local recurrence in patients with a N1 resection,[17] although margin status failed to reach significance when adjustment was made for lymph node status. Margin status did not significantly impact upon pattern of recurrence in a study of 360 patients treated by PD which reported locoregional recurrence in 16.7% of R0 versus 13.4% of R1 resections.[15] In addition to the prognostic value of peripancreatic fat invasion, it was found to be associated with the pattern of recurrence. Specifically, the presence of pancreatic fat invasion was associated with an increased incidence of local and regional recurrence as the primary site of recurrence. To our knowledge, the present study is the first to examine the influence of peripancreatic fat invasion upon the pattern of disease recurrence following potentially curative PD in patients with PDAC. Our data illustrate that invasion into the surrounding adipose tissue resulted in a significantly increased proportion of locoregional recurrence compared to those with no involvement of the peripancreatic fat (51.0% versus 28.9%, respectively [$p=0.002$]). While many of the clinicopathological factors including resection margin status and lymph node status were not found to be associated with the site of primary recurrence, high-grade tumors were associated with recurrence at a distant site. This finding is in contrast to the findings of Asiyanbola et al. who identified high-grade tumors being associated with local recurrence.[17] Regarding resection margin status, we note that within the current study, the margin involvement criteria of >1 mm minimum clearance as a gauge of complete resection is based upon those of the RCPath[19] (http://www.rcpath.org/). However, this definition is often not explicit within other guidelines, and in this, our work differs from much of the previous literature. This has resulted in a margin involvement rate of 73.1% in this series, which is becoming increasingly accepted as the norm[7,20,31] and which we have previously shown is independently associated with poor outcome following resection.[10] This discrepancy in resection margin involve-

ment rate may influence the association between patterns of failure and therefore requires reassessment in future studies.

Correlating macroscopic fat invasion with histological invasion will always be challenging; however, the identification of peripancreatic fat invasion at the time of assessment of resectability would identify a group at high risk of locoregional recurrence and poor survival. In terms of preoperative imaging, magnetic resonance imaging (MRI) is a valuable tool in the assessment of the full spectrum of pancreatic disease, including effectively detecting, diagnosing, and staging PDAC.[32] Dynamic enhanced MRI has a sensitivity and specificity equal to or better than that of helical CT for the detection of local tumor extension and vascular involvement.[33,34] MR imaging was recently shown to demonstrate extrapancreatic neural plexus invasion successfully in patients with PDAC undergoing resection.[35] Eight percent of patients with pathological proof of extrapancreatic neural plexus invasion by PDAC had abnormal signal intensity in background fat on MR imaging, which included streaky and strand-like signal intensity structures in fatty tissue in 50% and irregular masses adjacent to lesions in the remaining 30%. In addition to the evaluation of peripancreatic spread in PDAC, a recent study assessed the imaging characteristics of solid pseudopapillary tumor of the pancreas with multidetector row CT in comparison to pathological findings following resection. Peripancreatic regional spread including peripancreatic fat invasion was identified preoperatively in all patients which subsequently had spread found at pathological examination.[36] We unfortunately do not have MR imaging available for all patients to enable correlation between the preoperative macroscopic and microscopic appearance. However, these data would certainly be of interest in terms of influencing resectability based on cross-sectional imaging.

In a recent study, the term "isolated solitary ductal unit" has been used to describe clusters of adenocarcinoma cells forming solitary ducts completely surrounded by adipose tissue without any accompanying acini, islets or fibrosis, and which appear to be a reliable indicator of adenocarcinoma.[37] The identification of these clusters of cells distant from the bulk of the tumor in the adipose tissue has implications for the characterization of tumor size and margin extension. Their presence was identified in approximately 50% of resections; however, no attempt was made to correlate this pathological finding with outcome. As a result, however, a number of tumors were subsequently upstaged from T1 to T3. We did not assess our cohort for the presence of "isolated solitary ducts", but as the reported rate was certainly greater than the direct extension of tumor into the adipose identified in the present study, recognition of these structures may further stratify outcome.

Evidence that the presence of increased intrapancreatic fat is associated with poor outcome and disseminated disease was recently demonstrated in a case controlled analysis of 40 PDACs.[38] The authors claim that increased pancreatic fat may itself be a contributing factor to the aggressive phenotype associated with pancreatic cancer. There has been great focus placed recently upon the role of the tumor microenvironment in PDAC tumorigenesis including inflammatory mediators, stellate cells, and myofibroblasts.[39] Potentially adipocytokines including leptin and adiponectin produced by adipose tissue as a result of tumor infiltration may influence the inflammatory milieu and contribute to the tumor microenvironment, enhancing PDAC tumorigenesis, as has been demonstrated in other tumor types including colorectal cancer.[40,41]

Total body adiposity has been suggested as being associated with lymph node status following resection. This study identified that an elevated BMI greater than 35 was correlated with an increased incidence of lymph node positivity.[42] However, this finding was not corroborated in a recent larger study of 795 patients,[43] and so the influence of adipose tissue on tumor progression remains unclear. Clearly BMI is a crude measure of adiposity, and therefore, more accurate assessments of total body fat, such as cross-sectional imaging techniques, are required to fully answer whether total body adiposity compared to peritumoral or intratumoral adiposity influences tumor aggressiveness.

We acknowledge that the present study has a number of limitations. Notably our cohort had a relatively low rate of adjuvant chemotherapy compared to many other institutes. This may explain why the presence of pancreatic fat invasion was associated with locoregional recurrence. Clearly this is also the first study to identify the independent prognostic significance of pancreatic fat invasion and therefore the findings of the current investigation require validation in a further cohort, in particular the study should be repeated in a cohort of patients all receiving standardized adjuvant therapy to identify whether the prognostic value is preserved.

Conclusion

The results of the present study demonstrate that the presence of peripancreatic fat invasion assessed by histological examination following PD for PDAC in 189 patients provides independent prognostic information in addition to the categorization of T3 disease and other clinicopathological factors including resection margin status. Additionally, the presence of peripancreatic fat invasion, but not resection margin involvement, was associated with locoregional disease as the primary site of recurrence. Modification of future staging systems to improve outcome stratification

may be justified if these findings are replicated. Furthermore, there is potential for this poor prognostic factor to be identified preoperatively by advanced cross-sectional imaging techniques.

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ORIGINAL ARTICLE

Computed Tomography Reflected Endocrine Function of the Pancreas

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Abstract
Backgrouns/Aims There are few studies about the assessment of pancreatic function using computed tomography (CT) volumetry. In this study, we examined the correlation between CT volumetry and endocrine parameters (blood glucose and HbA1c) of the pancreas.
Methods A total of 68 patients underwent enhanced CT for pancreatic disease from January to December in 2008. In particular, we analyzed the correlation of diabetic status and pancreatic CT parameters at 1 year after pancreatoduodenectomy in 32 patients. CT parameters including volume, volume/body weight, arterial phase density, the arterial phase to portal phase density ratio (A/P ratio) of the pancreas, and size of pancreatic duct were also analyzed. Correlation between CT parameters and diabetic status was analyzed preoperatively and postoperatively by ANOVA test.
Results The preoperative diabetic status and parameters correlated well with arterial phase density ($p=0.004$), A/P ratio, and pancreatic duct size ($p<0.0001$). In the patients who underwent pancreatectomy, two out of 25 patients without preoperative diabetes mellitus (DM) had DM, and two out of seven patients with preoperative DM recovered from DM. Postoperative CT parameters correlated with the DM status 1 year after pancreatectomy.
Conclusion CT is a useful modality for evaluation of the pancreatic endocrine function and could be used for the prediction of postoperative diabetic outcome.

Keywords Computed Tomography (CT) · Volumetry · Density · Endocrine · Pancreas

This study was presented at the Joint Meeting of the International Association of Pancreatology and the Japan Pancreas Society 2010 on July 12, 2010 in Fukuoka, Japan.

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Introduction

Computed tomography (CT) imaging is the most commonly used imaging procedure for pancreatic diseases including cancer,[1] acute and chronic pancreatitis,[2,3] intraductal papillary mucinous neoplasm (IPMN),[4] pancreatic endocrine tumor,[5] and cystic pancreatic neoplasm.[6]

Three-dimensional (3D) imaging has become a popular modality because it can provide 3D views of organs, vessels, or diseases.[7] With 3D imaging, it is easier to understand the disease extent and anatomy than with two-dimensional (2D) imaging. 3D images can be obtained with CT, ultrasonography, and magnetic resonance imaging and are widely used for the evaluation of hepatobiliary and pancreatic disease.[7] It has been used for preoperative evaluation of hilar cholangiocarcinoma,[7] the detection of hepatobiliary abnormalities in the hepatic hilum,[8] evaluation

of the vascular invasion of pancreatic cancer,[9] preoperative staging of gallbladder cancer,[10] and evaluation of the vascular anatomy for liver transplantation.[11,12]

3D-CT is also reported as a useful modality to calculate the liver volume for transplantation[13,14] and cancer treatment.[15] Preoperative liver volumetry can predict postoperative residual liver function that is a major factor influencing the outcome of the patient after hepatectomy.[16] While liver volumetry using 3D-CT has been performed widely, there are no studies about pancreatic volumetry, especially about the pre and post-operative assessment of pancreatic endocrine function using CT. We considered that the volume of the pancreas may be useful for the assessment of pancreatic function.

In this study, we examined the correlation between CT parameters (volume, volume/body weight, arterial phase density, portal phase density, the arterial phase to portal phase density ratio (A/P ratio) and size of main duct) of the pancreas in enhanced CT and endocrine parameters of the pancreas (blood glucose and HbA1c) preoperatively. In some patients, we analyzed the correlation between CT parameters and the diabetic outcome at 1 year after pancreatectomy. Finally, we attempted to confirm the usefulness of CT for the assessments of pancreatic endocrine function.

Patients and Methods

Ethics

This study was approved by the Ethics Committee of Tohoku University School of Medicine. All data were obtained from clinical records while protecting the confidential information of the patients.

Patients

This is a single-institutional retrospective review of 68 patients who underwent enhanced CT using 16 raw multi-detector with 1 mm slice thickness with nonionic contrast agent (i.e., Iohexol and Iopromide) for pancreatic surgery from January to December in 2008 in Tohoku University Hospital. Out of 68 patients, 26 had pancreatic cancer (head, 14; body, 10; tail, 1; head and body, 1), 10 had IPMN, eight had lower bile duct cancer, seven had cystic pancreatic tumor, six had chronic pancreatitis, four had pancreatic endocrine tumor, three had gallbladder cancer, two had cancer of the Vater papilla, one had metastatic pancreas tumor, and one had arteriovenous malformation of pancreas (Table 1). All 68 patients underwent partial or total pancreatectomy (39: pancreatoduodenectomy (PD), including subtotal stomach preserving pancreatoduodenectomy (SSPPD), and hepatopancreatoduodenectomy (HPD); 24, distal pancreatectomy; two, total pancreatectomy; three, others). Eighteen patients were diagnosed as having preoperative diabetes mellitus (DM) and 50 were normal (Fig. 1). Definitions of DM were over 126 mg/dL of fasting blood glucose level, over 200 mg/dL of blood glucose level, or over 6.5% of HbA1c. History of the diabetic drug use or insulin treatment also meets the definition of DM.

Table 1 Characters of 68 patients before operation

Age	61.5 (16–81)
Gender (male/female)	38:30
Disease	
Neoplastic	61
Pancreatic cancer	26
IPMN	10
Bile duct cancer	8
Cystic pancreas tumor	7
Endocrine tumor	4
Gallbladder cancer	3
Cancer of Vater papilla	2
Metastatic tumor	1
Non-neoplastic	7
Chronic pancreatitis	6
Arterovenous malformation of pancreas	1
Volume of pancreas (cm^3)	45.3 (4.9–103.6)
Volume of pancreas/body weight (cm^3/kg)	0.78 (0.12–2.03)
Arterial phase density	97.9 (50.3–139.9)
Portal phase density	91.5 (49.8–128.4)
A/P ratio (arterial phase/portal phase)	1.04 (0.44–1.39)
Parenchymal rate (in neoplastic lesions, %)	79.7 (11.4–98.7)
Size of pancreatic duct (mm)	3.65 (0.87–32.06)
Blood glucose (mg/dL)	118.3 (76.3–233.3)
HbA1c (%)	5.6 (4.4–12.6)

Median (min–max)

Parameters of CT Findings

3D-CT volumetry was performed using OsiriX imaging software (OsiriX v.3.6.1, OsiriX Foundation, Geneva,

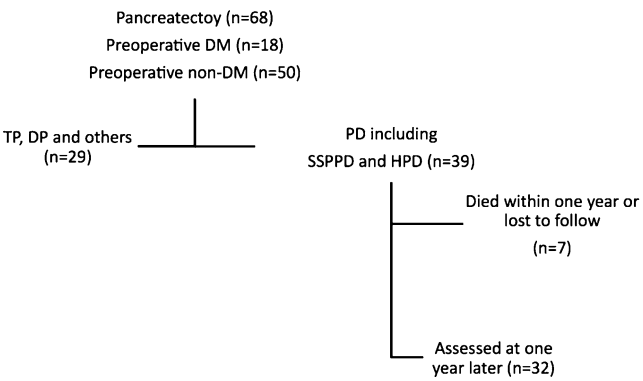

Fig. 1 Scheme of this study

Switzerland). In detail, CT images were downloaded as digital imaging and communication in medicine files into a Macintosh platform with the Mac OS X operating system and analyzed with OsiriX imaging software. Pancreatic areas were selected as regions of interest (ROI) in each slice, and the ROI volume was calculated (Fig. 2).

For a preliminary assessment, the correlation between age and endocrine parameters was assessed to see whether age might affect the endocrine function. Tumors, cysts, enhanced vessels, calcification, and dilated pancreatic ducts were excluded from the volume of pancreas. On the other hand, tumor sizes (including cystic tumor) were measured independently and parenchymal rate (=pancreas volume/(pancreas+tumor volume)) were calculated in patients with neoplastic disease (pancreatic cancer, metastatic tumor, and cystic tumor). Since there was a positive correlation between volume of pancreas and body weight (R^2=0.11, p=0.005, data not shown), the volume of pancreas (cm^3) was divided by body weight (kg) in the assessment.

We also measured the mean arterial phase density (45 s after infusion of contrast agents) and mean portal phase density (70 s after infusion) of ROI. Furthermore, to detect the change of enhancement, we calculated the ratio between the arterial phase and the portal phase density described as the A/P ratio. If the ratio is nearly 1, it indicates the enhancement effect did not change between the arterial and portal phase and, if over 1, the enhancement effect is diminished at the portal phase. An A/P ratio of <1 indicates a delay of enhancement.

As another CT parameter, maximum diameter of the main pancreatic duct (size of pancreatic duct) was also measured. Tumor or pancreatitis can cause duct dilation together with fibrotic change. Dilated pancreatic duct can be a representative parameter of primary or secondary pancreatitis and may have a possible correlation with hyperglycemia.

CT Parameters in Patients with PD at 1 Year After Operation

The CT images were available in 41 of the patients at 1 year after the operation. Other 19 patients could not be examined

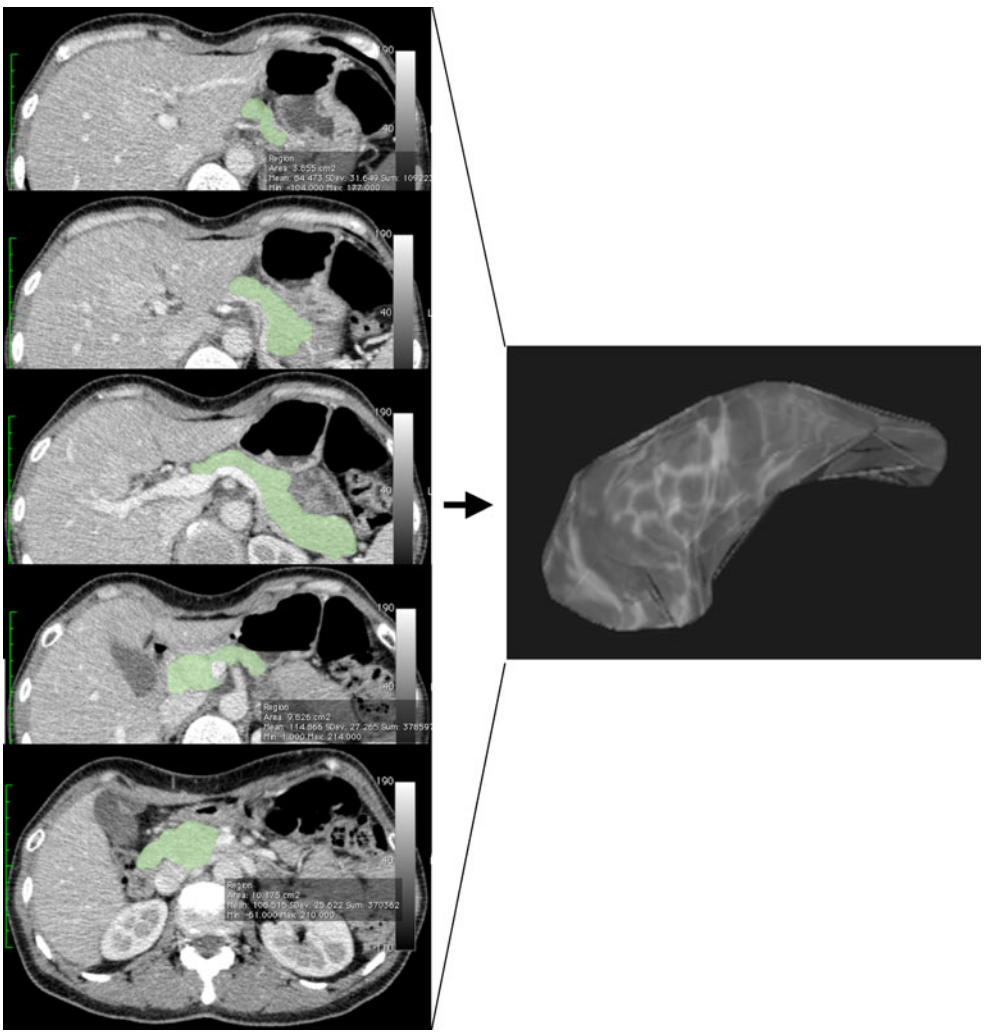

Fig. 2 Scheme for calculating the volume of the pancreas. Pancreas areas were selected as regions of interest (ROI, *green area*) in each slice and the ROI volume was calculated using OsiriX imaging software. Tumors, cysts, enhanced vessels, calcification, and dilated pancreatic duct were excluded from the pancreatic volume

because of transfer to other institutions after operation or death within 1 year. We focused on the patients who underwent PD, including SSPPD and HPD and measured CT parameters that we mentioned previously and the volume of the residual pancreas and calculated the residual rate of the pancreas (=[volume of the pancreas at post-operation]/[volume of the pancreas at preoperation]×100 (%)) in PD patients. Thirty-two out of 39 PD cases were used in this assessment (Fig. 1).

Endocrine Parameters

Blood glucose and HbA1c were applied as endocrine parameters. Blood samples were taken in the morning before meal. Blood glucose is indicated by the mean of three values obtained at three different days including the same day of CT examination and HbA1c was measured one time at the same day of CT. Serum insulin and C-peptide values were not available in most of the patients.

Assessment of Correlation Between Preoperative CT Parameters and Endocrine Parameters

CT parameters (volume of pancreas, volume of pancreas/body weight, pancreas parenchymal rate, arterial phase density, A/P ratio, and size of pancreatic duct) were compared between DM group and non-DM group. The correlation between each CT parameter and blood glucose and HbA1c were examined by correlation coefficient (R^2).

Assessment of Correlation Between Preoperative CT Parameters and Postoperative Endocrine Parameters in PD Patients

We evaluated the condition of DM in PD patients 1 year after the operation to certify how many patients in the preoperative non-DM and DM group were turned to DM or not. We compared CT parameters at 1 year after the operation between the two groups stratified by preoperative DM condition. In order to predict postoperative outcome, we searched cutoff values of preoperative CT parameters in PD patients using receiver operating characteristic (ROC) curve.[17] The nearest point from sensitivity=1 and false positive rate=0 was defined as cutoff line.

Statistical Analysis

Analysis of variance was applied in all the assessment except the rate of DM and non-DM between over and under the cutoff line of CT parameters at 1 year after PD. All the statistical analysis was performed with JMP 5.0.1J (SAS Institute Inc. Cary, NC, USA) and $p<0.05$ was defined as significant.

Results

CT Parameters

The median volume of the pancreas excluding tumor was 45.3 cm^3 (4.9–103.6) and the volume of pancreas/body weight was 0.78 cm^3/kg (0.12–2.03). The median arterial phase density was 97.9 (50.3–139.9), while the median portal phase density was 91.5 (49.8–128.4). The median A/P ratio was 1.04 (0.44–1.39). The median size of pancreatic duct was 3.65 mm (0.87–32.06; Table 1). The median of parenchymal rate in patients with neoplastic disease was 79.7% (11.4–98.7). The medians of preoperative blood glucose and HbA1c were 118.3 mg/dL (76.3–233.3) and 5.6% (4.4–12.6; Table 1).

Assessment of Preoperative DM Indicators

Figure 3 shows comparison in blood glucose and HbA1c between preoperative DM and non-DM patients. Although insulin and/or oral anti-diabetic drugs had been already

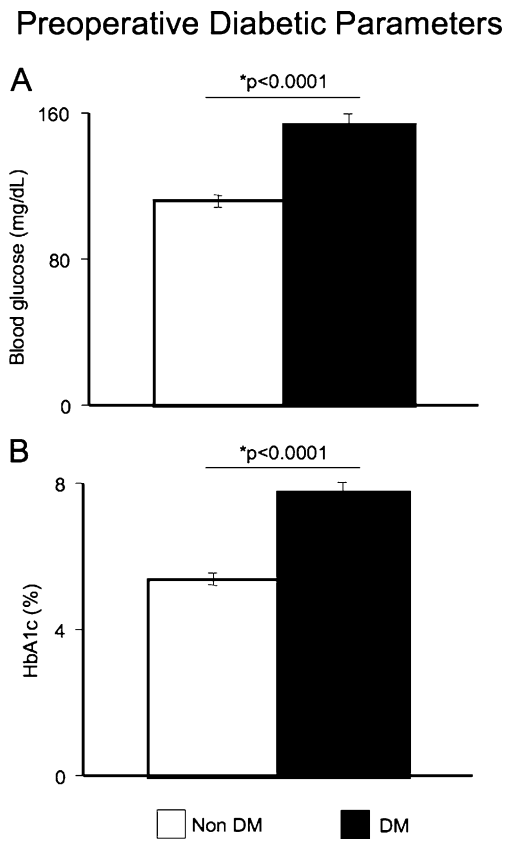

Fig. 3 Comparison in blood glucose and HbA1c between preoperative DM (*black bar*) and non-DM (*white bar*) patients. Both parameters were significantly lower in non-DM than in DM (**a** blood glucose 111.3±3.5 vs. 153.1±6.1 mg/dL, $p<0.0001$; **b** HbA1c 5.4±0.2% vs. 7.8±0.2%, $p<0.0001$). Significant difference was $p<0.05$ (indicated with *asterisk*)

administered in some diabetic patients, both parameters were significantly lower in non-DM group than in DM group (A: blood glucose 111.3±3.5 vs. 153.1±6.1 mg/dL, $p<0.0001$; B: HbA1c 5.4±0.2% vs. 7.8±0.2%, $p<0.0001$). Thus we postulate that preoperative blood glucose and HbA1c could be representative parameters of DM.

CT Parameters According to the Preoperative DM Status

Volume of pancreas, volume of pancreas/body weight, pancreas parenchymal rate, arterial phase density, and A/P ratio tended to be higher in non-DM group than DM group in spite of no significant difference (Fig. 4a–e). Size of pancreatic duct was significantly smaller in non-DM group (4.4±0.7 vs. 7.7±1.1 mm, $p=0.009$, Fig. 4f). These results suggest that higher volume parameters (i.e., volume of pancreas, volume of pancreas/body weight, and pancreas parenchymal rate), higher density parameters (arterial phase density and A/P ratio) and smaller size of pancreatic duct indicate pancreas with good function.

Diabetic Indicators According to CT Parameters

Since diabetic indicators (blood glucose and HbA1c) and CT parameters were already shown to correlate with diabetic status, it was expected that CT parameters and diabetic parameters may have positive correlation. Table 2 shows the R^2 of blood glucose and HbA1c in each CT parameters. We can detect the significant correlation between blood glucose and arterial phase density ($R^2=0.12$, $p=0.004$), A/P ratio ($R^2=0.11$, $p=0.006$) and size of pancreatic duct ($R^2=0.21$, $p<0.0001$).

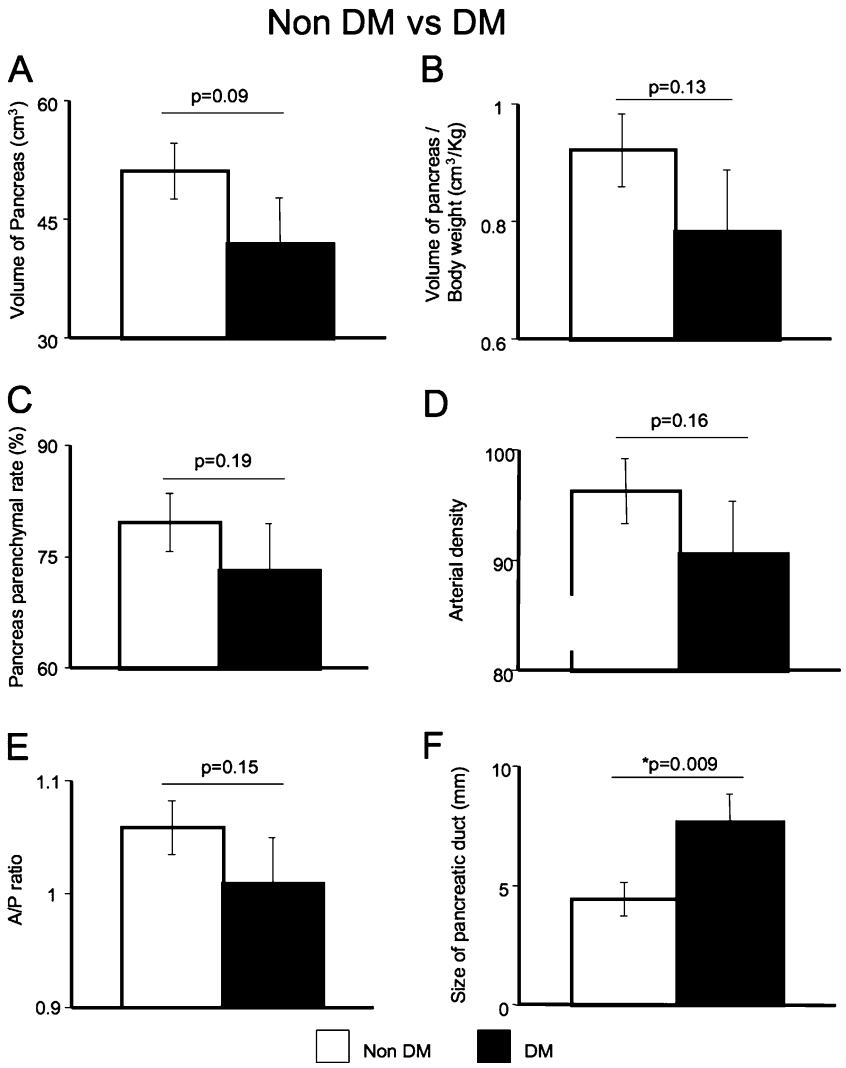

Fig. 4 Comparison of CT parameters between preoperative DM (*black bar*) and non-DM (*white bar*). Volume of pancreas (**a**), volume of pancreas/body weight (**b**), pancreas parenchymal rate (**c**), arterial density (**d**), A/P ratio, (**e**) tended to be higher in non-DM than DM in spite of no significant difference. Size of pancreatic duct was significantly smaller in non-DM (4.4±0.7 vs. 7.7±1.1 mm, $p=0.009$, **f**). Significant difference was $p<0.05$ (indicated with *asterisk*)

Table 2 Correlation of DM indicators with the CT parameters

CT parameters	Blood glucose (mg/dL)		HbA1c (%)	
	R^2	p value	R^2	p value
Volume of pancreas	0.005	0.58	0.02	0.25
Volume of pancreas/body weight	0.0002	0.92	0.01	0.45
Arterial phase density	0.12	0.004*	0.01	0.42
A/P ratio	0.11	0.006*	0.004	0.63
Size of pancreatic duct	0.21	<0.0001*	0.04	0.13

*$p<0.05$, significant difference

Endocrine Function and CT Parameters at 1 Year After PD

Out of 32 patients whose CT at 1 year after PD including SSPPD and HPD was evaluated, seven patients suffered from diabetes (Table 3). Out of 25 patients who did not have DM before operation, two patients developed DM after PD. On the other hand, two out of seven patients with preoperative DM, recovered from DM after PD. CT parameters of the residual pancreas correlated with the endocrine function.

Mean residual rate of the pancreas after PD was 37.2±3.6% and there was no significant difference between preoperative DM status (37.2±11.5% in DM group vs. 37.8±3.3% in non-DM group, $p=0.47$; data not shown). The correlation between CT parameters at 1 year after operation and the postoperative diabetic outcome was analyzed. In the patients of who did not have DM preoperatively, the volume of pancreas and volume of pancreas/body weight were significantly higher in postoperative non-DM subgroup than in postoperative DM subgroup ($p=0.01$) and the arterial density ($p=0.28$) and A/P ratio ($p=0.06$) tended to be higher in postoperative non-DM subgroup. The size of pancreatic duct was narrower in postoperative non-DM subgroup ($p=0.14$), suggesting that postoperative long-term diabetic outcome also associates with the favorable CT parameters as well as the preoperative condition (Table 3). Out of seven patients who had DM preoperatively, two patients resumed the endocrine function. The CT parameters of these two patients were very close to those of patients in pre- and postoperative non-DM subgroup (Table 3).

We applied preoperative CT parameters to ROC curve to define the best cutoff value to predict the postoperative DM status. As a result, the cutoff lines of each parameter were defined as follows; volume of pancreas, 69.2 cm^3; volume of pancreas/body weight, 1.16 cm^3/kg; arterial density, 109.0 (data not shown); portal density, 97.9 (data not shown); A/P ratio, 1.04; and size of pancreatic duct, 3.05 mm (Table 4). The ratio of postoperative DM occurrence according to each cutoff line regardless of preoperative DM condition is shown in Table 4. If the patient had pancreas larger than 69.2 cm^3, the probability of postoperative DM after standard Whipple resection (residual rate 37.2±3.6%) is quite low. Similarly, pancreas volume/body weight >1.16 cm/kg body weight, A/P ratio >1.04, and main pancreatic duct narrower than 3.05 mm are favorable factors for avoiding postoperative diabetes.

Of course the postoperative diabetes depends of the rate of residual pancreas. When over 80% of the pancreas was resected, four out of six patients had DM (66.7%, $p=0.12$) and all the patients who underwent over 90% pancreatectomy had DM (100%, three out of three, $p=0.02$).

Table 3 Outcome of endocrine function and CT parameters at 1 year after PD

Preoperative DM	Non-DM (N=25)		p value	DM (N=7)		p value
Postoperative DM	Non-DM (N=23)	DM (N=2)		Non-DM (N=2)	DM (N=5)	
Residual rate of pancreas (%)	38.0±3.6	36.5±6.3	0.45	24.8±0.1	43.4±16.4	0.27
Residual volume of pancreas (cm^3)	60.1±4.7	17.5±3.5	0.01*	59.6±30.4	29.4±6.0	0.14
Residual volume of pancreas/body weight (cm^3/kg)	1.0±0.1	0.35±0.1	0.01*	0.9±0.4	0.6±0.1	0.23
Arterial phase density at 1 year	101.7±4.2	92.5±11.7	0.28	95.1±8.8	84.5±10.3	0.31
A/P ratio at 1 year	1.1±0.0	0.8±0.0	0.06	1.1±0.1	0.9±0.1	0.11
Size of pancreatic duct (mm) at 1 year	4.0±0.6	6.4±1.0	0.14	2.5±0.6	5.2±1.0	0.11

Median±SD

*$p<0.05$, significant difference

Table 4 Validity of cutoff lines of preoperative CT parameters to predict postoperative DM

Preoperative CT parameters	Cut-off value	Rate of postoperative DM (%)		
		Over cutoff line	Under cutoff line	*p* value
Volume of pancreas	69.2 cm^3	0	31.8	0.04*
Volume of pancreas/body weight	1.16 cm^3/kg	0	31.8	0.04*
A/P ratio	1.04	7.1	35.3	0.06
Size of pancreatic duct	3.05 mm (the less the better)	7.7	31.6	0.11

*$p<0.05$, significant difference

Discussion

CT is the most widely accepted modality for the assessment of hepatobiliary and pancreatic disease and is useful for deciding the strategy of treatment. However, there has been no study about the evaluation of pancreatic function using CT imaging. We tried to reveal the usefulness of CT for assessment of the pre- and postoperative pancreatic endocrine function in this study. Since the perioperative management has been improved, long-term functional assessment is becoming more important. If the function can be estimated using preoperative CT examination, supplemental treatment for diminished function before and after operation can be decided more easily. This estimation may make it possible to decide the appropriate resection line to prevent postoperative DM beside the tumor extent. Thus, CT imaging for evaluation of the pancreatic function should be considered.

At first, we decided CT parameters for the assessment of endocrine function in volume aspect (volume of pancreas, volume of pancreas/body weight, and pancreas parenchymal rate), in density aspect (arterial and portal phase density and A/P ratio), and size of pancreatic duct. Volume parameters are postulated to represent the number of islets. On the other hand, density parameters were aimed to indicate the condition of blood flow in pancreas. Moldovan and Brunicardi[18] reported that glucose administration significantly increased blood flow of islet mass and islet during hyperglycemia received 85% more than during the basal condition. Thus, islet with good blood flow can be hypothesized to have a good function. In our study, arterial high density and high A/P ratio correlated well with endocrine function suggesting that well-established vascularization in pancreas with good islets. Though the CT parameters after PD were obtained 1 year after the operation, they revealed over 100 of arterial phase density and over 1.0 of A/P ratio seems to prevent postoperative DM onset (Table 3).

It is ideal for a surgeon to preserve pancreatic parenchyma as much as possible to maintain a good endocrine and exocrine pancreas if the sufficient tumor eradication was achieved. Johhanson et al. revealed that islets could secrete enough of insulin to maintain the blood glucose level in 50% pancreatomized rat model.[19,20] If the residual pancreas has good arterial phase density and higher A/P ratio, the postoperative diabetic control may require less insulin. Even in the patients with duct dilatation, the higher arterial phase density could contribute less insulin requirement. ROC curve for CT parameters to predict postoperative DM gave us the cutoff lines as shown in Table 4. Patients did not develop DM when the residual pancreas satisfy the parameters as follows; residual volume of pancreas >69.2 cm^3, volume of pancreas/body weight >1.16 cm^3/kg, arterial phase density >110, and A/P ratio >1.04 with narrower duct size (<3.05 mm; Table 4). Interestingly, the preoperatively diabetic patients who resumed endocrine function after operation showed similar CT parameters suggesting that these values are the sufficient requirement to keep the endocrine function. The limitation of this study is that the postoperative CT parameters at 1 year are a consequence of various postoperative factors. All patients underwent modified Child method duct-to-mucosa pancreaticojejunal anastomosis using 6-0 absorbable monofilament suture with or without pancreatic duct stent. The influence of postoperative morbidity including pancreatic fistula and the patients who died of the primary disease within a year were not included in the analysis. Also the dietary intake, supplemental pancreatic enzymes, adjuvant, or therapeutic chemotherapy greatly affects the postoperative diabetic condition of these patients.

The patients who underwent over 80% of pancreatectomy had high DM rate (66.7%) and all the patients who underwent over 90% of pancreatectomy became DM (data not shown). If the residual pancreas has sufficient ability to control blood glucose, the minimal residual rate could be assigned to 20% according to the current study.

CT parameters were very useful data for evaluating endocrine function. Higher volume of pancreas with or without correction by body weight could be detected in preoperative and postoperative non-DM patients (Fig. 4a, b and Table 4). Regarding density parameters, we also knew that pancreas with higher arterial density of the pancreas and rapid enhancement (=higher A/P ratio) have the better endocrine function than that with lower (Fig. 4). We conclude that volume and density are the important factors in order to decide the endocrine condition. Pancreatic parenchymal rate did not correlate with endocrine parameters

in this study. This may be because there were large ranges in endocrine data at the same level of occupying rate.

It is difficult to quantify the parenchymal texture even after the operation. We are using the diameter of pancreatic duct as a representative parameter for parenchymal texture. Dilated pancreatic duct is often detected in the patients with chronic pancreatitis and pancreatic head cancer. Pancreatic tissue in primary or secondary pancreatitis becomes hard due to fibrosis and may be of harsh environment for islets to control blood glucose. In the current data, blood glucose and HbA1c level tended to be higher in wider pancreatic duct group in spite of no significant difference.[21] Thus the size of pancreatic duct may be another predictor of postoperative diabetic onset.

This is the first report that describes preoperative CT volumetrical analysis, volume of pancreas, volume of pancreas/body weight, arterial phase density, A/P ratio, and size of pancreatic duct are very useful to estimate the pancreatic endocrine function with combination with blood glucose and HbA1c CT volumetrical analysis also helps to estimate the postoperative onset of DM by revealing the condition of pancreas before operation. In conclusion, CT is a useful modality not only for the evaluation of pancreatic disease but also for evaluation of the pancreatic endocrine function. In order to achieve function preserving operation with appropriate perioperative diabetic management, preoperative evaluation of CT volumetry and pancreatic endocrine function are warranted. Also, assessment the correlation between preoperative CT findings and postoperative DM in prospective study will be necessary as the next step.

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GI IMAGE

Laparoscopic Appendectomy for Amyand's Hernia: A Modern Approach to A Historic Diagnosis

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Abstract
Objectives This study seeks to discuss the management and diagnosis of Amyand's hernia, an exceedingly rare diagnosis.
Methods The case of a 60-year-old female found to have inguinal appendicitis on preoperative computed tomography imaging is presented.
Results The patient underwent concomitant laparoscopic inguinal hernia repair and appendectomy.
Discussion Laparoscopic management of Amyand's hernia should be strongly considered for repair and resection.

Keywords Appendicitis · Inguinal hernia · Laparoscopy

Clinical History

A 60-year-old female presented with a 1 day history of abdominal pain accompanied by anorexia, nausea, and vomiting. She denied fevers or chills at home. During the time preceding her presentation to our institution, she reported that her pain was becoming increasingly intense in her right lower quadrant. On physical examination, her abdomen was minimally tender in the right lower quadrant but without evident peritoneal signs. Additionally found on clinical exam was a tender right inguinal mass. Laboratory work was significant only for a leukocytosis of 12,500.

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Concern was immediately for incarcerated femoral hernia. However, given her history of periumbilical pain migrating to the right lower quadrant in the face of leukocytosis, there was great concern for an intraperitoneal process; namely appendicitis. For this reason, a high resolution helical computed tomography (CT) scan was performed of her abdomen and pelvis with imaging to mid-femur. The images clearly demonstrated a right inguinal hernia containing the distal portion of the appendix (Fig. 1).

The patient was taken to the operating room for laparoscopic appendectomy. At this time, the appendix was found to be passing through the internal inguinal ring (Fig. 2). An appendectomy and inguinal hernia repair were completed laparoscopically without complication. Final pathology confirmed acute appendicitis with necrosis of the distal appendix. The patient was discharged home on postoperative day 1.

Discussion

The first described appendectomy was performed in 1735 by Claudius Amyand at St. George's Hospital in London.[1–8] The patient was a small boy who presented with the apparent symptoms of an incarcerated inguinal hernia. Upon exploration, it was immediately evident that the patient instead suffered from appendicitis in an unusual location. Authors, since this initial description, note that appendicitis in the inguinal canal occurs in

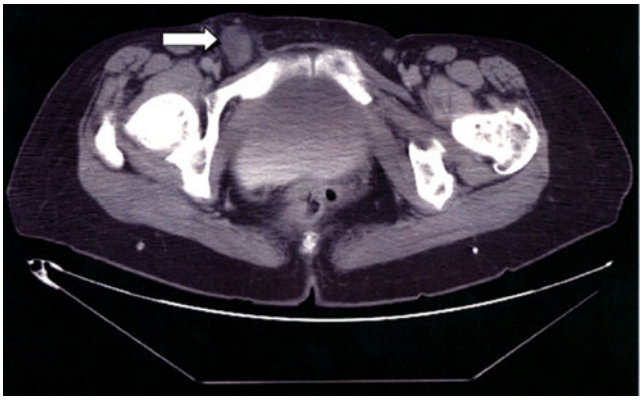

Fig. 1 Representative axial slice of CT scan. Inflamed appendix is noted within inguinal canal (*white arrow*)

approximately 0.1–0.3% of cases of acute appendicitis.[4] It is thus an exceedingly rare culmination of the two most common general surgery diagnoses.

The presentation of Amyand's hernia is generally quite similar to our patient. The natural history begins with typical visceral symptoms, many of which are ignored by patients. As the course progresses, the patient generally reports intense groin pain that does not abate rather than acute abdominal pain. The physical exam findings are nearly always consistent with incarcerated inguinal hernia.

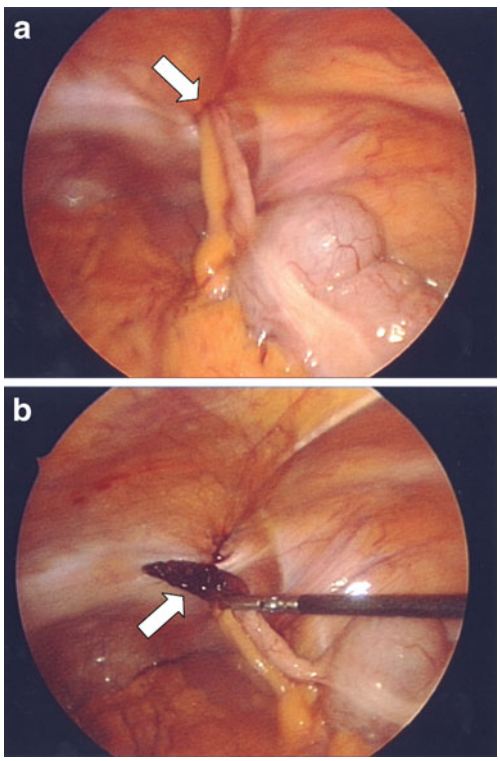

Fig. 2 Intraoperative view of pathology. **a** Appendix coursing through internal inguinal ring (*white arrow*). **b** Having successfully reduced the appendix from the inguinal canal, the *tip* is clearly necrotic (*white arrow*)

With the widespread use of helical CT scans in current practice, the diagnosis of appendicitis in the inguinal canal is made increasingly preoperatively.

Operative management and postoperative course should not differ from patients with the separate diagnoses of appendicitis or incarcerated inguinal hernia. The approach to operative repair of Amyand's hernia has historically been described as via a groin incision; in essence, a standard open herniorraphy approach. This approach remains from a time when helical CT scans were not as widely available and thus the preoperative diagnosis was nearly always incarcerated inguinal hernia. This is not the case today. With the widespread availability of high quality CT scans, there should not be uncertainty regarding the preoperative diagnosis. Herniorraphy and appendectomy may safely be undertaken simultaneously, and several authors report preference for non-mesh repair as the field is nearly always contaminated.[1, 8, 9] In one large series, risk of recurrence was no greater in those patients with a non-mesh, tension-free repair, but the incidence of postoperative infection was nearly 50% in those with mesh repair.[6]

In our case, we chose to perform laparoscopy initially to confirm the diagnosis, insure no other intraperitoneal pathology, and finally, as a method of operative repair. We believe that laparoscopy can be an efficacious and safe method for undertaking an operation for Amyand's hernia. Initially, this approach allows for identification of potentially more serious intraperitoneal processes. Should the preoperative diagnosis be confirmed, resection and repair can be undertaken with minimal morbidity to the patient. With current widespread experience in laparoscopic hernia repair and appendectomy, it seems only reasonable to address such a historic diagnosis with such a modern technique.

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LETTER TO THE EDITOR

Comments on the Article About Recurrence After Surgical Management of Liver Hydatid Cyst

Enver Zerem · Nermin Salkic · Alma Sušić · Bahrija Haračić

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Dear Editor,

We read with great interest the article by El Malk HO et al.[1] published in issue 7 of the *Journal Gastrointestinal Surgery* 2010, in which the authors presented a substantial experience in surgical treatment of liver hydatid cyst (LHC). They retrospectively analyzed 672 consecutive patients that underwent surgery due to LHC during a 15-year period and also evaluated predictive factors associated with hepatic recurrence of LHC after surgery.

The authors specified that:

"surgery remains the basic treatment for liver hydatid cyst (LHC). The intended goals of this surgical treatment are to ensure complete elimination of the parasite and prevention of recurrent disease with lower morbidity and mortality. Recurrences can occur after all therapeutic methods including percutaneous treatment, chemotherapy with benzimidazole compounds, or surgery...Surgery for recurrence of LHC is technically more difficult due to adhesions arising from previous surgeries, which increase considerably the morbidity and mortality rate of this procedure."

In their study, 56 patients (8.5%) had LHC recurrence after surgery. They concluded that the surgeon's practice and level of experience are the most important factors for both success of the surgical treatment and prevention of complications and recurrences.

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However, we would like to point to several important issues regarding the LHC recurrence reported in this study. From our point of view, after a long experience in performing a percutaneous method (PAIR) in the treatment of LHC,[2,3] we believe that confirmed recurrence of vital LHC after devisceration (regardless of the applied PAIR or surgical method) seldom occurs and the reported recurrence rate of 8.5% in this study is too high. We have performed over 400 PAIR interventions for LHC and had only one confirmed recurrence of live LHC after PAIR treatment.

There are several possible reasons for this. Firstly, the authors did not confirm that recurrent LHC was indeed the vital cyst. This may be the consequence of the somewhat vaguely described criteria that defined the recurrence. Additionally, in Table 1 the authors specified that over 80% of recurrent cysts after surgery were larger than 10 cm in diameter (median duration of recurrence diagnosis was 24 months (IQR, 10–48 months) and in Table 2 they presented that 26 recurrent cysts had a longest diameter over 10 cm 2 years after the procedure, but if it is well-known that hydatid cyst growth is a rather slow process (about 2 cm per year)[4] which raises questions regarding the reliability of the above-mentioned data. Moreover, Gharbi's morphologic type I of the cyst was diagnosed in 16.1% cysts only. This may suggest that some of recurrent cysts were misdiagnosed as the recurrent hydatid cysts rather than the residual cavity or cystic formation due to biliocystic communication after initial LHC surgery.

The role of albendazole in the prevention of recurrence remains an additional factor that may explain high rates of recurrence. Still, the authors did not present the data regarding

the use of albendazole in treated patients in the periods both before and after the initial surgery for LHC. It is also not clear why the authors omitted such an important factor from the Cox analysis.

At the end, as firm advocates of non-surgical approach in the treatment of LHC, we feel it is necessary to emphasize that surgery can be considered as basic treatment of LHC only in areas where less invasive modalities such as ultrasound-guided PAIR method are not available.

Conflict of Interest All authors have no conflict of interest.

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LETTER TO THE EDITOR

Response to the Comment: Surgery of Liver Hydatid Cyst's Recurrence is Always More Difficult

Hadj Omar El Malki · Amine Souadka · Belkacem Zakri · Yasser El Mejdoubi · Raouf Mohsine · Lahcen Ifrine · Redouane Abouqal · Abdelkader Belkouchi

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Dear editor,

We read with great interest the Letter to the Editor: Comments on the article about recurrence after surgical management of liver hydatid cyst by Enver Zerem et al. regarding our manuscript[1] published in the *Journal of Gastrointestinal Surgery*. We first want to thank you for allowing us to answer this letter to the editor and to explain our point of view.

Surgery remains the treatment of choice of liver hydatid cyst (LHC).Our rate of 8.5% of WTC recurrence is not high, it is an average rate of recurrence in all surgical studies ranging from to 4.5% to 30%.[1]

This rate may be explained by the fact that our center is a tertiary center which receives patients from all over the country with no selection of patients as reported in previous papers.[2,3] Our series is a retrospective study of all LHCs surgically managed in our department. At the opposite, in the Zerem study[4] performing ultrasound-guided puncture, aspiration, injection, and reaspiration (PAIR) method in the management of highly selected patients with types I and III Gharbi's classification LHC, it is possible to assess this very low rate of recurrence, excluding initially complicated cysts.

The diagnosis of LHC recurrence was assessed preoperatively during routine surveillance ultrasonography and then confirmed by a 6-month control ultrasonography associated with an abdominal CT scan showing either the same image or the worsening of the lesion. In doubtful cases, fine needle aspiration was performed to confirm the diagnosis.

During surgery, after covering and isolating the area, the cyst was incised at its most accessible part and all its content was aspirated. We insured a total removal of germinative membrane with forceps which definitely proved the real nature of the recurrence and eliminated any other differential diagnosis (residual cavity or biliary collection) even for the 16% of Gharbi's type I of LHC.

Surgery for recurrences is always more difficult due to structural and anatomical modifications. Based on these general conclusions, we can easily imagine that the development of LHC recurrence may be perturbed and may escape the rule of 2 cm per year process.[5] This may explain our 80% rate of cysts with a diameter greater than 10 cm. Moreover, new cysts can arise in free hepatic parenchyma but merge with old residual cavities, which can enhance the diameter of these new cysts. Finally, some small cysts may be unapparent at the first surgery and have fully the time to grow and to appear as a recurrence.

Indeed, the use of Albendazole perioperatively can help to lower this rate of recurrence, but in Morocco, it was only recently introduced during these last 4 years. This factor was analyzed in univariate analysis, but we

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did not find any significant statistical difference, thus, the reason why we did not include these patients in our COX regression multivariate analysis. We did not report these data in the manuscript because we think that it is a weak result due to our poor experience with the use of this chemotherapy.

At the end, as a firm advocate of the conservative surgical approach in LHC management, we feel that it is necessary to point out that surgery remains the treatment of choice of complicated LHC. Moreover, even if it seems to be an easy procedure for a benign disease, it should be performed in specialized centers accustomed to liver surgery to ensure the patients' better outcomes all over the world. Ultrasound-guided PAIR method may still be an option, in well-equipped centers, in the primary management of noncomplicated cysts in some highly selected patients.

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ERRATUM

Erratum to: Predicting Organ Space Surgical Site Infection with a Nomogram

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

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Erratum to: J Gastrointest Surg
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An author's name was inadvertently misspelled in this paper, the first author should be:

Luiz F. de Campos-Lobato.
The corrected author list for this paper is:
Luiz F. de Campos-Lobato, Brian Wells, Elizabeth Wick, Kevin Pronty, Ravi Kiran, Feza Remzi and Jon D. Vogel

The online version of the original article can be found at http://dx.doi.org/10.1007/s11605-009-0968-6.

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